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 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 different explanation of IgE-mediated reactions to drugs is proposed: These are a) A natural role of reduced mast cell (MC) reactivity in developing IgE-mediated reactions to drugs. This MC-unresponsiveness is antigen-specific and covers the serum drug concentrations, but allows reactivity to locally higher concentrations. b) Some non-covalent drug-protein complexes rely on rather affine bindings and have a similar appearance as covalent hapten-carrier adducts. Such drug-protein complexes represent so-called "fake antigens", as they are unable to induce immunity, but may react with and crosslink preformed drug-specific IgE. As they are formed very rapidly and in high concentrations, they may cause fulminant MC degranulation and anaphylaxis. c) The generation of covalent hapten-protein adducts requires hours, either because the formation of covalent bonds requires time or because first a metabolic step for forming a reactive metabolite is required. This slow process of stable adduct formation has the advantage that it may give time to desensitize mast cells, even in already sensitized individuals. The consequences of this new interpretation of IgE mediated reactions to drugs are potentially wide-reaching for IgE-mediated drug allergy but also allergy in general.

Anaphylaxis to drugs: overcoming mast cell unresponsiveness by fake antigens

Short title: mast cell unresponsiveness and fake antigens

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Abbreviations: antigen-presenting cell: APC; basophil activation test: BAT; dendritic cell: DC; drug hypersensitivity reactions: DHR; fake antigen reactions: FAR; high affinity receptor for IgE: FcεRI; human leukocyte antigens: HLA; human serum albumin: HSA; mast cell: MC; neuromuscular blocking agents/muscle relaxants: NMBA; Proton pump inhibitor: PPI; radiocontrast media: RCM; sulfamethoxazole: SMX; T cell receptor for antigen: TCR

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a) A natural role of reduced mast cell (MC) reactivity in developing IgE-mediated reactions to drugs. This MC-unresponsiveness is antigen-specific and covers the serum drug concentrations, but allows reactivity to locally higher concentrations.

b) Some non-covalent drug-protein complexes rely on rather affine bindings and have a similar appearance as covalent hapten-carrier adducts. Such drug-protein complexes represent so-called "*fake antigens* ", as they are unable to induce immunity, but may react with and crosslink preformed drug-specific IgE. As they are formed very rapidly and in high concentrations, they may cause fulminant MC degranulation and anaphylaxis.

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The consequences of this new interpretation of IgE mediated reactions to drugs are potentially wide-reaching for IgE-mediated drug allergy but also allergy in general.

I Introduction:

Drug hypersensitivity reactions (DHR) are immune or inflammatory reactions elicited by a small molecule and occasionally proteins etc.. DHR can be sub-classified as a specific immune reaction against the drug acting as antigen with drug-specific stimulations of antibodies or T-cells (*drug allergy*), as off-target *p* harmacological activity of drugs with *i* mmune receptors (human leukocyte antigens, HLA or T cell receptor for antigen, TCR) leading to T-cell mediated immune stimulations (*p-i-concept*) and as pseudo-allergic reactions where the drug therapy results in activation of inflammatory cells or mediators without the involvement of the specific immune system ("*pseudo-allergy* ") (1).

The clinical picture of DHR is very heterogeneous as different cell types (T-cells, mast cells (MC), basophils, eosinophils, neutrophils, etc) and inflammations are involved (2,3,4). This report focusses on IgE-mediated adverse reactions to small molecules, normally <1000 D. They represent an uncommon, but potentially dangerous complication of drug therapy. Symptoms appear rapidly after drug exposure and include generalized urticaria, angioedema, bronchospasm and anaphylaxis with respiratory and/or gastrointestinal symptoms, cardiac arrest and even death. Indeed, drug elicited anaphylaxis is considered to be particularly dangerous with a high rate of deadly outcomes (5).

The underlying mechanism of IgE-mediated drug allergy is based on the hapten concept. It was developed more than 80 years ago by K Landsteiner and others, stating that small molecules like drugs or other chemicals are too small to function as antigen for the immune system (6). Only if the drug acts as a "hapten" and binds to a protein and thus forms a larger drug-protein adduct, it functions as antigen to which immune reactions, including IgE, may develop. This hapten-protein (or hapten-carrier) concept relies on the ability of the drug (or metabolite) to bind via *covalent* bonds *stably* to a protein. The immunity may persist for

years after stopping therapy. Importantly, the potentially severe symptoms mentioned above do not happen during the sensitization and only may become apparent through a new exposure/re-challenge.

This hapten-carrier concept was validated in an endless number of experiments. It was used to investigate immunity in animal models of autoimmunity, cancer immunology, allergy, and specific immunity to small molecules, etc.. It also served as an explanation for IgE-mediated drug allergy in the clinic: For example, allergy and clinical manifestations after beta-lactam therapy were explained by the hapten-carrier concept (7).

Since only haptens were considered as potential elicitors of drug allergy, drugs in development were assessed for their ability to covalently bind to proteins (8, 9). To reduce the risk for DHR only the development of drugs *not* capable of forming covalent bonds with proteins was pursued. Nevertheless, drug-induced allergy and in particular IgE-mediated anaphylaxis remained a substantial clinical problem. Anaphylaxis to beta-lactams, to proton-pump inhibitors (PPI), to neuromuscular blocking drugs (NMBA), to disinfectants like chlorhexidine, to radiocontrast media (RCM) and many more still occurs (2, 7, 10-11). Additionally, other ways of mast cell stimulation and degranulation by drugs were recently described, such as reactions triggered by mast cell-specific G protein-coupled receptors (MCGPR) (12).

A critical evaluation of patients with IgE-mediated allergy/anaphylaxis to drugs reveals some inconsistencies in the prevailing concepts, in particular regarding the symptoms during re-challenges: many of the drugs causing anaphylaxis are per se not haptens, but inert chemicals, not able to form covalent bonds; some might have acquired hapten characteristics by metabolism (e.g. sulfamethoxazole, 13). Most importantly, some of the reactions occur very fast before covalent binding or metabolism can occur. Of note as well, the immediate reaction in skin tests or a positive in-vitro basophil activation test (BAT) to an inert drug can not be explained by the hapten-concept. The involved drugs are not "haptens" - leaving open how cross-linking of specific IgE, MC degranulation, and symptoms of IgE-mediated reactions are elicited by the drug (1).

This paper addresses some of these inconsistencies comparing clinical observations to accepted features of IgE-mediated reactions. Such observations comprise the rapidity of the appearance of symptoms, in- vitro and in-vivo diagnosis of drug allergy, experience with desensitizations, pharmaceutical features of drugs and speed of covalent vs non-covalent drug binding to proteins. The result is *a new interpretation of IgE mediated drug allergy* : It extends the hapten concept and postulates: i) When IgE is induced, the antigen simultaneously induce an MC-unresponsiveness. ii) Some non-covalent bindings of drugs to proteins are affine enough to allow IgE cross-linking by the formed complexes; iii) the formation of covalent hapten-protein adducts *in vivo* is slow and may allow MC-unresponsiveness both during sensitization and even during re-exposure: No symptoms occur.

The new concept is radical as covalent hapten-protein adducts are considered to be "good" (controlled) antigens, which, although they induce an unwished immunity, do simultaneously induce MC-unresponsiveness; In contrast, non-covalent drug-protein complexes are taking the role of "fake antigens" responsible for harmful effects. The consequences of this new interpretation are potentially wide-reaching both for IgE-mediated drug allergy but also for IgE reactions and symptoms in general.

II Drugs and the immune system

Non-covalent precedes covalent interaction between drug and protein

Small molecules like drugs bind to proteins, e.g. human serum albumin (HSA). The attachment of a drug to a protein occurs first via non-covalent bonds (table 1 and figure 1) (14-16). These are the dominant type of intermolecular forces in supra-molecular chemistry and rely on van der Waals forces, electrostatic interactions, ion pairs, and hydrogen pairs. Even though they are weak individually, their cumulative energies of molecular interactions can be significant. The bindings are formed very rapidly, are reversible and the molar concentrations of the protein [P], ligand [L], and complex [LP] respectively are determined by the dissociation constant: Kd= [L][P]/ [LP]. Thus, the affinity of interaction and the concentrations of drug and protein determine the number of complexes formed.

After an *initial* non-covalent binding the drug may bind by a covalent bond to a certain amino acid within the protein (figure 1). This feature depends on the chemical property of the drug. Other drugs cannot bind directly, but gain this property by metabolism (13).

A drug or drug metabolite able to bind by covalent bonds to a carrier molecule/protein is called a hapten. Covalent bonds involve the sharing of electron pairs between atoms. The formation of such bonds may take some time as studies with penicillin revealed (17-19). Under optimized in-vitro conditions (pH 10.2), the first bond of a beta-lactam like penicillin G (as penicilloyl or penicillenic acid) to lysine 199 of human serum albumin (HSA) can be observed at 20 min. At physiological pH 7.4 bonds are seen after 60 min or later and the process continues in the following hours. It is not readily reversible. The resulting hapten-modified protein ("adduct") represents a new antigen, to which an immune response can be developed.

Of note, the drug-protein complexes or adducts based on non-covalent or covalent bindings have a very similar "appearance", as the location of binding and the orientation of the drug versus protein may be the same in non-covalent and covalent bindings (14). Consequently, an antibody initiated by the covalent hapten-protein adduct may recognize both, the complex formed by covalent bonds and the complex formed by non-covalent bindings (figure 1).

Drug-protein adducts based on covalent bonds are necessary to stimulate the immune system.

To initiate an IgE-immune response to a small drug-like a beta-lactam, a complex interplay of antigen-presenting cells (APC), T cells and B cells takes place (1,7). Neither the drug itself (too small) or the protein (often a self protein, to which tolerance exists) elicit a reaction. It is the novel hapten-protein adduct, which represents the antigen, which stimulates B- and T cells. For T-cell stimulation, the hapten-protein adduct is processed inside APC into smaller peptides and then presented on HLA. These immunogenic peptides keep the drug bound to the amino-acid only if the bonds between peptide and drug are covalent and stable.

For instance, amoxicilloyl- albumin is taken up by dendritic cells (DC) and/or B-cells acting as APCs. This hapten-protein adduct is processed inside the APC to peptides. Due to the covalent link between the hapten and protein/peptide, the peptides resulting from processing and presented to T cells still carry the amoxicilloyl group. A non-covalent bond between drug and protein would be disrupted by intracellular processing. This presentation of new (drug- modified) peptides stimulates T-cells, which secrete IL-4/IL-13 to provide help for B-cell maturation into IgE-producing plasma cells. The secreted specific IgE binds immediately to the high affinity Fc-receptor for IgE (FcεRI) on mast cells (MC) and basophils the individual is sensitized (figure 3). But even if therapy is continued, no symptoms appear.

During sensitization (IgE), desensitization of IgE/MC reactivity to the hapten-carrier complex develops

A cornerstone of the new interpretation of (drug) allergy is the hypothesis that the sensitization of MC by a *gradual* increase of specific IgE in the presence of antigen reduces MC reactivity to the specific antigen (1) (table 2, figure 2). The underlying mechanism of this *antigen-specific unresponsiveness* is not clear. It could be similar or identical to the process of drug desensitization: In*in-vitro* models it has been shown that this antigen-specific process blocks calcium flux, impacts the antigen/IgE/FcεRI complex-internalization and prevents the acute and the late-phase reactions as well as mast cell mediator release (20). Importantly, this unresponsiveness of MCs is specific for the antigen-IgE complex, while MC-reactivity to other antigens by IgE cross-linking persists. The MC unresponsiveness just covers the antigen concentration used for inducing unresponsiveness, normally determined by the serum concentration of the drug. When the MCs that carry specific IgEs are confronted with a suddenly higher concentration of specific antigen than the tolerizing dose, the unresponsiveness of MCs is broken and the MCs react/degranulate. This phenomenon is known as "breakthrough reaction" in drug desensitizations. It occurs, when the last increase of the drug concentration was too large (21). When no antigen exposure occurs, this MC-unresponsiveness is decreasing over time and an allergic reaction may re-appear to previously tolerated antigen concentrations: MC-unresponsiveness can be re-adjusted by a natural exposure: e.g. the first bee stings in spring in already sensitized beekeepers may cause urticaria, but these generalized reactions to stings disappear in the following weeks (table 2) (22) and

may also disappear by intended antigen-exposure ("immunotherapy") (22,23).

Importantly, this MC-unresponsiveness may represent the *normal*response when IgE is formed to protein antigens (allergens) and the antigen is still present (table 2). It is different from the long-lasting T cell-based tolerance mechanism (24), as it is based on the unresponsiveness of MCs and probably also basophils, both carrying IgE-FcεRI. The concept of MC-unresponsiveness also implies that at least one scope of allergen-specific IgE is to react via MC to a*locally* relatively high allergen concentration, but *not* to normal, systemically available levels of allergen.

MC-unresponsiveness could also explain the high number of sensitized but not allergic individuals in various studies on the prevalence of allergic diseases (25). Sensitization is often identified by positive skin tests (prick, i.d.), where locally an excess amount of allergen is applied (26). The concentrations used for skin tests break MC-unresponsiveness and results in a local wheal and flare reaction. Since epidemiological studies revealed that about half of the skin test-positive individuals do not show symptoms to pollens (seasonal rhino-conjunctivitis), they may be unresponsive to the usual concentrations of pollen allergens reaching the tissue (26, 27). However, they react to the high local allergen concentrations applied in skin tests. This suggests that the difference between allergic (sensitized and symptomatic) and sensitized (but asymptomatic) individuals is that the amount of allergen reaching the tissue is higher in allergic individuals. More likely seems that in allergic individuals the IgE coated MC react to lower local concentrations of allergen; IgE mediated allergy is thus a) defined by the formation of antigen-specific IgE, *and* b) the MC-(un)responsiveness to the antigen/allergen reaching the tissue is not well adjusted.

Drug-protein complexes based on non-covalent bindings can cause degranulation of sensitized MC.

The necessity of covalent bonds between drug and protein is a prerequisite for initiating an immune response to the drug/hapten, both in animal models as well as in humans. It is also observed for eliciting an MC degranulation in previously sensitized animals using *in vitro* prepared hapten-protein adducts. However, it has not been established, whether a non-covalent bound complex is sufficient for interaction and cross-linking specific IgE in already sensitized animals or not. One reason might be, that the experiments with relatively labile drug-protein complexes did not deliver consistent, reproducible results. It is an exception that some of the non-covalent bindings between drug and protein reach an affinity which makes cross-linking of IgE/FcεRI possible. Thus, symptomatic IgE mediated drug allergy is rare.

The main arguments for the role of non-covalent drug-protein complexes in drug re-exposure reactions are summarized in table 3. Immediate skin test reactivity to drugs like beta-lactams (<15 min) occurs before covalent binding between beta-lactam and protein takes place (17-19). The necessity of covalent bonds cannot explain the immediate reactivity seen in BAT (<5 min) and the clinical setting (anaphylaxis <5 min).

For some drugs, metabolism is required to form the reactive metabolite: E.g., sulfamethoxazole (SMX) is metabolized in the liver to sulfamethoxazole hydroxylamine (SMX-NHOH), which is further oxidated in the tissue to the reactive hapten SMX-NO, able to undergo covalent bonds (13). This metabolism lasts >6-10 hrs (13). But skin test reactivity and in-vitro BAT can be observed within 15min with SMX itself, which does not have hapten characteristics. The IgE detected is cross-reactive with covalently bound SMX-NO and non-covalently bound SMX. Such a cross-reactivity of the immune system has already been observed in T-cell reactions to SMX and SMX-NO (28).

It's all about drug-protein binding: fake antigen in drug allergy

A comparison of covalently and non-covalently linked drug-protein complexes points out that the *kind* of drug-protein binding may determine whether "*silent" immunity* or *symptomatic allergy* evolves.

Hapten-protein adducts, based on covalent bonds, represent novel antigens, which can induce a complex immune response, including IgE. IgE reactions are not per se "harmful", even if we associate them with the very common, annoying allergies. The first encounter with the antigen is often unspectacular and

sensitization remains unnoticed since sensitization goes along with MC-unresponsiveness. In most IgE-reactions, this tight control may persist and no symptoms appear.

Not only the induction of immune response but also the reaction at re-exposure may be mitigated by the type of antigen. The formation of covalent bonds is a comparatively slow process, and for some drugs, even a metabolic step is intermingled, before complex-formation can start. Moreover, the number of antigenic epitopes are limited to those protein sites able to accommodate covalent binding. Thus, if the IgE reactivity is directed to haptens exclusively, the formation of antigen-complexes is slow (hours) and the amount of antigen is limited. Both conditions would favor the induction of MC-unresponsiveness at re-exposure again and no symptoms would occur.

The situation is different if the non-covalent drug-protein complexes are

1. relatively stable,
2. can interact with preformed IgE, and
3. even cross-link the FcεR-bound IgE:

Such complexes *pretend* to be relevant antigens but are in reality "**fake antigens"** . They are formed very rapidly and in high concentrations (see table 3) and thus overrule MC-unresponsiveness: an uncontrolled MC-degranulation/**"fake-antigen reaction" (FAR)**with urticaria, angioedema and anaphylaxis may occur (figure 3).

Fake antigens and FAR/anaphylaxis

It is unclear what the clinical benefit of anaphylaxis may be. Perhaps there is none, and systematic MC-degranulation is not a valuable option in immune defense as it should not happen. If it occurs, it may be by mistake, as the immune system recognizes a fake antigen as a true antigen.

Consequently, drugs or drug metabolites causing anaphylaxis (e.g. beta-lactams, chinolones, chlorhexidine,metamizole, muscle relaxants, PPI, RCM, SMX/SMX-NO, etc.) are characterized by two features:

1. ability to bind covalently to proteins and to form an antigen, which is needed to induce IgE;
2. ablility to form a sufficiently affine non-covalent complex (*fake antigen*), which reacts with and cross-links the preformed IgE.

In up to 50% of patients with drug induced anaphylaxis, a prior exposure to the drug is not documented ("anaphylaxis at first sight") (2, 11): Some reactions might be IgE independent (12). But if IgE was involved, the IgE might have been induced by a different compound, which happen to (cross-)react with the newly formed fake antigen. Under these special conditions even a drug, which is per se *not* able to form an antigen and to induce IgE, may cause anaphylaxis.

The following conditions may additionally favor anaphylaxis by fake antigens (FAR): providing a high amount of drug; this may compensate for moderate affinity (Ka); And administration of the drug by bolus injection. The short-lasting, high drug concentration may generate a tsunami of fake antigen; Example for highly-dosed and fast delived drugs causing anaphylaxis (often at first sight) may be RCM or NMBA (2, 12);

Since fake antigens can bind and cross-link IgE/FcεRI, they could also be used for desensitization to induce MC-unresponsiveness: However, they need to be applied very cautiously, in small, stepwise increasing concentrations to avoid anaphylaxis related side effects. It is uncertain, whether the duration of induced MC-unresponsiveness caused by fake or true antigen differs.

In this context, one should re-consider the meaning of DHR-diagnosis by immediate skin tests (prick, i.d.) as well as by the in-vitro basophil activation tests (BAT) (table 3) using the drug alone: Both tests rely on drug-specific IgE and FcεRI cross-linking and are evaluated within 15 min. Although both tests need to be interpreted with caution, as they may be false positive for various reasons, these tests detect in principle the ability of the drug to form a fake antigen. They do*not* detect reactivity to the hapten-protein adduct. In

contrast, a serological assay like "ImmunoCAP" just detects drug-specific IgE but does not give indications on the ability to elicit a FAR.

The occurrence of atopic allergies like pollinosis, allergic asthma, etc. is not associated with drug allergy (29). Only the clinical manifestation of drug allergy symptoms might be aggravated in acute drug allergy, e.g. if anaphylaxis involves the lung in a patient with allergic asthma. One might conclude that the regulation of MC-unresponsiveness is not impaired in patients with drug allergy and that the clinical problems of immediate drug allergy are mainly due to the sudden formation of fake antigen.

III Conclusion:

DHRs are interesting diseases on one hand, as the eliciting cause (drug) is well defined with exact data on dose, duration of exposure, availability, kinetic, metabolism, and serum concentrations in humans. On the other hand, DHR is clinically difficult. It occurs only rarely and unexpectedly, and many reactions may represent an exception and not the rule. As iatrogenic diseases, they are hard to reproduce for ethical reasons. Additionally, some DHRs are a result of a series of weak non-covalent and reversible reactions, but not a very strong, quasi irreversible, covalent reaction. Moreover, and maybe partly because of this, for most DHRs we do not have animal models.

The methodological approach taken in this paper is unusual: The novel conclusions and alternative explanation of IgE-mediated drug reactions are based on well known facts and neglected inconsistencies. By combining clinical observation and history, skin and in-vitro tests, pharmacological features of drugs and their protein-binding ability as well as immunological concepts of IgE response, the old dogma that only covalent drug-protein complexes can *induce* IgE, remains. But the*effector* phase, which is elicited by IgE and MC, cannot be reduced to antigens formed by covalent bonds. When considering the different kinetic of forming non covalent drug-protein complexes or covalent hapten-protein adducts, the speed of clinical reactivity, particularly of anaphylaxis, and insights from drug-desensitizations, a new concept of IgE-mediated drug-reactions emerges: It's three main concepts are summarized in figure 3:

1) Inducing IgE goes along with silencing MC-reactivity to the same antigen. This is a natural and normal process in IgE-mediated reactions, both for drugs, but also for normal protein allergens. It combines non-reactivity of IgE-coated mast cells to small concentrations of drug/allergen while permitting reactivity to high local levels.

2) At re-exposure, anaphylaxis causing drugs form fake antigens fast and in high quantity. They are dangerous as they can react and cross-link preformed drug-specific IgE and cause MC degranulation with urticaria/anaphylaxis.

3) If at re-exposure only covalent hapten-protein complexes react with drug-specific IgE, the reaction may remain asymptomatic, as the slow generation of such stable antigens may re-establish MC-unresponsiveness.

The beauty of this concept is its simplicity. The involved components are drug concentrations, type of bonds (covalent or non-covalent), and affinity of drug-protein bindings: together they result in a slow or fast formation and +/- high amount of drug-protein complexes, which then determine MC-unresponsiveness with silent immunity vs. MC-reactivity with allergy.

It should be emphasized that part of the concepts proposed here apply to allergy in general. The IgE antibody is evolutionarily very old (30) and cannot and should not be seen from an allergy perspective alone. IgE may represent a normal, potentially beneficial immune response to*local antigen accumulations* . Anaphylaxis to drugs is a rare event, which only appears when various exceptional conditions occur together: some rely on the drug, others are due to the individual (e.g. prior exposure to IgE-inducing antigens, drug metabolism).

The possible consequences of this new interpretation of IgE-mediated drug allergy would be far-reaching for the clinical practice, risk estimation and prevention of drug allergy, and our concept of IgE-mediated

allergy in general. It is hoped that these ideas promote discussions to further shed light on the topic, and consequently prompt new research confirming or disapproving the theories discussed.

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Table 1

Covalent hapten-protein adducts and non-covalent drug-protein complexes

Covalent bonds between Non-Covalent bindings hapten and protein between drug and protein **(hapten protein adducts) (drug-protein complexes)**

stable **Stability** reversible

high affine **Affinity** low to medium affine

>20 min to hours **Duration** Very rapid < 5 min

Hapten protein adduct is as new **Stimulation** As labile drug-carrier complex not antigen stimulatory for T and B cells**Behaviour** stimulatory for B and T cells, but (IgE) may be able to interact with

preformed IgE.

The slow formation and gradual **MC- unresponsiveness** The rapid formation of increase of hapten-carrier protein complexes able to bind to adducts allows induction of preformed drug-specific IgE (= fake unresponsiveness of mast cells: antigens) may overcome MC-

No symptoms appear unresponsiveness and elicit MC degranulation with symptoms of anaphylaxis.

Table 2:

Examples where the presence of IgE and antigen elicits no reaction	Examples where the presence of IgE and antigen elicits no reaction	Examples where the presence of IgE and antigen elicits no reaction	Examples where the presence of IgE and antigen elicits no reaction
Fact		**Explanation**	**Explanation**
Drug tolerance during initial IgE sensitization		During an e.g. 10d therapy with amoxicillin, no symptoms appear, although specific IgE is already formed and MC are sensitized	During an e.g. 10d therapy with amoxicillin, no symptoms appear, although specific IgE is already formed and MC are sensitized
Bee keepers tolerate bee stings when they carry IgE to bee venom.		Bee keepers with IgE (and often IgG) to bee venom react with urticaria and (often mild) anaphylaxis in springs after the first bee stings, which subsides as the season carries on.	Bee keepers with IgE (and often IgG) to bee venom react with urticaria and (often mild) anaphylaxis in springs after the first bee stings, which subsides as the season carries on.
Bee or wasp allergic individuals tolerate 50µg venom already after 3,5hrs of immunotherapy (23).		Protocol: S.c injection of increasing concentrations of venom (0.1 µg - 1 µg - 10 µg - 20 µg - 30 µg - 50 µg (>111mg)) within about 3.5 h. Transient MC-unresponsiveness is achieved in IgE-sensitized individuals after 3.5 h with a tolerance of 50µg venom; Further injections (100µg) are well tolerated at day 7, 21 (22, 23).	Protocol: S.c injection of increasing concentrations of venom (0.1 µg - 1 µg - 10 µg - 20 µg - 30 µg - 50 µg (>111mg)) within about 3.5 h. Transient MC-unresponsiveness is achieved in IgE-sensitized individuals after 3.5 h with a tolerance of 50µg venom; Further injections (100µg) are well tolerated at day 7, 21 (22, 23).

Drug desensitizations can be achieved within a few hrs.	Multiple schemes of desensitizations of IgE-mediated reactions to drugs exist (20, 21). Desensitization is achieved by starting with very low drug concentrations and increasing stepwise (30min intervals) until the normal daily drug dose is achieved in 4-6hrs. This desensitization is tolerated without symptoms. It is repeated after ca. 4 weeks before the next drug therapy.	Multiple schemes of desensitizations of IgE-mediated reactions to drugs exist (20, 21). Desensitization is achieved by starting with very low drug concentrations and increasing stepwise (30min intervals) until the normal daily drug dose is achieved in 4-6hrs. This desensitization is tolerated without symptoms. It is repeated after ca. 4 weeks before the next drug therapy.
Sensitization without symptoms in spite of allergen exposure is frequent.	Many sensitized individuals (IgE, skin test reactivity) do not show allergy symptoms. E.g. in the pollen season, the sensitized but not allergic individuals tolerate the pollen exposure without symptoms (ca 20µg inhaled major allergen/season). The skin tests are positive, as the local allergen concentration in skin testing is high (also ca. 10-20µg/ml major allergen) (26,27).	Many sensitized individuals (IgE, skin test reactivity) do not show allergy symptoms. E.g. in the pollen season, the sensitized but not allergic individuals tolerate the pollen exposure without symptoms (ca 20µg inhaled major allergen/season). The skin tests are positive, as the local allergen concentration in skin testing is high (also ca. 10-20µg/ml major allergen) (26,27).

Table 3: IgE Reactivity and cross-linking by non-covalent drug-carrier complexes

1. **Positive skin prick tests** (SPT, within 15 min) to amoxicillin, cefuroxim, etc., before covalent drug-carrier complexes are formed
2. **Positive SPT/i.d. tests to drugs (15 min) like sulfamethoxazole,** which per se does not form covalent bonds; SMX needs **metabolism** to generate reactive metabolites (SMX-NO), which needs > 6-10 h (13)
3. **Positive BAT** to drugs: it occurs fast, before covalent bonds are formed (amoxicillin, cefuroxim, etc.) or reactive metabolites (e.g. SMX-NO) are generated in the *in- vitro* conditions
4. **Anaphylaxis** with mast cell degranulation to drugs occurs <5 min after injection, before covalent links can be formed

Legends:

Figure 1: non-covalent and covalent drug binding to protein

Typical examples of drugs acting as haptens are beta-lactams like penicillinG or amoxicillin. Their beta-lactam ring conjugates spontaneously to lysine groups within proteins.

E.g. amoxicillin binds first via non-covalent interactions to certain regions of the protein. These initial and fast non-covalent bindings positions the drug favorably to facilitate subsequent covalent binding of the beta-lactam to lysines: The beta-lactam ring opens and binds as amoxicilloyl covalently to lysins in position 190, 432, 525, 541 of the human serum albumin (HSA) (16). The configuration of the bound amoxicillin-HSA (non-covalent) and amoxicilloyl-HSA (covalent) is similar/identical for the reactive IgE antibody.

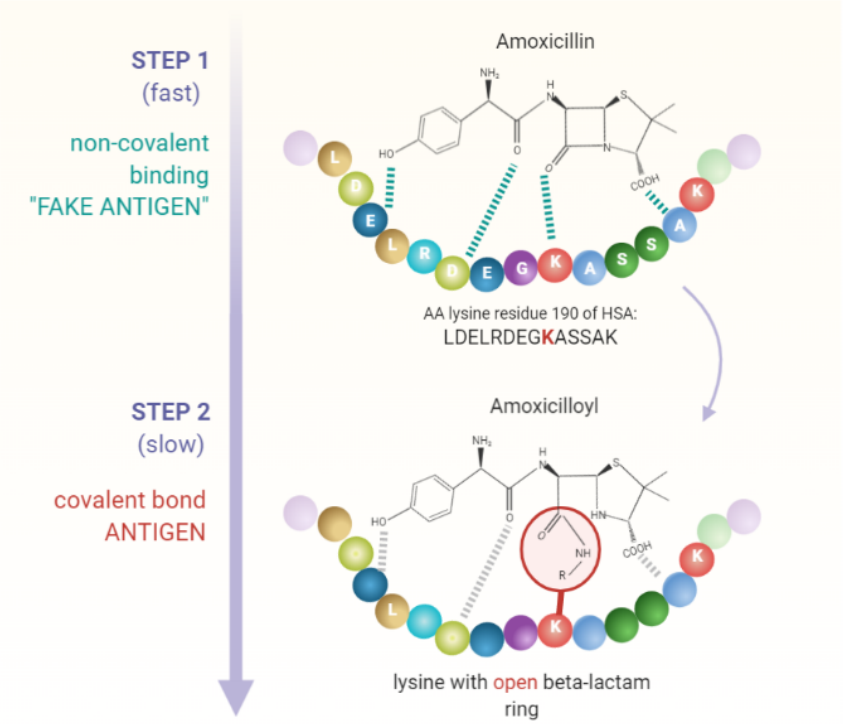

Figure 2: induction of mast cell unresponsiveness by IgE-antigen complexes

*Δυριν**γ σενσιτιζατιον** ωιτη ηαπτεν-ςαρριερ αδδυςτς, α γραδυαλ ινςρεασε οφ δρυγ-σπεςιφις ΙγΕ οςςυρς, ωηιςη βινδς το ΦςεΡΙ ον Μ˜. Τηε Μ˜-βουνδ σπεςιφις ΙγΕ βινδς τηε ηαπτεν-ςαρριερ αδδυςτς. Τηε σλοω ινςρεασε οφ ΙγΕ ανδ ΙγΕ-αντιγεν ςομπλεξες λεαδς το α γραδυαλ δεσενσιτιζατιον οφ μαστ ςελλς, ωηιςη ις σπεςιφις φορ τηις αντιγεν/αλλεργεν. Ιν ινιτιαλ σενσιτιζατιον, τηε λιμιτινγ φαςτορ ις ΙγΕ·*

In already sensitized MC with already high specific IgE, the procedure of ***"desensitization"*** *occurs by using initially low antigen (drug) concentrations. The limiting factor would be the antigen/drug concentration.*

In both, sensitization and desensitization, the slow process of antigen formation based on covalent bonds may also contribute to MC-unresponsiveness (see text)

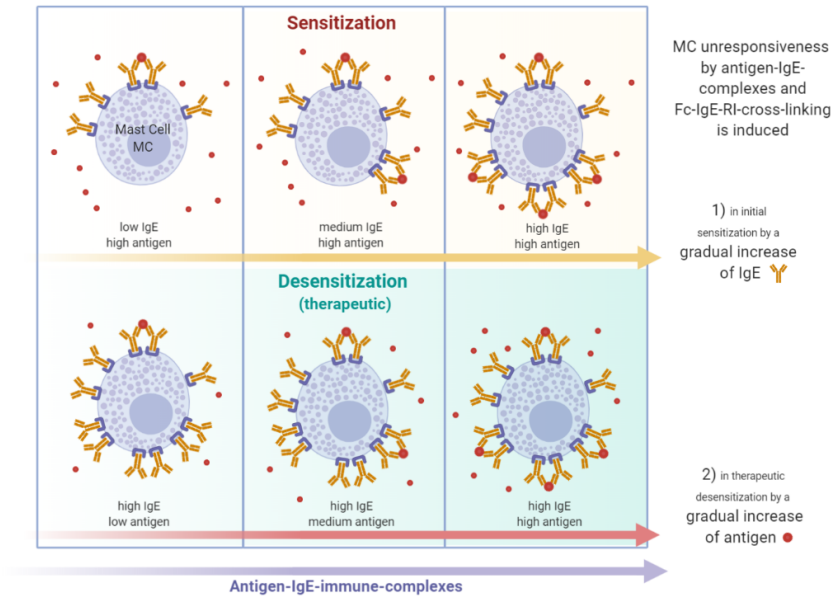

Figure 3: Induction of IgE by hapten-protein adducts, elicitation of MC degranulation by fake antigen

Induction of IgE- and MC-unresponsiveness by hapten-protein adducts and of MC degranulation by fake antigen.

Covalently bound hapten-carrier adducts (=true antigen,) are taken up by APC like DC, processed and presented as drug-peptide on HLA to T cells. Some specific T cells react and secrete cytokines like IL-4, IL-13 , which booster B cell maturation to plasma cells secreting hapten-carrier specific IgE. These IgE bind to high-affinity FcεRI on mast cells (MC), where they are cross-linked by hapten-carrier complexes. The increasing amount of immune complexes (hapten-carrier/IgE antibodies/FcεRI) make MC unresponsive to the specific antigen. The sensitization phase remains asymptomatic, although antigen (continued use of drug), IgE and mast cells are present.

After a drug free interval the patient may be re-challenged by the drug : Some drugs are able to form non-covalent drug-carrier complexes [ ] rapidly and in high amount ("fake antigens"); they look similar/identical to true antigen and can bind to the preformed drug specific IgE on MC: the quasi immediately and ubiquitously available, large amount of fake antigens can overcome MC-unresponsiveness and elicit a generalized MC-degranulation with anaphylaxis.

IgE-mediated Hypersensitivity