

REVIEW ARTICLE

The important role of non-covalent drug-protein interactions in drug hypersensitivity reactions

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Abstract

Drug hypersensitivity reactions (DHR) are heterogeneous and unusual immune reactions with rather unique clinical presentations. Accumulating evidence indicates that certain non-covalent drug-protein interactions are able to elicit exclusively effector functions of antibody reactions or complete T-cell reactions which contribute substantially to DHR. Here, we discuss three key interactions; (a) mimicry: whereby soluble, non-covalent drug-protein complexes (“fake antigens”) mimic covalent drug-protein adducts; (b) increased antibody affinity: for example, in quinine-type immune thrombocytopenia where the drug gets trapped between antibody and membrane-bound glycoprotein; and (c) p-i-stimulation: where naïve and memory T cells are activated by direct binding of drugs to the human leukocyte antigen and/or T-cell receptors. This transient drug-immune receptor interaction initiates a polyclonal T-cell response with mild-to-severe DHR symptoms. Notable complications arising from p-i DHR can include viral reactivations, autoimmunity, and multiple drug hypersensitivity. In conclusion, DHR is characterized by abnormal immune stimulation driven by non-covalent drug-protein interactions. This contrasts DHR from “normal” immunity, which relies on antigen-formation by covalent hapten-protein adducts and predominantly results in asymptomatic immunity.

KEYWORDS

allo-immunity, drug hypersensitivity, fake antigen, heterologous immunity, virus reactivation

1 | INTRODUCTION

Drug hypersensitivity reactions (DHR) are defined by an immune-mediated reaction toward a drug (small molecule or protein) which elicits an inflammatory response.^{1,2} The mechanisms underlying immune stimulation by drugs and immune reactions are heterogeneous, which explains the observed variability of DHR in the clinic. Inflammatory

manifestations of DHR include anaphylaxis, blood cell dyscrasia, contact dermatitis, maculopapular exanthema (MPE), Stevens-Johnson syndrome and toxic epidermal necrolysis (SJS/TEN), and drug reaction with eosinophilia and systemic symptoms (DRESS/DiHS) among others.^{3–7} Indeed, any physician observing a severe hypersensitivity reaction following the administration of a normally well-tolerated molecule is perplexed and horrified. One instinctively questions the

Abbreviations: (cyto-)LTT, cytokine-based lymphocyte transformation test; APC, antigen-presenting cell; BAT, basophil activation test; CMV, cytomegalovirus; DC, dendritic cell; DHR, drug hypersensitivity reactions; DITP, drug-induced immune thrombocytopenia; DRESS, drug reaction with eosinophilia and systemic symptoms; EBV, Epstein-Barr virus; Fc-εRI, high-affinity receptor for IgE; HHV6, human herpes virus 6; HLA, human leukocyte antigens; MC, mast cell; MPE, maculopapular exanthema; PPI, proton-pump inhibitor; SJS/TEN, Stevens-Johnson syndrome and toxic epidermal necrolysis; SMX, sulfamethoxazole; TCC, T-cell clones; TCR, T-cell receptor for antigen.

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biological benefit of such a fulminant and catastrophic immune reaction. Therefore, the question arises whether these severe DHR are excessive variants of a regular immune reaction to an antigen, or are they the result of a qualitatively different immune reaction?

For many years, DHR was exclusively explained via the hapten hypothesis.⁸⁻¹¹ It was assumed that drugs were too small to be an antigen per se, but an antigen feature was necessary to elicit an immune reaction. Thus, only if drugs, or their metabolites, interacted covalently with proteins to form larger, stable drug-protein complexes, would they represent new complete antigens. These drug-protein adducts were considered necessary to stimulate an immune response. Consequently, the "hapten-dogma" influenced and governed the interpretation of any explanation for DHR.

However, the all-encompassing nature of this hypothesis was disputed over 20 years ago following the analysis of drug-specific T-cell clones (TCC) derived from patients with DHR, and later T-cell receptor (TCR) transfected hybridoma cell lines.¹²⁻¹⁸ This induced a paradigm shift in the field. The authors observed unorthodox T-cell stimulation in vitro, which had no strict human leukocyte antigen (HLA) restriction and high alloreactivity. Moreover, they identified CD4+ and CD8+ T cells that were uncharacteristically reacting to HLA-class I and HLA-class II drug presentation, respectively.¹⁹⁻²¹ The take-home message being drug-specific T cells do not follow strict rules.²¹ Most importantly, these in vitro analyses revealed that drugs interacted with antigen-presenting cells (APC) and/or T cells in a labile manner, and drugs without hapten features were stimulatory, even when intracellular processing or metabolism was blocked.^{12,13,22,23} These findings were incompatible with the usual hapten concept and were the basis for the p-i concept (pharmacological interaction of drugs with immune receptors). This meant that some drugs may non-covalently bind to immune receptors such as the HLA or T-cell receptor (TCR), inducing a T-cell response.²⁴⁻²⁶ Since this initial observation, the localization of drugs such as abacavir, carbamazepine, oxypurinol, dapsone, vancomycin etc. to the peptide-binding groove of HLA, and sulfamethoxazole to TCR-V β chains has been demonstrated.²⁷⁻³² These in vitro findings were also supported by ex vivo analysis of the blister fluid cells and elevated cytokine concentrations from patients with SJS/TEN.³³⁻³⁵

A role for non-covalent drug-protein interactions in antibody-mediated DHR was also proposed. In classical IgE-mediated reactions, the term fake antigen describes non-covalent drug-protein complexes that mimic covalent drug-protein adducts. They can bind to and cross-link IgE and may cause fulminant mast cell degranulation (anaphylaxis), but are unable to induce IgE.³⁶

Other non-covalent drug-protein complexes are involved in various blood cell dyscrasias. For example, in the quinine model of drug-induced immune thrombocytopenia (DITP), drugs are thought to get trapped between the thrombocyte-glycoprotein (GP) and antibodies. In drug-induced hemolysis, the drug binds to the antibody and Rh complex. Ultimately, this drug binding increases the affinity of the antibodies for the GP or Rh complex.³⁷⁻⁴⁰ The antibody-covered platelets/erythrocytes are then directly removed by mononuclear phagocytes.

In contrast, drugs forming hapten-protein adducts are well tolerated if not used at very high concentrations.⁴¹⁻⁴³ Indeed, hapten-like drugs can form new antigens and induce immunity, but this is mostly asymptomatic. This suggests that the real danger arises from the non-covalent drug-protein interactions which cause abnormal immune stimulations and symptomatic DHR. Here, we discuss the main non-covalent drug-protein reactions leading to DHR.

2 | ASYMPTOMATIC AND SYMPTOMATIC IMMUNITY TO DRUGS (HAPTENS)

Small molecules generally interact with larger proteins via transient and unstable non-covalent links (electrostatic interactions, OH-bonds, hydrogen bonds). Due to the transience of such drug-protein complexes, they are often ignored by both the innate and adaptive immune systems, and this ignorance by the immune system applies to most current drugs on the market.

Conversely, some drugs (including metabolites and other chemicals) represent haptens, as they share their electron pairs with proteins and form stable covalent bonds. The engagement in such bonds requires certain features of the shared atoms, namely from the site of the drug as well as from the accepting region in the protein. These stably formed drug-protein adducts are formed at the same site of the protein where prior non-covalent drug-protein interactions have taken place; however, their formation can take many hours (reviewed in^{36,41}). This newly formed drug-protein adduct is then seen by the immune system as a single novel antigen, and a protein to which tolerance once existed can then become immune stimulatory.

However, an isolated new protein/drug-protein adduct, if not linked to some danger signal, is not sufficiently stimulatory to the immune system.⁴⁴ Co-stimulation can be provided by the drug, but also by simultaneously applied adjuvants capable of stimulating immunity.⁴⁵⁻⁴⁷ In contact dermatitis, which is a localized skin reaction to a hapten-drug, chemical, or metal, the small compound provides not only the antigen but also some co-stimulation, which is often linked to the local toxic effect of the contact sensitizer.⁴⁷⁻⁵⁰ Thus, increasing the dose enhances immunogenicity, symptoms of contact dermatitis, and skin irritation. Although occasionally strong, this is still a non-systemic and controlled immune response.

The immune response to newly formed drug metabolites generated in the liver appears to be a special case. Many metabolites have hapten characteristics, but both detoxification (eg, by glutathione binding) and the tolerance-favoring intrahepatic environment may prevent an immune response.^{51,52} Only if the metabolites are available in substantial amounts outside the liver, may they become immunogenic. An example is being the circulating sulfamethoxazole (SMX)-metabolite SMX-NHOH, which is oxidized in the peripheral tissue to the hapten SMX-NO, which occasionally may be responsible for DHR.⁵³

Many hapten-drugs are well-tolerated and do not cause DHR.⁴¹⁻⁴³ An example is omeprazole. This and other proton-pump inhibitors (PPI), such as pantoprazole and lansoprazole, form adducts with the drug binding site within the hydrogen-potassium adenosine

triphosphatase enzyme system (H⁺/K⁺-ATPase).⁵⁴ Only rarely do individuals develop IgE to PPI and develop anaphylaxis to the drug.⁵⁴

The best-studied examples of drugs acting as haptens are beta-lactams. They can form covalent bonds spontaneously, and all persons treated with beta-lactams have beta-lactam modified proteins, such as albumin or transferrin.⁵⁵ However, only ~50% of beta-lactam-exposed persons develop (asymptomatic) IgG antibodies to beta-lactams.⁵⁵ It is assumed that some covalent protein modifications provide co-stimulation and that high concentrations of beta-lactams favor IgG formation. In beta-lactam-treated individuals without IgG, this co-stimulation may be too weak or absent. Moreover, there may be differences in the immunogenicity of hapten-drugs, dependent upon whether they bind to easily accessible amino acids (eg, like lysine in beta-lactam-albumin interactions⁵⁵;) or preferentially to a selective binding site of a target protein (eg, omeprazole⁵⁴). Only a small group of beta-lactam-treated patients develop symptomatic IgG reactions with blood cell dyscrasias. Of note, the majority of thrombocytopenia is due to non-covalent drug binding of beta-lactams^{39,56} (see below). Therefore, the ability of a drug to act as hapten does not necessarily mean that an eventually appearing DHR is due to this hapten feature. Even with hapten-drugs, the DHR can still be due to non-covalent binding (eg, thrombocytopenia,⁵⁶).

An appearance of other systemic DHR (mostly macular, maculopapular, or urticarial exanthems) after therapy with beta-lactams is higher than with other drugs (2–8% incidence⁵⁷), but this is still the exception. Observations made in cystic fibrosis patients suggested that the dose, duration, and repeated treatments may enhance the likelihood of symptomatic reactions.⁵⁸ Another factor enhancing symptomatic T cell-mediated DHR are viral infections such as Epstein-Barr virus (EBV) and human immunodeficiency virus (HIV).^{59,60} Recently, the use of checkpoint inhibitors was also linked to more frequent delayed DHR.⁶¹

In conclusion, most drugs are ignored by the immune system, since their binding to proteins is labile or the binding site is irrelevant for immune stimulations. A minority of drugs can form stable adducts with proteins, but these are mostly well tolerated.^{41–43,55} They do not induce immunity (no co-stimulation), or their immunity remains asymptomatic (IgG). When a damaging effect like thrombocytopenia occurs, it may well be due to non-covalent drug binding that elicits effector mechanisms.⁵⁶ Indeed, if a DHR to beta-lactams appears, which represents the classical hapten-drug class, it is mostly due to non-covalent binding.

3 | NON-COVALENT DRUG BINDING INDUCES ANTIBODY-MEDIATED EFFECTOR MECHANISMS

3.1 | Fake antigen model

This recently proposed model³⁶ refers to the effector phase of IgE-mediated reactions to drugs, namely IgE-mediated mast cell or basophil degranulation, and acute symptoms such as anaphylaxis. It is

a hypothesis based on the kinetics of covalent binding and clinical observation. Only the non-covalent interactions explain the rapid reactivity in skin tests or of basophils to inert drugs which are unable to form covalent bonds, but still elicit mast cell or basophil degranulation.³⁶ Moreover, it postulates that clinical symptoms, or positive skin and in vitro basophil activation tests (BAT), can only be due to non-covalent drug-protein complexes.

Drugs bind to albumin, transferrin, immunoglobulin, or other soluble proteins by transient, non-covalent bindings. Some of these drugs have hapten features. They are able to form a stable drug-protein adduct over time (hours) and represent new antigens, which can induce IgE. Simultaneously, the newly formed IgE-{drug-adduct} immune complexes induce unresponsiveness of Fc-εRI-positive cells mast cells/basophils.³⁶ If the patient encounters the drug again, non-covalent drug-protein complexes are formed again in seconds/minutes and in high amount. If these primarily formed non-covalent drug-protein complexes are rather stable and able to cross-link the IgE on Fc-IgεRI+ mast cells and basophils, they can overcome mast cell unresponsiveness immediately (Figure 1A). This would explain the instantaneous and significant anaphylaxis to a small drug which has had insufficient time to form covalent bonds. These non-covalent drug-protein complexes are called “fake antigens” as they are not able to elicit IgE production themselves but can still react with preformed IgE.³⁶

3.2 | Drug-induced blood cell dyscrasias

Drug-induced immune *thrombocytopenia* (DITP) represents thrombocytopenia following drug treatment. It is mostly mediated via drug-dependent antibodies, which elicit clearance of platelets by mononuclear phagocytes or cause direct platelet destruction.^{37–39,56} Thrombocytopenia may occur about 1 week after exposure, which may lead to mucosal bleeding (purpura or epistaxis). More serious clinical complications, such as intracranial or intrapulmonary hemorrhages, are rare.

There are hapten-induced antibodies (eg, beta-lactams), where the drug binds to the platelet membrane and promotes antibody responses. However, this process is rare.⁵⁶ In quinine-type DITP, antibody binding to the thrombocyte membrane occurs only in the presence of the drug, and this effect can be removed by cell washing. Aster and colleagues analyzed quinine-type DITP and its underlying immune mechanism in detail.^{37,38,62–64} Affected patients already have low-affinity antibodies for platelet GPs. It is assumed that the drug binds to the complementary-determining region (CDR) of the antibody,⁶⁴ which increases antibody affinity for a specific epitope expressed by a platelet glycoprotein (Figure 1B). Therefore, only in the presence of the drug do antibodies bind to and destroy platelets. The diagnosis of DITP relies on clinical criteria and laboratory tests, which demonstrate the presence of drug-dependent platelet antibodies.^{38,39,56}

Many other drugs have been reported as causes of DITP via similar mechanisms. These drugs encompass non-steroidal anti-inflammatories,

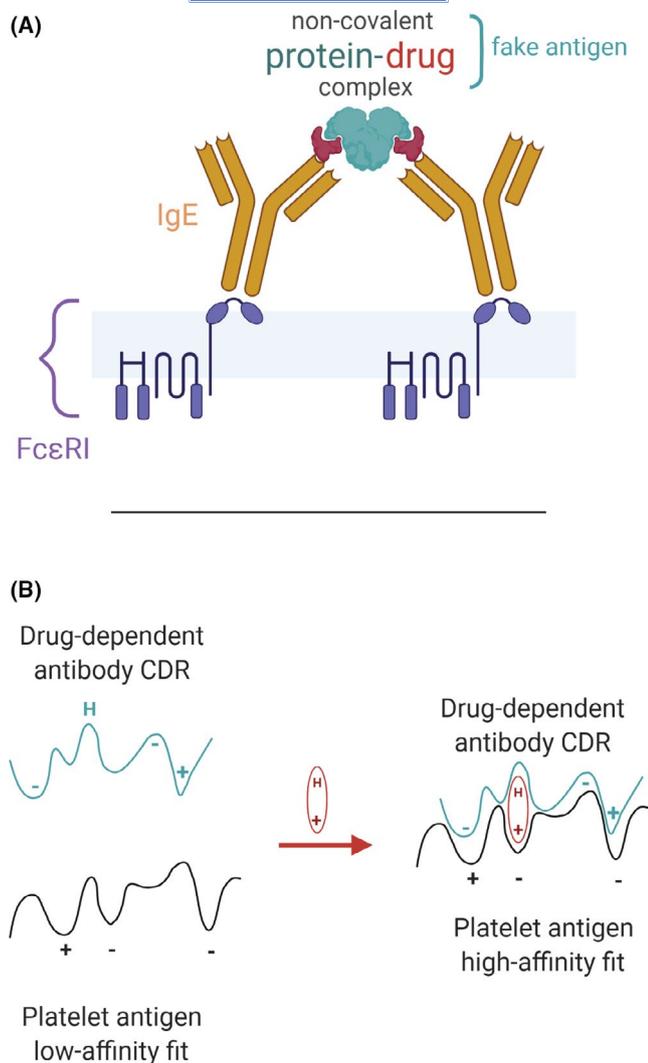


FIGURE 1 (a) Fake antigen model: Drug-specific IgE responses are induced by covalent drug-protein adducts; fake antigens are non-covalent, but still rather affine drug-protein complexes, which react with preformed drug-specific IgE on Fc-IgE RI-expressing cells. This cross-links the bound IgE and may induce immediate anaphylaxis.³⁶ (b) Enhanced affinity model (quinine induced DITP): Low affine antibodies (IgG, IgM) directed to membrane components such as GP on thrombocytes, or the Rh system on erythrocytes, may exist in many individuals and remain asymptomatic. Therapy with quinine or other substances can (rarely) induce a blood cell dyscrasia such as immune thrombocytopenia or hemolytic anemia. The drugs bind non-covalently to the CDR of anti-GP or anti-Rh antibodies (labile, can be washed away) and increase the affinity of the antibody for the target structure. The antibody/drug bind to the blood cells, which are removed from the circulation (adapted from³⁸)

antibiotics (ceftriaxone, piperacillin, vancomycin, rifampicin, trimethoprim/sulfamethoxazole, and teicoplanin), and anticonvulsants (phenytoin and carbamazepine). Of note, even in beta-lactam-induced DITP, the effector mechanism seems to occur more frequently via this quinine-like mechanism, rather than hapten modification.⁵⁶

Besides DITP, other forms of blood cell dyscrasias such as hemolytic anemia may be due to a similar mechanism. Some red blood cell

membrane proteins are targeted by antibodies (preferentially the Rh complex), whose affinity is enhanced by prior drug binding. The antibody-coated red blood cells are then removed from the circulation or lysed. Notably, some of the hemolysis inducing drugs are also involved in DITP (cephalosporin, diclofenac, quinidine^{40,56}).

In conclusion, antibodies which are directed to certain blood cell membrane structures can be modified by non-covalent binding of drugs. This drug binding enhances the antibody affinity and can cause blood cell destruction.⁶²⁻⁶⁴

4 | NON-COVALENT DRUG BINDING TO HLA OR TCR: THE p-i CONCEPT

Non-covalent drug binding to immune receptors involved in T-cell stimulation (HLA, TCR) is known as the p-i concept and is peculiar as it can initiate a complete T cell-restricted immune reaction.²⁴⁻²⁶ During a normal, protein-specific immune response $\alpha\beta$ -TCR interact with HLA-immunogenic peptides. As both the TCR and HLA proteins are highly variable structures, the chances are high that some contain drug binding sites. Structural, computational, and functional studies have shown that drugs (eg, abacavir, carbamazepine, and oxypurinol) may indeed have an off-target activity, as they directly bind with substantial affinity to the peptide-binding groove of certain HLA molecules.^{27-32,65-67} For example, abacavir binds exclusively to HLA-B*57:01, carbamazepine interacts mainly with B*15:02, and allopurinol/oxypurinol preferentially binds to B*58:01.²⁷⁻³² Most selective drug binding to HLA or TCR occurs on the cell surface. Only abacavir was shown to bind to HLA inside the cell as well, whereby this can alter the peptide repertoire presented by B*57:01.^{65,66,68}

The majority of data refer to p-i HLA which is better studied compared with p-i TCR. Some drugs, such as sulfamethoxazole, may preferentially bind to TCR^{69,70} or interact with both HLA protein and TCR structures.^{67,69-72} Drug binding to TCR is similar to p-i HLA and displays increased alloreactivity and non-HLA-allele-dependent drug stimulation.^{19,20,25} Importantly, full stimulation requires that the drug-modified TCR/T cell still interacts with HLA on antigen-presenting cells (Figure 2).^{12-15,24-26} The drug, trapped in between TCR and HLA/peptide, may exert a similar modification of the interacting immune receptors such as p-i HLA, since the TCR has a high degree of adaptability.^{69,73} A main difference to the exclusive p-i HLA involvement may be the expansion of oligoclonal, or sometimes monoclonal T-cell reactions (when the drug binds to TCR-V β family proteins or specific TCR determinants.^{71,72} Whether $\gamma\delta$ TCR and other HLA structures (eg, HLA-Ib) are also affected by drugs in an immune stimulatory way is not known.

4.1 | Allo-like stimulation by p-i reactions

The transformation of a self-HLA to an allo-HLA-structure by non-covalent drug binding elicits unorthodox stimulation of T

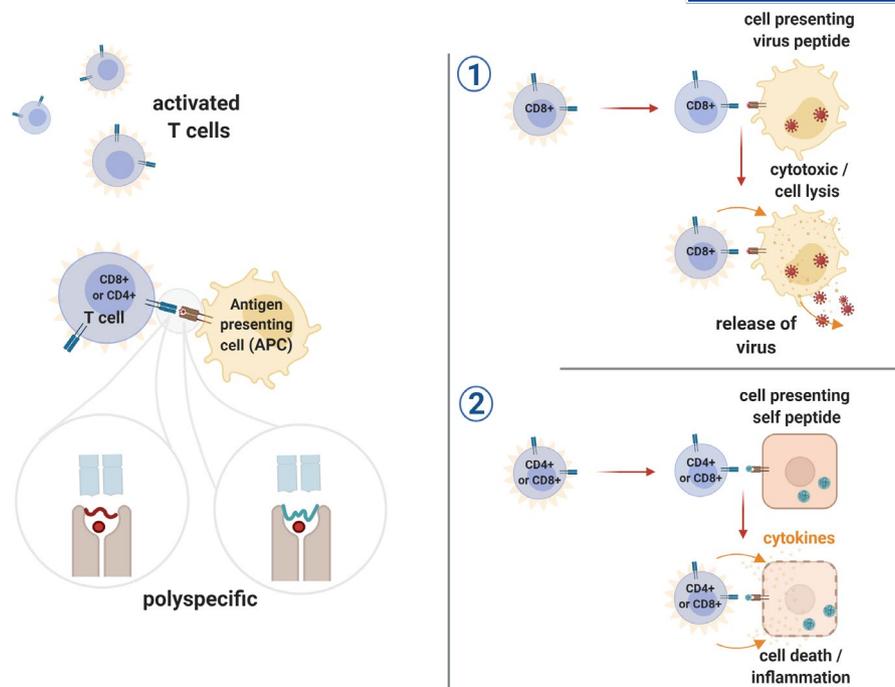


FIGURE 2 p-i stimulation results in an acute drug-dependent disease (acute DHR), often followed by late reactions in the absence of drug: *Acute reaction*. The drug binds to immune receptors, for example, to a peptide presenting cleft of a HLA molecule (p-i) and thereby makes the self-HLA look like an allo-HLA. This provides a strong, allo-like, stimulatory signal to a substantial portion of CD4 and CD8 cells, which are poly-specific (= one TCR/T cell is able to react with different peptides). The reactive T cells expand (over weeks) and cause a cytotoxic reaction with a severe DHR, such as MPE or DRESS. If CD8/NK cell reactions dominate, this can result in SJS/TEN. *Late reactions*: Further symptoms appear in the absence of drug over the following weeks. The p-i activation induces a change in the memory and naïve T cells to a more alert state. When the TCR/T cell of p-i activated cells cross-reacts with a peptide presented by the unmodified self-HLA, the T cells exert effector mechanisms (eg, cytotoxicity, cytokine release). (1) As a substantial portion of memory T cells are devoted to the control of herpesviruses (HHV6, CMV, EBV), the p-i activation includes many T cells directed against herpes virus peptide-expressing cells. This causes their destruction and the release of herpes viruses into the circulation. Virus reactivation occurs in the first 3–6 weeks after the onset of DRESS. (2) The p-i activation may also include self-peptide reactive T cells. Their encounter with self-peptides continues their activation, they expand and attack the self-peptide presented cells, thus autoimmunity ensues. Self-peptide-specific T cells have a low precursor frequency, and the symptoms appear after >4–6 weeks. The late complication of viral reactivations and autoimmunity occurs primarily in DRESS but not in SJS/TEN, where exhaustion of the activated CD8+ T cells may block further reactivity

cells and contributes to the peculiar clinical presentation of DHR. The number of reactive T cells in p-i stimulation is high because the whole scaffold for peptide presentation, namely the peptide-binding groove of HLA, is altered by the drug^{26,65,66} (Figure 2). Therefore, potentially every HLA expressing cell, independent of the presented peptides, is susceptible to the off-target activity of the drug (Box 1).

The p-i modification of immune receptors also has functional features of an allo-stimulation.^{19,20,26,65,66} P-i stimulation recruits the T cells from both naïve (CD45RA+) and memory (CD45RO+) pools, similarly to direct allo-like stimulation.^{74,78,79} Intermediate processing and presentation by dendritic cells is not necessary.⁷⁸ P-i-stimulated T-cell clones react with different peptides (poly-specific) and show limited HLA restriction, high alloreactivity, and a high tendency to develop into cytotoxic T cells.^{18–20,23,26,73,75} This initial strong allo-like T-cell stimulation is responsible for the initial clinical manifestations of DHR, namely MPE, DRESS, or SJS/TEN (Figure 2).

4.2 | p-i mechanism: The drug modification of the immune receptors is transient

The clinical implications of p-i stimulations are very broad and range from mild to life-threatening. However, most p-i DHR do not have clinically severe symptoms, such as those observed in acute transplant rejection or graft-versus-host disease. This might be due to some features of p-i, which indicate a lower stimulatory potential of p-i compared with allo-transplant reactions: The drug therapy lasts just days or weeks. This is in contrast to the permanent exposure to allo-HLA in allo-transplantation. Even if the drug is given for a longer period, it may still not elicit a strong enough stimulation, as the formation of drug-immune receptor complex is labile and reversible. Thus, the process of T-cell activation by the “allo-” like (drug-HLA or drug-TCR) complex is often interrupted. Indeed, one hallmark of severe T cell-mediated DHR is a long period between the start of therapy and the appearance of symptoms (>10–50 days).⁵ This long drug exposure may be required both for T-cell stimulation and

BOX 1 Alloreactivity

T cells bearing $\alpha\beta$ TCR are selected by a subset of peptide-laden HLA in the thymus and in the periphery and therefore are restricted to recognising host or "self-" HLA. However, some T cells show a direct T-cell alloreactivity: Their TCR directly engages a peptide-*allo*-HLA complex and activates the T cell (without intermediate processing and presentation by APC). It manifests itself clinically as transplant rejection and graft-versus-host disease.

The direct *allo*-stimulation is strong⁷⁴:

1. The frequency of this alloreactivity is higher than a peptide specific response: depending on the degree of difference self vs. *allo*, more than 10% of circulating T cells may be activated.
2. These *allo*-activated T cells are often polyspecific, which means that they are able to react with multiple different peptides presented and that many T cells react.⁷⁴
3. The *allo*-stimulated T cells have an additional specificity for peptides presented by self HLA as well,⁷⁴⁻⁷⁶ which may explain late reactions with viral or self-peptides (Figure 2)⁷⁷

expansion if drug-immune receptor complexes are unstable. It would also explain why drugs given for a longer period or constantly (eg, anti-epileptics) are the main inducers of severe p-i DHR, like DRESS or SJS/TEN.^{1,3-5} Therefore, p-i reactions may range from clinically asymptomatic courses to mild self-limited MPE and severe DHR like long-lasting MPE, DRESS, and SJS/TEN.

4.3 | The p-i initiated immune reaction may be dominated by expansion vs exhaustion of T cells

The two main clinical outcomes of acute *allo*-like p-i reactions are MPE/DRESS and SJS/TEN.^{2,29,31,33,34} In MPE/DRESS patients, high numbers of activated lymphocytes and increased concentrations of various cytokines are found in the circulation.⁸⁰ This high reactivity is persistent for a long time period (weeks to months); reactivation of viruses such as human herpes virus 6 (HHV6) or cytomegalovirus (CMV), or the appearance of autoimmunity is common in DRESS.^{5,80} The identification of the eliciting drug is possible by skin tests or in vitro assays, which reveals proliferation of cytotoxic CD4 and CD8 T cell with increased cytokine secretion to the incriminating drug, which is sometimes detectable for many years after the event.⁸¹

In stark contrast, patients with SJS/TEN may show lymphopenia and only limited signs of general cell activation/inflammation.^{3,82} It looks, as if the self-destructive process was suddenly stopped, and the killer cells had left the scene: They escaped into the blisters, where the fluid is filled with predominantly CD8/NK+cytotoxic T cells.^{3,33,34,83} Cytotoxic molecules (granzyme B, perforin, granzulin)

can be detected in vivo in blood and the blister fluid in the first days of the disease.^{83,84} During the acute phase (~1-2 weeks), the circulating T cells still react in in vitro assays with the eliciting drug. But after 3-4 weeks, the CD8 T cells (the main cell population contributing to disease) appear to be exhausted and are refractory to drug stimulation.^{85,86} How CD8 exhaustion occurs is unknown, but apoptosis of these cells has been reported in the hapten system. This is based on changes in the presenting peptide or when inflammatory signals are lacking.^{87,88} Occasionally, a small fraction of activated CD4 cells seem to escape this exhaustion, as in vitro IFN γ secretion by CD4 cells following drug stimulation can be detected in some SJS/TEN patients long after the acute disease.⁸⁶

4.4 | Late reactions: A shift from *allo*-like to a peptide/self-HLA-specific reaction

Late complications of p-i DHR occur in the absence of drug and are due to viral reactivations and autoimmunity.⁵ Two principles of T-cell immunity are involved:

- a. p-i activates a substantial proportion of the CD4+ and CD8+ T cells as documented by the many atypical lymphocytes in the circulation, a hallmark of acute DRESS and persisting for weeks.^{4,5,89} This changes the activation status of these T cells from unresponsive/naïve and resting/memory, to activated T cells with a bias to cytotoxic responses.⁷⁵
- b. The p-i-activated T cells have an additional/"natural" specificity for foreign or self-peptides on self-HLA. This special type of cross-reactivity (peptide-{drug-HLA} and peptide/self-HLA) is also called "*heterologous immunity*".⁷⁷ Although the structure of drug-modified or unmodified HLA with distinct peptides differs substantially, they are similar as perceived by the TCR.⁹⁰

The polyclonal T-cell response arising from the memory T-cell pool includes T cells which are primed by prior immune responses. Herpes viruses are permanently harbored in various cell types after infection.⁹¹⁻⁹³ A large amount of T cells are involved in the control of these herpes viruses (ie, limiting herpes replication, with no cell destruction), and it is logical that the p-i stimulation also includes these herpes-peptide-specific T cells. Their activation by p-i alters their function from "control" to cytotoxicity against the herpes virus peptide-expressing cells.^{26,75,86} The attacked cells release virus particles into the circulation, which induce symptoms of viral reactivation (high virus load, increased liver enzymes, and activated lymphocytes)^{5,94} (Figure 2). Indeed, herpes virus reactivation in DRESS was described already in 1997⁹⁵ and is so common that it is part of the Japanese definition of DRESS/DiHS.⁹⁴ Since the precursor frequency of herpes virus-specific T cells is high (>10% of the CD8+ T cells in the elderly),⁹⁶ symptoms evoked by such T cells can appear after ~2-6 weeks.

The second wave of peptide reactivity may result in autoimmunity (Figure 2). Self-peptide reactive T cells may stem from the naïve

TABLE 1 Rules on the stimulation of specific immune system by drugs

(1) Principle	It is all about drug binding to proteins: how and where: How: is it covalent or non-covalent? How affine is the non-covalent binding? Where: <i>Covalent</i> : how many and for immune receptor accessible/relevant sites of the protein are modified? <i>Non-covalent</i> : does it bind to immune receptor (HLA, TCR or preformed antibody/CDR) → DHR or does it imitate a drug-protein adduct (fake antigen) → DHR? If no relevant binding → ignorance
(2) Stimulation	There are <i>classical</i> immune stimulations by antigen (hapten-protein adducts) and <i>unorthodox</i> stimulations by drugs, which directly interact with immune receptors, but are not acting as antigen: Such drugs may interact with <i>preformed</i> antibodies (rapidly formed “fake antigen,” which imitate true antigen) or enhance affinity of antibodies (blood cell dyscrasia) or may initiate an allo-like T-cell response with late reactions.
(3) Effect	Drug interaction with the immune system can result in a. ignorance (no or no functionally relevant drug-protein binding) b. unresponsiveness (antigen present, co-stimulation missing) c. silent immunity to antigen (mostly IgG); clinical symptoms of antigen-driven immunity appear at excessive concentrations or if the drug/chemical transmits co-stimulation as well (contact dermatitis) d. symptomatic DHR due to unorthodox immune stimulations: p-i, fake antigen, enhanced antibody affinity
(4) Initiation vs effector	Differentiate initiation/effector phase of immune response a. antibody reactions: – covalent drug-protein adducts can initiate and elicit antibody production (mostly asymptomatic) – non-covalent drug-protein complexes cannot induce antibodies – non-covalent drug-protein complexes can be stimulatory in the effector phase as fake antigen or if the drug binding enhances antibody affinity b. T-cell reactions: – covalent drug-protein adducts can initiate and elicit T-cell reactions (mainly contact dermatitis) – non-covalent drug-protein bindings can initiate and stimulate various effector mechanisms if they bind directly to the immune receptors (TCR-HLA complex). An unusual, allo-like stimulation can result (p-i) with MPE, DRESS, SJS/TEN as acute symptoms and potentially late complications (viral reactivation, autoimmunity)
(5) Clinic	Asymptomatic immunity ↔ bizarre DHR a. The immune stimulation by drugs able to form covalent bonds with proteins is similar to protein-specific immunity. It is mostly asymptomatic b. The immune stimulation by non-covalently binding drugs is unusual: – The classical antigen stimulation is bypassed by taking place on the cell surface which bypasses the regulatory mechanism exerted by antigen handling – P-i (TCR/HLA) stimulation has functional and clinical similarities to graft-versus-host/transplant reactions and superantigen stimulations – The clinical pictures are unusual/bizarre; for example, anaphylaxis, blood cell dyscrasia, DRESS, and SJS.

and memory T-cell pool. The p-i stimulation changes the activation status of T cells from unresponsive/naïve and resting/memory, to activated T cells. After finishing drug therapy, this p-i activation is slowly diminishing, but not completely. Some activated T cells may interact via their TCR with self-peptides, which can promote cytokine release, cytotoxicity, and potentially, autoimmunity.⁹⁷ As such, self-peptide reactive T cells are present in relatively low amounts and they may need >6–8 weeks to expand to cell numbers which can cause damage. Consequently, autoimmunity occurs in only a minority of patients (<20%) and appears later than the virus reactivation. It mainly manifests as autoimmune polyendocrine syndrome, autoimmune thyroid disease, and type 1 diabetes, but other autoimmune reactions can also appear.^{97,98}

4.5 | Multiple drug hypersensitivity (MDH)

An additional consequence of p-i stimulations is multiple drug hypersensitivity (MDH). Patients with MDH develop an additional DHR to

a structurally different drug, with the same or different clinical manifestations.⁹⁹ MDH occurs in over 25% of patients with DRESS¹⁰⁰ and can occur any time, from the onset of DRESS (often in response to combination therapy), during the initial activation, and can even appear years after the first DHR.^{99–101} Daubner et al observed that even after the acute phase of DRESS, permanent T-cell activation can persist for months to years in the absence of the causative drug and that those T cells reactive to the new drug stem from this pre-activated cell pool.¹⁰²

5 | DISCUSSION

A clue to comprehend systemic DHR is to link their unusual clinical presentation to their unorthodox immune stimulations. A substantial part, if not the majority of DHRs occur when drugs interact and affect immune receptors (immunoglobulins, TCR, and HLA) by non-covalent bindings. These rather weak drug-protein interactions, unable to form new antigens, can be sufficient to elicit abnormal,

non-antigen-driven, immune stimulations which vary from mild-to-severe DHR. Table 1 summarizes some rules on the stimulation of specific immune reactions by drugs.

Let's first discuss the *limitations*. The concept of a fake antigen is still a hypothesis, as are the increased affinity models in blood cell dyscrasia. The proof of p-i reactivity in daily practice is difficult,²⁵ and the link between p-i reactivity and DRESS, SJS/TEN^{2,26} is not generally accepted. The delayed complications of p-i (eg, herpes virus reactivation or autoimmunity) with cross-reactivity of p-i-activated T cells with peptide/self-HLA have not been proven and are extrapolated from allo-reactive T cells which recognize EBV virus peptides.^{75,76}

With these caveats in mind, what are the consequences of putting non-covalent drug-protein binding at the center of the DHR mechanism (Table 1)? It is quite clear that it is the type of drug binding which is decisive as to whether the immune stimulation is antigen driven or not. This makes a big difference for all areas of DHR: history, clinic, cross-reactivity, role of drug metabolism, diagnosis and causality assessment, genetic, and the risk assessment of new drugs. Each of these aspects will require further in-depth analyses and enhanced communication with DHR researchers, as it would represent a shift in the basic concepts of DHR. To illustrate the complexity, here are just a few examples:

5.1 | History

If the immune stimulation is not due to an antigen, no sensitization is needed. This applies to the p-i model, where just the amount of reactive T cells (some even from the naïve T-cell pool) may determine the strength of the reaction (Table 1).²⁶ In antibody-mediated reactions,

the antibody must first be generated and expanded to the drug presented in immunogenic form (as hapten); it is also possible that some chemical, etc., may have induced the initial antibodies, which cross-react with the non-covalent drug-protein complexes.³⁶ The sum of reactive T cells and antibodies, their affinity, and region of interaction will determine the clinical outcome.

5.2 | Diagnosis

It is not covalently bound drug-protein adducts that are detected during diagnosis, but the result of non-covalent drug-protein bindings. For example, skin tests or BAT detect the fake antigen involved in anaphylaxis³⁶; and in the diagnosis of drug-dependent reactions in quinine-type DITP, the drug can be washed away.^{38,39} The patch tests and in vitro (cyto) LTT with drugs such as phenytoin and carbamazepine are due to non-covalent drug-protein interactions. In patients with severe DHR, a polyclonal and poly-specific T-cell response against the allo-like, drug-modified self-allele, but not the drug as an “antigen”, is detected (Figure 2). These T cells do not directly interact with drugs such as abacavir and oxypurinol.^{32,65,66} Some of these tests are quite sensitive (eg, abacavir patch test), but these tests are often negative, particularly in milder reactions. This may be due to significant variation in the affinity of the drugs binding non-covalently to protein. Low affine interactions may result in mild reactions. Moreover, some DHR may only occur if cofactors are present which stabilize the labile non-covalent interactions (eg, virus infection). Under such conditions, the diagnosis of DHR may be difficult as the clinical manifestation may not be reproducible.

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

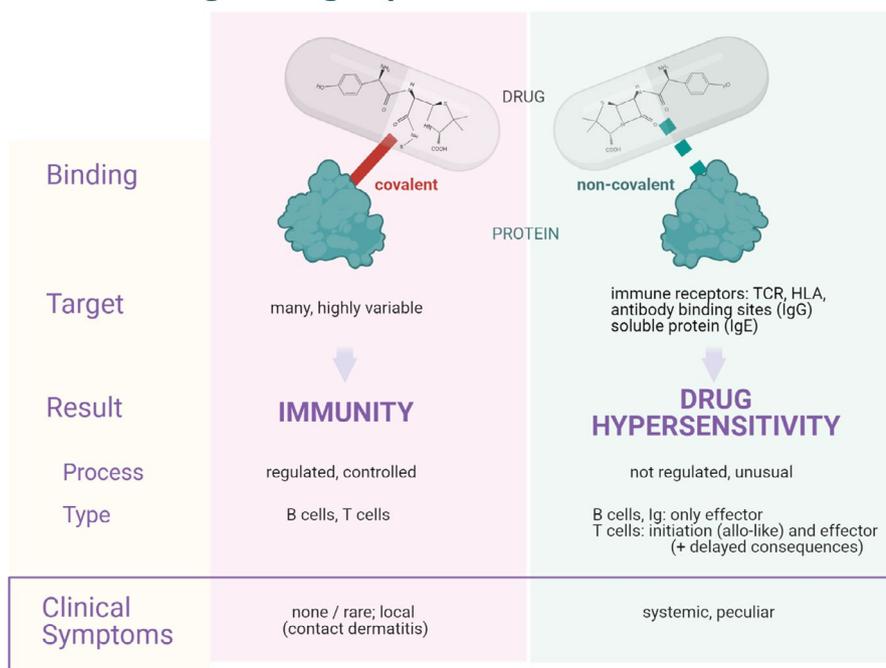


FIGURE 3 Immune reactions to drugs are determined by drug binding to proteins—how and where. The type of binding (covalent or non-covalent) and whether selected proteins are targeted (immune receptors, antibody binding sites) determine whether the functional consequence is (silent) immunity or drug hypersensitivity. In drug hypersensitivity, the non-covalent drug-protein interactions can lead to abnormal immune stimulations based on allo-immune-like reactions (p-i), formation of fake antigens or enhancement of antibody affinity. This unusual immune stimulation makes drug hypersensitivity reactions peculiar, which is also mirrored in the clinic, difficulties in drug hypersensitivity analysis and prediction (see text)

5.3 | Booster reactions

In antibody reactions (fake antigen and DITP), the non-covalently bound drug can elicit effector functions, but would not boost antibody production. In p-i-mediated T-cell reactions such as DRESS, exposure can result in T-cell expansion and, upon re-exposure, trigger even more severe reactions.¹⁰³

5.4 | Risk estimation

Strategies to incorporate certain non-covalent interactions in DHR risk estimations need to be developed. This raises various problems. Discrimination between harmless and potentially harmful drug-protein interactions by in vitro tests is difficult, as such interactions happen everywhere and with high frequency. It may also only be relevant if the drug binding involves immune receptors (HLA and TCR), or pre-existing antibodies with drug (IgE) or blood cell membrane specificity (IgG), or if a functional consequence of drug binding can be demonstrated. As the antibodies involved are generated due to the hapten characteristics of the drug, screening for these characteristics in preclinical evaluation remains relevant. In T-cell reactions to the drug (p-i), screening may reveal reactivity in vitro, but one has to keep in mind that the manifestation of DHR in vivo is substantially lower. For example, all HLA-B*57:01+ individuals react to abacavir in vitro with T-cell activation and expansion, but only ~55% in vivo.^{104,105} This gap between the propensity for DHR and real DHR is often large and may rely on immune-regulatory cell interactions.¹⁰⁶

The great impact of non-covalent binding on many aspects of DHR and the limitations mentioned call for more proof of the concepts in general, as well as for different drugs and diseases. Research supporting DHR has been perceived with skepticism for a long time, as many reactions were classified as “idiosyncratic” and thus not predictable or quantifiable in prospective studies. However, the strong association of certain HLA alleles with manifestations of DHR made some DHR to avoidable diseases and a big success for personalized medicine.^{104,107} The recently described DHR models in B*15:02 or B*57:01 transgenic mice have helped to elucidate the interplay between different cells and clinical manifestations.^{72,106} This highlighted the strength of these models in the preclinical analysis of new drugs. Most importantly, various researchers have been successful in generating drug-specific T-cell lines and TCC from drug naïve individuals in vitro, making DHR research less dependent on rare patients.^{32,78,105,108} Thus, while the concepts discussed are still hypotheses, the chances are good that more data are generated and the potential roles of non-covalent drug binding in DHR will be better understood.

6 | CONCLUSION

DHR arises at the encounter of an endless number of newly designed small molecules (drugs) and an extremely polymorphous, highly sophisticated and balanced immune system. DHR is clearly different

from natural immunity to viruses, bacteria, fungi, and proteins, which are all antigen-driven reactions (Figure 3). The interaction of drugs with the immune system can be due to predominantly asymptomatic hapten formation, or result in DHR, which is linked to non-covalent drug-protein complexes and is accompanied by clinical symptoms. The manifestation of these new diseases at this crossroad highlights the limitations of modern medicine. Drugs, which although beneficial, can interact with our natural immune system in an unprecedented and unexpected way. Therefore, DHR are not only unexpected for the patient and physician, but are “unexpected” for the immune system also. It appears that DHR arises when the immune system is somehow deceived by drugs. Interestingly, drugs which stimulate by non-covalent bindings have exploited classical weak spots of the specific immune system, namely cross-reactivity (fake antigens and mimicry), autoimmunity (increased affinity of self-reactive antibodies), and allo-immunity (allo-like stimulation by p-i). At present, we see the negative sides of such interactions, and we do not (yet) have the knowledge to control them. However, if we could fully understand the mechanisms underlying how drugs elicit cross-reactivity beyond structural similarity, autoimmunity, and allo-immunity, it may not only help to improve our understanding of such diseases, but even open possibilities to use drugs to control and direct immune reactions.

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CONFLICT OF INTEREST

Prof. Pichler has nothing to disclose.

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