Consequences of drug binding to immune receptors: Immune stimulation following pharmacological interaction with immune receptors (T-cell receptor for antigen or human leukocyte antigen) with altered peptide-human leukocyte antigen or peptide

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Abstract

Drugs may stimulate the immune system by forming hapten–carrier complexes or via their pharmacological features, namely by noncovalent binding to proteins such as immune receptors. The latter type of immune stimulation is called the p-i concept, meaning pharmacological interaction with immune receptors, which implies stimulation of the immune system by noncovalent binding of a drug to T-cell receptors for antigens (p-i TCR) or human leukocyte antigens (p-i HLA). The functional consequences of these interactions are heterogeneous: clinically, it can lead to T-cell mediated reactions such as Stevens–Johnson syndrome/toxic epidermal necrolysis, drug rash with eosinophilia and systemic symptoms, acute generalized exanthematous pustulosis, and maculopapular eruptions. If the drug binds to the TCR, it can become stimulatory, and an additional interaction with HLA/peptide complexes is necessary for full stimulation. The T-cell reaction can be oligoclonal or polyclonal. Binding of drugs to an HLA molecule can have two consequences: if the drug can modify the HLA molecule, a distinct repertoire of peptides might be presented: this is the altered peptide model. However, peptide exchange is not necessary to make the peptide-HLA complex immunogenic: if the drug binds to HLA, already the complex of altered HLA and normal peptide is immunogenic and able to stimulate T-cells (altered peptide-HLA model). The immunological and clinical consequences of different forms of the p-i concept are described with typical p-i binding drugs such as abacavir, carbamazepine, flucloxacillin, allopurinol, and sulfamethoxazole. Thereby the role of drug binding to HLA or TCR, the affinity of drug binding, additional TCR binding, and potential oligoclonality are described and compared.

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Introduction

Drug hypersensitivity (DH) reactions are a modern and largely man made (iatrogenic) heterogeneous group of diseases, linked to the wide use of chemicals that are orally, parentally, or locally/topically applied. DH represents only a part of drug-related side effects. It is usually described as unpredictable and the clinical picture is not explained by the drug action or underlying disease: these unexpected clinical manifestations and the sometimes fulminant course make it an enigmatic area for clinicians and researchers. In addition, in DH, two highly variable systems meet: on one hand the endless number of novel small molecules, the majority chemically synthesized; and on the other, the highly variable immune system with > 10^{11} different T-cell receptors (TCRs) and antibodies per individual, and a large number of human leukocyte antigens (HLA) molecules (> 9300) in the population (http://www.ebi.ac.uk/imgt/hla/stats.html).

The predominant antigens for both T-cells/TCRs, as well as B-cells/immunoglobulins, are proteins; in particular structural or sequential epitopes [mostly 8–20-amino acid (AA) long peptide stretches] within or derived from larger proteins. The highly variable immune receptors are supposed to not interact with small molecules (< 1000 Da), as the high specificity of the immune receptors requires a certain size of its antigens to be recognized as antigen and be differentiated from other structures. If the antigen was very small, e.g. methanol (CH_{3}OH), it could bind to many different regions within the protein receptor. However, even if it would fit into the receptor binding site, it would probably not

Keywords:
- abacavir
- altered peptide concept
- altered pHLA concept
- carbamazepine
- flucloxacillin
- hapten
- p-i concept
- p-i HLA
- p-i TCR
- sulfamethoxazole

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provide enough surface interactions to cause signal transduction. A further checkpoint to exclude small molecules from activating the immune system is the need for cross-linking immune receptors to elicit an effector mechanism. Small molecules are too small to cross-link two adjacent immunoglobulins and their Fc-IgE receptors; thus no cellular signaling occurs.

In spite of these limitations, there are enough examples of drugs eliciting DH, drug-induced autoimmunity, and drug-induced immunodeficiency. Until recently, all these immune interactions were explained by the hapten features of the drug or drug metabolite (see below). However, during the last 15–20 years it has become clear that drugs have more possibilities to interact with the immune system than just by inducing an immune reaction by forming hapten–carrier complexes: drugs are able to stimulate the innate immune system by binding to TLR (imiquimod), bind to major histocompatibility complex (MHC) molecules and interfere with peptide loading onto MHCs (MHC loading enhancer, MLE), which is similar to the altered peptide hypothesis, where peptides presented by a certain allele.8

In the context of drug-induced immune reactions, antibody production and secretion and stable hapten-binding modifications are of particular cross-reactivity and autoimmunity.15 This may enhance and explain why hapten-specific IgE reactions are often fulminating and occasionally even fatal. Later, the work of Landsteiner et al showed that the delayed reaction to haptons (later shown to be T-cell mediated) is also very specific.14 Proteins are processed and presented as small 8–20-AA long peptides by MHC-encoded molecules, which as proteins (HLA) appear on the cell surface. Thereby the 14–18 HLA molecules expressed per individual present different peptides (mostly 8–10 AA for HLA class I, ~14–16 AA for HLA class II), which fit into the peptide binding groove of HLA-molecules. Further work by Wetzstein et al demonstrated that the location of the hapten modification (in the middle or at the end of the 9-mer peptide) may influence the functional consequence of the evolving immune response, in particular cross-reactivity and autoimmunity.15

It is important to realize that hapten-specific immune responses are complete immune responses, involving stimulation of antibodies and T-cells. Actually, if a drug is able to elicit both B- and T-cell immune responses, it is most likely to have hapten-like characteristics. Haptns are immunogenic and antigenic: their immunogenicity is linked to the ability to activate the innate immune system, mostly by binding to molecules that cause cell activation or damage. For quite a number of molecules it has been shown that haptenization leads to the activation of dendritic cells (DCs) in vitro,16,17 and this capacity of haptons is used to identify contact allergens by in vitro tools. The immunogenicity is supplemented by antigenicity, which is the provision of antigenic determinants for the specific immune receptors (B- and T-cell receptors).

Not every hapten modification may result in an efficient immune response: if, for example, the hapten modifies a peptide sequence, which is not presented by the available HLA alleles, the hapten modification remains unnoticed by the immune system. If the hapten induced modification does not simultaneously activate the innate immune system it may remain ignored, as no efficient immunity will be developed.

An unexplained issue of the hapten (or prohapten) theory is the fact that hapten-formation is common for given drugs such as penicillin and happens in the majority of treated patients. IgG antibody formation to penicilloyl-determinants seems to be frequent. Why only a minority of patients develops an allergic, clinically symptomatic immune reaction is unclear.

### The hapten concept

The limited interactions of small molecules with the immune system have been recognized by studying the (humoral) immune system—and soon it became clear that there are ways to overcome it. The hapten concept goes back to the 1930s; a small molecule can gain antigenicity if it is bound to larger proteins:11,12; stable, covalent binding is required to modify the larger protein structure. Thereby the modified protein could be a foreign or an endogenously produced protein, to which tolerance has been developed. In both instances, the modified protein becomes a new antigen, as the stable hapten-binding modifies the protein. The modified protein (hapten–carrier complex) can under certain circumstances elicit B-cell reactions, antibody production and secretion and—after processing to small peptides presented by MHCh–T-cell reactions. The antibody specificity is often predominantly directed to the small hapten itself as even a small modification of the hapten can already abrogate the recognition of the whole hapten–carrier complex (Table 1).12

Haptns are chemicals that are chemically reactive and have a tendency to build covalent bonds to some AAs within a protein. For example, at least 13 lysine groups within the albumin molecule have been shown to bind piperacillin and were processed to different modified epitopes within the same protein.13 For the immune system, accessible modifications may elicit antibody responses to these hapten-modified epitopes. If the antibodies react with the hapten bound to different sites on the same protein, cross-linking of the bound antibodies can occur. This requires a certain sterical distance between these hapten-modified epitopes, otherwise the rather large antibody molecule (their Fab part) would interfere with binding. If the antibody response is predominantly directed to the hapten and distant enough to allow two antibody bindings, cross-linking of the hapten-specific antibodies (including antibodies with the same specificity!) can occur by a single protein. This may enhance and explain why hapten-specific IgE reactions are often fulminating and occasionally even fatal.

Later, the work of Landsteiner et al showed that the delayed reaction to haptons (later shown to be T-cell mediated) is also very specific.14 Proteins are processed and presented as small 8–20-AA long peptides by MHC-encoded molecules, which as proteins (HLA) appear on the cell surface. The 14–18 HLA molecules expressed per individual present different peptides (mostly 8–10 AA for HLA class I, ~14–16 AA for HLA class II), which fit into the peptide binding groove of HLA-molecules. Further work by Wetzstein et al demonstrated that the location of the hapten modification (in the middle or at the end of the 9-mer peptide) may influence the functional consequence of the evolving immune response, in particular cross-reactivity and autoimmunity.15

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### The prohapten concept

Many drugs are not chemically reactive but are still able to elicit immune-mediated side effects. The prohapten hypothesis reconciles this phenomenon with the hapten hypothesis by stating that a chemically inert drug may become reactive upon metabolism.18,19 Sulfamethoxazole (SMX) is a prototype of such a prohapten. It is

<table>
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<th>Table 1</th>
<th>Comparison of the hapten and p-i concepts.</th>
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<tr>
<td><strong>Hapten concept</strong></td>
<td><strong>p-i concept</strong></td>
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<tr>
<td>Chemical binding to proteins or peptides</td>
<td>Structural binding to HLA or TCR</td>
</tr>
<tr>
<td>Covalent interactions</td>
<td>Noncovalent interactions</td>
</tr>
<tr>
<td>Often dependent on processing and metabolism</td>
<td>Processing and metabolism not required</td>
</tr>
<tr>
<td>Activation of the innate immune system</td>
<td>Bypass of the innate immune system</td>
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not chemically reactive itself but gains reactivity and thus antigenicity by a cytochrome p450 dependent metabolism (CYP2C9) in the liver, where SMX-hydroxylamine is formed. This is also found extrahepatically, where it is easily converted to SMX-nitroso (SMX-NO) by oxidation. The latter is chemically highly reactive and binds stably to intracellular proteins (cysteins) creating neo-antigenic determinants; this binding to certain intracellular proteins may also result in cell damage, if SMX is given in high doses. Thus, SMX (actually SMX-NO) seems to have both antigenic and immunogenic features. Since many different proteins might be modified, the resulting clinical picture might be as variable as with haptnens and SMX is indeed known to cause many different types of diseases affecting many organs [exanthems, anaphylaxis, Stevens—Johnson syndrome (SJS), hepatitis, blood cell dyscrasia, etc.]. These side effects are mediated by antibodies and/or T-cells. In contrast, the conversion of a prohapten to the reactive hapten may occur exclusively in the liver or kidney and may thus cause an isolated hepatitis or interstitial nephritis. Importantly, the metabolite may or may not be a hapten. If it not transformed to a hapten, it may stimulate the immune system like the parent compound (see allopurinol/oxyipurinol).

The p-i concept

A completely different approach to view and analyze the interaction of small molecules with immune receptors is based on the pharmacological feature of drugs, meaning that small molecules have a tendency to interact with larger proteins. This feature is widely exploited in the design of drugs, which are often synthesized with the scope to fit into certain receptors or enzyme pockets and to block or stimulate them by noncovalent binding. Via this normal feature of drugs, they may also target immune receptors, whereby the enormous polymorphism of the highly variable immune receptors makes it rather likely that at least some of the TCRs, some of the antibodies, or of the HLA alleles bind a drug with a substantial affinity. While most of these interactions may remain functionally irrelevant, some data generated over recent years show that some of the drug bindings to immune receptors may lead to an unusual stimulation of the immune system (Table 1).

Definition of p-i

The concept of drug interaction with immune receptors is called the p-i concept, meaning pharmacological interactions of drugs with immune receptors. According to the p-i concept, chemically inert drugs, unable to covalently bind to peptides or proteins, can nevertheless activate certain T-cells, if they fit with a sufficient affinity into some of the various T-cell receptors or MHC-molecules available. This reversible interaction is similar to the one of a ligand to its receptor.

One has to emphasize that it is a (noncovalent) drug binding to receptors based on van der Waals, electrostatic forces, or hydrogen bonds. The consequences of drug binding to either HLA or TCR receptors based on van der Waals, electrostatic forces, or hydrogen bonds. The consequences of drug binding to TCRs or MHC-molecules available. This resembles the interaction of a drug with receptor proteins, whereby the enormous polymorphism of the highly variable immune receptors makes it rather likely that at least some of the TCRs, some of the antibodies, or of the HLA alleles bind a drug with a substantial affinity. While most of these interactions may remain functionally irrelevant, some data generated over recent years show that some of the drug bindings to immune receptors may lead to an unusual stimulation of the immune system (Table 1).

A short history of the p-i concept

TCCs were first generated to penicillin G, SMX, and lidocaine. These TCCs were very specific, could be generated within weeks from patients with respective allergies and small modification of the drug abrogated reactivity. Analysis of SMX and lidocaine-reactive T cells made it clear that the stimulation of T cells by some drugs did not follow the rules described for classical protein antigens or hapten—carrier constructs: (1) Aldehydes-fixed antigen-presenting cells (APC: Epstein—Barr virus transformed autologous B-lymphoblastoid cell lines) were still able to activate TCCs, which specifically reacted with a drug (lidocaine, SMX). Since fixed APCs are unable to take up the drug, it showed that no metabolism of the drug to a reactive compound nor processing of a drug modified protein antigen to a hapten-peptide was required to elicit TCC activation, as one would expect for hapten reactions or normal protein antigens. (2) Covalently bound haptenes (such as penicillin G) are not removed from the drug (e.g. penicillin) pulsed APC by washing. In contrast, washing of APC incubated with lidocaine and SMX abrogates the reactivity to these drugs: this was interpreted as illustrating the lability of drug binding to its immune-receptors. This feature of the p-i concept made certain biochemical studies or studies with radioactive labeled drugs impossible—and remains one of the main reasons for skepticism of the p-i concept; recent data with abacavir reveal that this particular drug binds with sufficient affinity to the F-pocket in HLA-B*57:01 and is not washed away. However, this high affinity binding of a drug by noncovalent bonds is the exception and not the rule. (3) Calcium influx in TCCs happens within seconds (20—100 seconds) after the addition of the drug to the mixture of drug-specific TCCs and APCs. This time interval is too short to allow uptake, metabolism, processing of modified proteins to immunogenic peptides and presentation of modified peptides. (4) Blocking of drug metabolism or processing to peptides within APCs does not interfere with TCC stimulation; e.g. in the case of abacavir stimulation of TCCs, abacavir metabolizing enzymes are not expressed in immune cells and inhibition of metabolism or of proteasome in APCs does not affect TCC reactivity. (5) Transfection of the TCR into hybridoma cells (not expressing human MHC) shows that: the drug specificity is due to a (transfected) specific TCR; both chains of the TCR are needed for signaling; and that interaction with HLA molecules presented on APC are required for full activation.

Functional analysis of TCC revealed some peculiar features: (1) Some CD4+ TCC are MHC class I restricted; some CD8-TCC are MHC class II restricted. (2) Quite a large proportion of drug-specific TCCs simultaneously express CD4 and CD8; while an effect of cell
culture cannot be ruled out on the generation of these double-positive T cells, the frequency of such reactions is substantially higher in drug compared to peptide specific cultures/TCCs. In addition to SMX and lidocaine, carbamazepine (CBZ)- and lamotrigine-specific TCCs were often double positive.\(^{26,37}\) (3) A high proportion of CD4+ TCCs are cytotoxic: killing is mediated by FasL, but also by granzyneB and perforin\(^{23,38,39}\); however, granulysin-mediated killing is rare in CD4+ T cells. (4) Elution and/or exchange of peptides presented by HLA-DR does not affect TCC reactivity in some SMX specific TCCs;\(^{36-39}\) and some lidocaine and SMX specific peptides have been shown to be HLA-dependent, but not HLA-allele restricted in direct drug stimulations: different alleles are sufficient to stimulate the TCC.\(^{26,35}\) (5) Of SMX specific CD4+ TCCs, 27% are alloreactive: these TCCs are stimulated by alloalleles without adding SMX, meaning that the TCC also has a certain peptide specificity. This occurrence of alloreactivity is substantially higher compared to peptide specific TCC (<5%).\(^{35}\) (6) Many TCCs are self-presenting, meaning that the activated T cells, which express MHC class II, are already sufficient to activate the TCCs (in the absence of exogenously added APCs).\(^{35}\)

These data prompted the development of the p-i concept\(^{8-10}\) to explain these puzzling features.

One typical clinical feature of p-i is that the clinical effect is restricted to T cell reactions: there are no descriptions of anaphylaxis to classical p-i reacting drugs such as CBZ, phenytoin, and lamotrigine, although these drugs have obviously a high potential to stimulate the immune system. It is doubtful whether B cells are activated by these drugs, and the activation of the innate immune system and activation of DCs is also often questionable: if it happens, it might occur as a consequence of prior, direct drug-induced T-cell stimulation. Other drugs such as SMX may act mainly via p-i, but may occasionally also elicit anaphylaxis, as hapten responses are also occurring (to SMX-NO).

**Direct p-i (p-i TCR)**

Full T-cell activation by the drug (measured by immediate Ca\(^{2+}\) influx into specific T-cells, cytokine synthesis and proliferation) requires the interaction of the TCRs with MHC (documented for MHC class II) on APCs. This was proven by testing hybridoma cells of mouse origin (devoid of human MHC), which were transfected with drug (SMX or quinolone) specific TCR: they did not mount a full response (IL-2 secretion) to addition of the drug. Only if APCs with human MHC were provided, did they react.\(^{33}\)

The data also raise the question of whether the drug binds first to the MHC molecule (p-i HLA), modifying its structure and thus leading to specific T-cell activation (= indirect p-i), or whether the drug binds primarily to specific TCRs, rendering the MHC interaction a necessary, but only supplementing signal (p-i TCR, direct p-i). Initial data suggest that the interaction of the drug happens first with the TCR, since in SMX and lidocaine models the MHC-bound peptide could be exchanged or removed without affecting CD4+ T-cell activation.\(^{33,39}\) Moreover, some TCCs react to the drug even in the presence of the allogeneic MHC molecules, indicating that no strict HLA restriction for SMX or lidocaine presentation exists.\(^{13,35}\) Indeed, a recent study showed that in certain SMX specific TCCs, other, structurally related sulfanilamides may block the stimulation of SMX.\(^{10} \) Docking studies have revealed that these blocking sulfanilamides bind to the same site as SMX (CDR3), but without signalling. Other TCRs/TCCs are also reactive with SMX, but the cross-reactivity with sulfanilamides differs by: (1) combining the reactivity and cross-reactivity to SMX and 11 related sulfanilamides (proliferation and Ca\(^{2+}\) influx) of two TCRs/TCCs; (2) comparing the structure of the reactive TCR; (3) evaluating blocking of SMX stimulation by other sulfanilamides; and (4) docking studies of SMX and sulfanilamides to different TCRs whereby a rather clear picture emerged which identified the involved sites on the SMX-specific TCR.\(^{30}\) They were on the variable region of the CDR2 or CDR3 region and were absent on other TCRs, which were not SMX reactive. Moreover, comparing the two model TCRs reveals that TCC 1.3, which is stimulated by SMX only, binds to CDR3, which is in the interphase of TCR and peptide-MHC. It directly activates the T cell. In contrast, in TCC H13 the binding site is located outside of the peptide-MHC interacting region. The SMX binding on CDR2 induced an allosteric modification, with structural changes enhancing TCR-peptide interactions.\(^{31}\)

Another example is the important role of a certain TCR clonotype in CBZ hypersensitivity. Patients with CBZ-induced SJS/toxic epidermal necrolysis (TEN) and HLA-B*15:02 background react to normal peptides, and not against a hapten-modified peptide.\(^{62}\) The T cells use the TCR V11-1ISGSY clonotype.\(^{64}\) This clonotype was present in 16 of 19 patients and absent in all 17 CBZ-tolerant patients. CBZ-specific cytotoxicity could be primed in vitro in the PBMCs of healthy individuals who are carriers of HLA-B*15:02 and VB11-ISGSY. These data show that, in addition to B*15:02, the TCR sequence may be crucial for disease manifestations. Although no direct interaction of the drug (CBZ) with the TCR was found, the data suggest that CBZ and its metabolites induced by CYP450 are involved in the selection and stimulation of T cells. It may be that only if both a certain HLA and a certain TCR sequence are present that a strong and disease-causing stimulation develops.

**Indirect p-i, (p-i HLA)**

A decisive step forward in understanding severe, T-cell-mediated DH reactions was the description of a strikingly high HLA-B-allele association\(^{44-46}\) for certain severe DH reactions. The HLA-allele associations are extremely high, with a negative predictive value close to 100%; this is linked to the reaction to a particular drug, and mostly linked to severe reactions. The frequency of the involved allele in the population is important for linkage associations.\(^{47}\)

The strong association raises the question of whether this linkage is due to the allele itself or the presented peptide. Elution of the peptide shows clearly that the peptides are not modified, neither in CBZ nor in abacavir hypersensitivity reactions.\(^{3,5,42}\) In the case of HLA-B*57:01 linked abacavir hypersensitivity,\(^{48}\) the binding of abacavir to the F-pocket can be shown by crystallography.\(^{49}\) A similar binding cleft can be identified for CBZ, which binds to HLA-B*15:02, an allele rather common in southeast Asia and probably responsible for the high occurrence of SJS/TEN due to CBZ in these regions.\(^{4,45}\) Thus, p-i (HLA) can be verified by various means and for different drugs.

**Altered peptide model**

What is the consequence of drug binding to HLA? In principle, two possibilities exist: the drug may alter the peptide presented (altered peptide repertoire),\(^{3-5}\) or the drug may, by binding to HLA, alter the whole immunogenic conformation (altered HLA).

Recent studies on abacavir hypersensitivity have not only shown that abacavir can bind by noncovalent bonds in the F-pocket of the HLA-B*57:01 groove, but also that this binding can even modify the peptide bonding properties of HLA-B*57:01.\(^{3-5}\) These in vitro studies have been done using rather high concentrations of abacavir (100 μg/mL). Three different groups showed that in the presence of abacavir more peptides harboring a small aliphatic anchor residue at the C-term are loaded, leading to an altered peptide repertoire. About 20% of eluted peptides were altered peptides. These studies also suggest that not abacavir but an altered
peptide is the antigen in abacavir hypersensitivity—thus postulating a possible link between DH and autoimmunity.

It should be noted that the concept of altered peptide presentation has already been approached from a different viewpoint. In the search to optimize MHC peptide presentation and to improve vaccination efficacy, Dickhaut et al described so-called MLEs, which are small catalytic compounds able to open up the MHC binding site by triggering ligand-release and stabilizing the receptive state of MHC class II molecules. One such MLE is adamantane ethanol, but abacavir may have a similar ability on MHC I (B*57:01).

**Altered pHLA model**

A separate extensive analysis of many abacavir reactive TCC challenges the altered peptide concept and came to the conclusion that peptide exchange is not necessary for immunogenicity (32). Altering the HLA-(self) allele without modifying the peptide sequence is sufficient for immunogenicity, as drug binding alters the whole conformation of the HLA-peptide complex (pHLA) as seen from the TCR and make the self-allele plus self-peptide antigenic for T-cells.

The crucial experiment is shown in Figure 1: abacavir binds to B*57:01 with high affinity and resists washing of abacavir incubated cells. When APC are pulsed with abacavir overnight, it is internalized and loaded in the endoplasmic reticulum (ER) on HLA-bated cells. When APC are pulsed with abacavir overnight, it is the whole conformation of the HLA-peptide complex (pHLA) as sequence is sufficient for immunogenicity, as drug binding alters the whole conformation of the HLA-peptide complex (pHLA) as seen from the TCR and make the self-allele plus self-peptide antigenic for T-cells. Altered pHLA model (Figure 1).

**Open questions for p-i**

The p-i concept is an nonimmunological approach to an immunological problem, namely how a small molecule interacts with the immune system. It postulates a pharmacological interaction without the multiple checkpoints typical for immune reactions (activation of DCs, costimulation). This feature of p-i could explain the self-destructive nature of some DH reactions. By contrast, the possibilities of the immune system to react are limited to immune reactions and while it appears bizarre, they are partly similar to normal immune stimulations and have some similarities to virus stimulations, or occasionally to graft-versus-host or superantigen stimulations. Differentiating p-i TCR or p-i HLA also raises several points (see below).

**p-i stimulation of preactivated cells**

Already by conceiving the p-i concept it was enigmatic how a small molecule might stimulate T cells. This refers mainly to p-i TCR stimulations, for which initially more data have been accumulated than for the later elaborated p-i HLA stimulations. Actually, it has been speculated that only effector/memory cells may react to the minor signal as drug binding to TCR (p-i TCR), since memory T cells have a substantially lower threshold for activation than naive T cells. This threshold of T-cell activation might be low in the skin, where sentinel T cells show indeed a lower threshold of activation than resting circulating T cells.

Altogether occurring massive immune stimulation of T cells, as it occurs during generalized herpes or human immunodeficiency virus infections, or during exacerbations of autoimmune diseases, may be a cofactor for lowering the threshold of T-cell activation by drugs; such immune processes go along with high cytokine levels and an increased expression of MHC- and costimulatory molecules on APC and other cells. Consequently, T cells are already preactivated by the immune stimulation and might be more ready to react to a minor signal such as binding of a drug to its TCR. This would explain the high occurrence of drug hypersensitivities in these diseases. However, experimental data supporting these clinical observations are still missing.

**Multiple DH**

About 10% of patients with severe drug allergies develop a second drug allergy; as recently shown, patients with multiple DH carry in their blood T-cells with a particular phenotype, similar to herpesvirus stimulated T-cells. However, these individuals were negative for human herpesvirus-6, Epstein–Barr virus, and cytomegalovirus infections. This peculiar cell fraction contains the precursor T cells for different drugs, while the normal cell fraction does not contain these precursor cells. This finding shows that some preactivation may play a role; and that a neglected risk factor for DH is the existence of a DH—either ongoing or in remission.

**Costimulation by metabolites**

While the p-i concept has been documented for many drugs (SMX, lidocaine, lamotrigine, CBZ, p-phenylenediamine, quinolones, radio contrast media, abacavir, fluoxacillin, and more), in some drug reactions, the metabolites of the parent compound are often also implicated in DH. Clonizations suggests that some of these TCCs/TCRs are reactive via p-i not only to the parent compound, but possibly also to a metabolite. Some of the implicated metabolites might be chemically reactive and cause hapten-like stimulations. Thus, immune reactions might evolve via p-i stimulations and hapten simultaneously. The reaction to a hapten raises the question of whether the hapten-characteristic of a drug, with its immunosimulatory consequences on innate immunity, might be a cofactor for some p-i mediated stimulations.

**Differences and similarities of p-i stimulations by various drugs**

In addition to maculopapular exanthems, drug rash with eosinophilia and systemic symptoms (DRESS), acute generalized exanthematous pustulosis, SJS/TEN, and some other severe hypersensitivity syndromes were described for drugs that stimulate in vitro via the p-i concept. Often positive patch tests or delayed
It is assumed that abacavir-induced T-cell clones (TCCs) react with a peptide and not abacavir itself, as abacavir binds to the F-pocket with no direct access to the T-cell receptor for antigen (TCR). However, the dose–response curve is at first sight suggestive of direct abacavir reactivity. How can one explain this? All abacavir-induced TCCs react to antigen-presenting cells (APCs), which were pulsed with abacavir over night. A fraction of these TCCs carry TCRs that have enough affinity for the human leukocyte antigens (HLA)-
Table 2  p-i stimulations: characteristics of clinical and immunological features of some well-defined drug hypersensitivity reactions.

<table>
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<tr>
<th></th>
<th>Abacavir</th>
<th>Flucloxacillin</th>
<th>Allopurinol</th>
<th>Sulfamethoxazole</th>
<th>Carbamazepine</th>
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<tr>
<td>HLA association</td>
<td>B*57:01</td>
<td>B*57:01</td>
<td>B*58:01</td>
<td>No</td>
<td>Han Chinese: B<em>15:02, Japanese &amp; European: B</em>31:01</td>
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<td>CD8 (predominant)</td>
<td>Mainly CD8</td>
<td>CD4, (CD8)</td>
<td>Mainly CD8</td>
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<td>TCC reactivity with pulsed APC</td>
<td>Immune response elicited</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
<td>No response</td>
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<tr>
<td>Characterization of binding to HLA</td>
<td>Stable binding (persistent after washing)</td>
<td>Labile binding (not persistent after washing)</td>
<td>Labile binding (not persistent after washing)</td>
<td>7; labile to TCR</td>
<td>Rather labile (?)</td>
</tr>
<tr>
<td>Activation of DC</td>
<td>No</td>
<td>Unknown</td>
<td>Unknown</td>
<td>?; controversial</td>
<td>No</td>
</tr>
<tr>
<td>Activation of TCC</td>
<td>Immediate (10 μg) and 10 ng after internal HLA loading</td>
<td>Immediate to fast (maximum after 5–7 min)</td>
<td>Immediate</td>
<td>Immediate</td>
<td>?</td>
</tr>
<tr>
<td>Proposed mechanism of T-cell stimulation</td>
<td>p-i, altered peptide or altered pHLA</td>
<td>p-i in HLA-B<em>57:01-individuals, hapten in HLA-B</em>57:01-individuals</td>
<td>Han Chinese, Thai: SJS/TEN; Caucasian: SJS</td>
<td>Rash, SJS/TEN, DRESS</td>
<td>SJS/TEN</td>
</tr>
<tr>
<td>Clinical manifestation</td>
<td>Abacavir hypersensitivity syndrome 55%</td>
<td>DILI in B*57:01 (but nephritis, exanthema with other alleles) 1–2% (?)</td>
<td>Han Chinese, Thai: SJS/TEN; Caucasian: SJS</td>
<td>1.52%</td>
<td>Not known</td>
</tr>
<tr>
<td>PPV for disease in risk population</td>
<td>Polyclonal</td>
<td>Polyclonal</td>
<td>Polyclonal (oligoclonal)</td>
<td>Polyclonal (oligoclonal)</td>
<td>VB11, VA22 more frequent (Han Chinese) → VB11-15GSV in 84% of patients with SJS/TEN</td>
</tr>
<tr>
<td>T-cell receptor repertoire</td>
<td>None</td>
<td>Hapten, p-i 77</td>
<td>No, not even with oxypurinol</td>
<td>Depends on the TCR: from very restrictive to extensive with other sulfonamides</td>
<td>With oxcarbazepine or metabolites</td>
</tr>
</tbody>
</table>

Appearances in Table 2. Abacavir (ABC) binding to B*57:01 differs from other drug binding by its high affinity to HLA-B*57:01. Abacavir is not washed away by incubating it with B*57:01+ cells. It is very selective for abacavir, but not for similar nucleoside analogues. B*57:01 has a high positive predictive value of 47.6% for abacavir hypersensitivity manifestations, if the patient is patch test positive. This high association of B*57:01 and hypersensitivity manifestations might be related to the high affinity interaction of abacavir with B*57:01. Abacavir reactivity may appear as early as 1–3 days after treatment started, and is detected after 2 weeks culture in every B*57:01+ individual, indicating a rather high precursor frequency. No other allele, even B*58:01, has been linked to abacavir hypersensitivity, where the difference between B*57:01 and B*58:01 is only one AA in the peptide binding groove.

apprising intradermal reactions and positive lymphocyte transformation tests could be found. The reactivity in skin or in vitro tests often appears very similar between p-i or hapten-stimulated drugs and cannot be differentiated by the clinical picture alone. The clinical symptoms by the p-i drugs may appear more rapidly and some severe reactions such as SJS/TEN, DRESS, or hypersensitivity syndromes are clearly p-i related. In addition, the concentration of the drug is—like in other pharmacological reactions—decisive for the clinic in p-i reactions. It is not infrequent that DRESS symptoms arise after increasing the dose.

A summary of the best defined p-i acting drugs and their peculiarities is given in Table 2. In some, the affinity of drug binding to HLA may be crucial (illustrated by abacavir), other drugs may be presented by various HLA-alleles but with different affinity and severity (CBZ, allopurinol), in B*58:01 associated allopurinol hypersensitivity the metabolism to oxypurinol is required, in SMX and CBZ, the binding to TCR is important, and in flucloxacillin not

the hapten, but the p-i stimulation may be decisive for liver damage. Altogether, the p-i stimulating drugs vary considerably (Table 3).

Abacavir (ABC) binding to B*57:01 differs from other drug binding by its high affinity to HLA-B*57:01. Abacavir is not washed away by incubating it with B*57:01+ cells. It is very selective for abacavir, but not for similar nucleoside analogues. B*57:01 has a high positive predictive value of 47.6% for abacavir hypersensitivity manifestations, if the patient is patch test positive. This high association of B*57:01 and hypersensitivity manifestations might be related to the high affinity interaction of abacavir with B*57:01. Abacavir reactivity may appear as early as 1–3 days after treatment started, and is detected after 2 weeks culture in every B*57:01+ individual, indicating a rather high precursor frequency. No other allele, even B*58:01, has been linked to abacavir hypersensitivity, where the difference between B*57:01 and B*58:01 is only one AA in the peptide binding groove.
The T-cell reaction to abacavir is polyclonal. The high-affinity binding of abacavir to B*57:01 can influence the peptide repertoire presented: if high abacavir concentrations are used (100 μg/mL, which substantially exceeds the normal plasma concentrations of abacavir of 3.3 μg/mL) up to 20% of peptides eluted from B*57:01/abacavir differ from the normal B*57:01 peptide repertoire. However, no convincing data are yet available that these altered peptides elicit a T-cell response. Moreover, as shown by Adam et al and discussed above (Figure 1), abacavir may simply modify the B*57:01 structure without removing the peptide: this altered pHLA is not sufficient to cause a massive immune stimulation, both when abacavir binding to B*57:01 occurred on the cell surface or in the ER (altered pHLA model). Re-exposure to abacavir in B*57:01+ individuals with prior hypersensitivity can lead to a fulminating, lethal hypersensitivity reaction with rapid destruction of liver, lung tissue, etc. Of note, there are also some minor, less severe hypersensitivity reactions in B*57:01 negative individuals as well. Their pathomechanism has not yet been deciphered.

CBZ can bind to HLA B*15:02, but also to B*15:11 and A*31:01. If the B*15:02 is present in the population, side effects are mainly B*15:02 associated. CBZ binding is not as exclusive as that of abacavir, as some cross-reactivity to CBZ-metabolites has been described in B*15:02. If the B*15:02 allele is not prevalent, association with other alleles such as A*31:01 or A*15:11 appear (Japanese, Europeans). Whether the immune reaction is similar to B*15:02 driven reactions is not yet clear.

The interaction with B*15:02 seems to be rather strong, but has less affinity than the one with abacavir. Docking studies have placed CBZ in the middle of the peptide binding groove, at the secondary anchor residue. It can also induce presentation of altered peptides, which was observed in about 15% of peptides presented (4). Thus, it might, like abacavir, actually induce a peptide response. The T-cell response to CBZ in B*15:02+ individuals seems to be oligoclonal. It has been reported that only if a certain TCR-clonotype is available, does a strong cytotoxic immune response to CBZ develop. This clonotype (VB-11-ISGVY) is also present in healthy individuals; such cells could be primed to develop into cytotoxic T-cells by CBZ exposure (59). The example illustrates nicely that some drugs may develop immune stimulations by binding to HLA and TCR.

McCluskey et al has also postulated a possible role of altered peptide presentation in CBZ-induced B*15:02-linked hypersensitivity reactions. However, no data demonstrating a T-cell reaction to the altered peptide have been obtained so far. Thus, while the possibility of altered peptide presentation is well documented (at least for abacavir), actual relevance and the in vivo role of altered peptide presentation is not yet well documented.

Flucloxacillin is a penicillin derivative and actually a typical hapten. Flucloxacillin binds covalently to selective lysine residues in albumin in a time-dependent manner. Multiple binding sites of flucloxacillin to various lysines within albumin have been found, and modifications of albumin by flucloxacillin could be detected in all treated patients studied to date. Multiple modified epitopes may induce immune reactions, which are presented by various HLA-alleles. Thus it is difficult to link the hapten characteristic to a single HLA-allele. While the clinical picture of flucloxacillin allergy is heterogeneous (exanthem, interstitial nephritis), flucloxacillin-induced liver injury is linked to B*57:01. Interestingly, exactly this allele has been shown to present flucloxacillin via p-i and not via hapten, suggesting a link between p-i presentation and severe flucloxacillin-induced liver injury. Thus, a hapten characteristic does not exclude p-i presentation as well.

Allopurinol is a main cause of SJS/TEN in Europeans. It is strongly linked to B*58:01 in Chinese and somewhat less in Europeans, where only 60% express this allele. Thus, other alleles are also involved. Allopurinol is rapidly metabolized to oxypurinol, and in vitro analysis of affected patients has shown that oxypurinol is the relevant antigen. Dose seems to be a crucial factor in developing these sometimes severe side effects. The immune response is due to p-i HLA, and is mainly directed to oxypurinol.

SMX is a drug that causes a variety of hypersensitivity reactions, which are not known to be HLA-allele linked. It is a typical p-i hapten, and some side effects appear to be linked to hapten feature of this drug (SMX-NO), while in other reactions like the DRESS syndrome by SMX or sulfapyridine (the relevant component of sulfasalazine) hypersensitivity reactions are caused by direct binding to TCRs. Thus, these reactions are p-i TCR mediated; the binding site of SMX (and other sulfanilamides) in two SMX-specific TCC has been identified by blocking and docking studies on the CDR2 and CDR3 regions of the TCR and by molecular dynamics modeling. Binding may result in signaling (with Ca influx and proliferation, if interaction with HLA is possible). Surprisingly, the T-cell reactions are often polyclonal at the start, but SMX, and other drugs (e.g. ioxidanol) may occasionally cause an oligoclonal or even monoclonal T-cell outgrowth.

**Conclusion**

Taken together, the p-i concept suggests that some drug allergies are pharmacological off-target reactions and not true allergies. Drugs are able to interfere with the human immune system not only as an antigen (namely as hapten coupled to a carrier molecule), but also by pharmaceutical means, merely by acting on (immune) receptors. The dogma that small chemicals are not full antigens is still valid and must not be refused, but DH-like reactions can occur also by simple pharmacological means, namely by stimulation of drug binding to (immune) receptors such as HLA or TCR.

From a general perspective, DH reactions and the underlying p-i concept demonstrate certain limitations of modern medicine: DH is, like graft-versus-host diseases or transplant rejections, an unusual (or, better, unnatural) way of immune stimulation, which was not foreseen in the natural evolution of the immune system. The immune system was neither prepared for the wide use of chemicals/drugs, nor was the immune system prepared that humans will transplant organs. In a certain sense, DH is, like transplantation...
References


