



Mechanisms leading to T-cell activation in drug hypersensitivity

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Purpose of review

Delayed-type or nonimmediate drug hypersensitivity reactions often involve the activation of drug-specific T cells. As such, the molecular initiating event is an interaction between HLA proteins, HLA-binding peptides and the drug. For many years, the formation of covalently modified drug protein adducts was assumed to be a prerequisite for T-cell activation. The purpose of this article is to review recent studies using human PBMC, T-cell lines and clones, which show that drugs are in fact loaded onto HLA molecules in different forms to activate T cells.

Recent findings

We now know that protein-reactive drugs such as β -lactam antibiotics activate T cells via direct noncovalent interactions with HLA or HLA-binding peptides, direct covalent modification of HLA-binding peptides and covalent binding of non-HLA associated proteins. Adducts formed inside and outside of the cells undergo protein processing to generate HLA-binding peptides that are assumed to contain the drug modification. Studies using synthetic stable (e.g. oxypurinol) and reactive (e.g. nitroso sulfamethoxazole) metabolites show that metabolites activate T cells via the same pathways. A variety of drugs with different structural features have also been shown to activate T cells through a direct HLA-binding interaction. Of note, abacavir behaves in an unexpected way, binding deep in the peptide binding cleft of one HLA, selectively activating CD8⁺ T cells.

Summary

In-vitro studies have revealed that a number of drug HLA-binding interactions lead to the activation of T cells. These can be categorized according to two hypotheses, namely hapten and pharmacological interactions. As we move forward with the development of diagnostic and predictive T-cell assays, it is critical to reach a consensus that direct drug HLA binding and the formation of drug protein adducts are important events for T-cell activation.

Keywords

altered self-antigens, hapten, pharmacological interaction

INTRODUCTION

Delayed-type drug hypersensitivity reactions targeting skin and internal organs remain an important clinical problem and an obstacle to the drug development process. The lack of effective tools to predict which compounds will be associated with a high frequency of reactions relates to the fact that susceptibility is a function of the chemistry of the drug and the genotype and phenotype of the patient. In terms of patient genotype, the expression of specific human leukocyte antigen (HLA) alleles are known to predispose individuals to certain forms of drug hypersensitivity. The association between flucloxacillin-induced liver injury and expression of the HLA class I allele B*57:01 is one of the most widely studied [1]. Flucloxacillin interacts with the protein encoded by HLA-B*57:01 to activate CD8⁺ T cells in

patients with liver injury and all healthy donors. Thus, 100% of HLA-B*57:01+ individuals have a T-cell repertoire for flucloxacillin [2–4]. Despite this, only 1 in 1000 HLA-B*57:01+ patients exposed to flucloxacillin develop liver injury. This indicates that there are additional yet to be defined genetic or phenotypic differences that overlay the HLA

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KEY POINTS

- Triggering of T lymphocytes from patients with drug hypersensitivity involves the formation of a complex between the drug, HLA molecule and HLA-binding peptide.
- Drugs interact with HLA molecules and HLA-binding peptides through covalent and noncovalent binding interactions.
- Herein we review advances in our understanding of the way in which drugs interact with HLA molecules to activate T cells.

association and determine the outcome of drug exposure be it immunological ignorance, tolerance or hypersensitivity. This topic is discussed in detail elsewhere in this edition of the journal.

One of the major controversies in the field of drug hypersensitivity is the mode of drug HLA binding that leads to the activation of T cells. Landsteiner and Jacobs [5] in the 1930s discovered a relationship between the reactivity of simple chemicals and their ability to sensitize experimental animals. This led to the evolution of the hapten hypothesis for drug hypersensitivity reactions, which states that reactive drugs or drug metabolites (haptens) must bind covalently to proteins to initiate immune reactions. This hypothesis has a body of supportive evidence. For example, IgG and IgE antibodies from plasma of patients with hypersensitivity reactions to drugs such as β -lactam antibiotics and halothane bind to drug-protein adducts, but not the native protein [6,7]. Although IgG antibodies are not always pathogenic [8], it does prove that the immune system is responding to drug-protein adducts. Expansion of the hapten concept to T-cell-mediated drug reactions was a logical step. Proteins are naturally processed into peptide fragments, the peptides bind to HLA molecules and are displayed to T cells on the surface of antigen-presenting cells. Hapten theory suggests that T cells are activated with HLA-bound peptides that contain the hapten bound covalently to nucleophilic sites on amino acids such as cysteine and lysine.

Our understanding of the chemical basis of drug hypersensitivity reactions improved, but became much more complicated, when researchers found that certain drugs interact directly with HLA molecules or HLA-binding peptides. This led to evolution of the pharmacological interactions hypothesis, which states that a direct, readily reversible interaction of drug with HLA will activate T cells (i.e. binding of drug to the immune receptor [HLA], results in unexpected and unwanted secondary pharmacology that manifests as hypersensitivity).

Herein, we summarize recent advances in our understanding of the hapten and pharmacological interactions hypotheses with reference to T-cell-mediated drug hypersensitivity. We now know that drugs and drug haptens interact with HLA in different ways, and this has resulted in exciting new concepts. However, these data should enhance and not confuse or complicate our mechanistic understanding. The fact remains that drugs bind to HLA via covalent or noncovalent bonds, and as such, only two fundamental hypotheses are required for drug-specific T-cell activation – hapten and pharmacological interactions. These are discussed below.

HAPTEN HYPOTHESIS

The consequences of drug-protein adduct formation

Haptens are small molecules that can covalently bind to proteins either directly or via drug metabolism. The major protein targets for the directly reactive haptens could be serum proteins such as albumin and haemoglobin, intracellular proteins or specific tissue proteins depending on the routes of administration and the accumulation in specific tissues. The detection of amoxicillin-modified intracellular proteins through use of a biotinylated antibiotic could be of particular importance [9]. In contrast, protein targets for the reactive metabolites could vary greatly depending on the sites of formation and their chemical reactivity, though liver proteins may be the major targets for many reactive metabolites because of its primary metabolic function. For example, the cytochrome P450 enzymes are targeted by many short-lived reactive metabolites, and haptening of these proteins can lead to both drug-specific and auto-immune responses [10]. Stable metabolites that are able to escape from the liver can also haptenate circulating proteins, for example, carbamazepine 10–11 epoxide was found to bind to albumin in patients receiving carbamazepine therapy [11]. Both haptenic structures and carrier proteins are vital for T-cell and antibody recognition, however, haptenic structures have a dominant role in both events. For instance, piperacillin forms two distinct haptens in patients by conjugation with the lysine residues of albumin, one formed directly from piperacillin and a second in which the dioxopiperazine ring has undergone hydrolysis [12]. These haptens are able to stimulate the T cells of patients with piperacillin hypersensitivity. Flucloxacillin also binds to the same lysine residues on albumin as piperacillin, but the resulting conjugates do not stimulate the piperacillin

hypersensitive patient-derived T cells, demonstrating that the drug conjugates have structural specificity [13]. In addition, no cross-reactivity was observed between antipiperacillin and antifucoxacin IgG antibodies, providing further evidence that the immune responses to drugs are highly structurally specific. It is, therefore, absolutely vital to characterize the exact haptenic structures if we are to understand the molecular mechanisms of drug hypersensitivity.

Nowadays, advanced mass spectrometric-based proteomic methods have allowed us to not only identify protein targets and the specific sites where the haptens bind to but to also characterize complex antigenic structures formed *in vivo*. For example, both amoxicillin and its dimer were found to form adducts on albumin in patients, and clavulanic acid formed multiple adducts on albumin through several degradation intermediates [14]. We and others have shown that clavulanic acid rapidly undergoes hydrolysis in plasma and forms multiple degradation products [14,15]. Adducts including those formed by direct binding of clavulanic acid to lysine residues, a cross-linking adduct, and pyrazine adducts were identified by mass spectrometric analysis. All these adducts could have potential to stimulate specific B and/or T cells. Considerable progress has been made on the characterization of haptened proteins, but the functions of these antigens in drug hypersensitivity remain largely unexplored. A limited number of drug-modified proteins have been shown to stimulate drug-specific T cells via a pathway dependent on protein processing [16,17²²]. Synthetic peptides designed to bind to specific HLA molecules and modified covalently with drugs also stimulate a T-cell response [18²²,19]. Furthermore, synthetic reactive drug metabolites activate T cells through the direct covalent modification of HLA-binding peptides or HLA itself and the formation of adducts with non-HLA-associated protein (Fig. 1) [20,21]. The latter is dependent on protein processing to generate HLA-binding peptides. It is worth noting that the formation of drug antigens may be important for initiating an immune response, but this process alone will not always result in hypersensitivity. Indeed, circulating drug-modified proteins have been detected in patients without hypersensitivity [17²²]. Furthermore, all individuals express T-cell receptors (TCRs) that detect drug-modified peptides [18²²]. Therefore, the critical question remains, what determines immunity? The consistent exposure to drug antigens, the maintenance of immune activation by costimulatory signals, or the inhibitory signals that regulate the immune responses? Any of these

factors may be important in determining the overall immune response.

Processing and presentation of haptened proteins

Intracellular processing of protein antigens to peptides followed by presentation of peptide-HLA complexes by professional antigen-presenting cells such as dendritic cells and B cells is essential for initiating T-cell responses to infectious pathogens. Comprehensive methods have been developed to evaluate the identities and densities of peptides presented by antigen-presenting cells to address the relationship between peptide display by HLA molecules and antigen-specific T-cell responses. For example, profiling of peptides presented by HLA-class I molecules has highlighted a mechanism through which a polymorphism within an HLA supertype can diversify immune responses by varying peptide length preferences [22]. However, how adduction of haptens to proteins affects the processing of proteins and the presentation of haptened peptides remains unknown. One possible pathway of presenting drug-peptide conjugates is through intracellular processing of haptened proteins. It is anticipated that haptening could have profound effect on the complex antigen-processing cascade. For example, covalent binding of haptens to specific amino acid residues, for example, cysteine, serine, lysine or arginine could block the enzymatic processing by cytoplasmic proteases, which could result in generating different peptides of various lengths compared with the counterpart without modification. In addition, haptening of basic amino acids could affect the peptide-binding affinity to the transporter associated with antigen processing (TAP) [23], thereby creating structurally distinct peptide-HLA complexes. Alternatively, direct haptening of the peptide-HLA complex could also occur. In this scenario, the selectivity of covalent binding will be determined by the surface exposure and intrinsic reactivity of an amino acid and the presentation of drug-peptide conjugates will be independent of peptide-HLA binding affinity. Finally, haptening of HLA itself could lead to altered peptide presentation through covalent binding to the peptide binding groove or even allosteric sites. It should be noted the position of hapten binding may alter the TCR contact sites on the HLA molecule, and thereby play an important role in T-cell activation. Despite extensive research in this field, drug-peptide conjugates presented by antigen-presenting cells have not been reported yet, possibly because of the technical challenges of identifying them. The lack of knowledge has greatly hindered the understanding of how

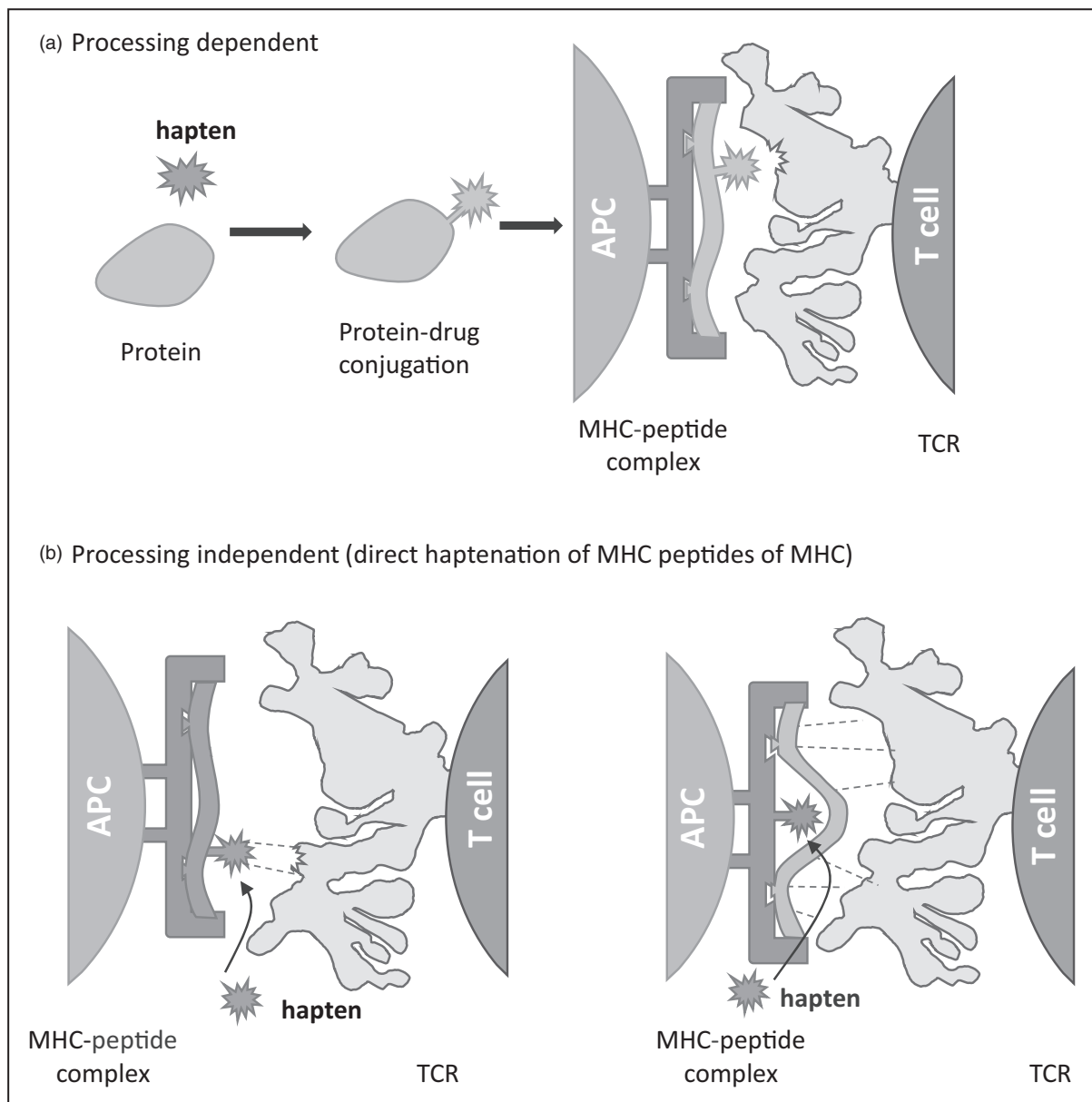


FIGURE 1. Possible pathways of presenting haptened peptides to T cells. (a) Processing dependent: haptens (small drugs and chemicals) can covalently bind to proteins that are processed intracellularly and presented by APCs in the context of MHC molecules; (b) Processing independent: they can directly bind to either MHC-peptide complex or MHC molecules, leading to alteration of either the conformation of MHC-peptide complex or the presentation of the self-peptide repertoire.

haptens initiate T-cell responses. Continuing efforts to characterize haptened peptides presented by specific HLA alleles and to examine their immunogenicity will certainly shed light on the molecular mechanisms underlying T-cell activation.

In addition to antigen presentation by direct cell-cell contacts, the intact peptide-HLA complex can also be transferred and presented intercellularly through extracellular vesicles. This process involves the acquisition of entire peptide-HLA complexes from a donor cell, supplementing cross-presentation pathways [24]. This can occur between

antigen-presenting cells or antigen-presenting cells and other target cells. We and others have recently shown that exosomes released by primary human hepatocytes and B cells contain peptide-HLA complexes and drug-modified proteins [25^{***}]. The exosomes can be up-taken by dendritic cells, providing an additional pathway for the transfer of tissue-specific drug-derived antigenic signals to immune cells (Ogese *et al.*, unpublished data). It is possible that the transfer of peptide-HLA complexes between target cells and antigen-presenting cells is of great importance and may play a role in drug-

specific T-cell-mediated tissue injury. Therefore, characterization of peptide–HLA complexes present in exosomes will certainly improve our fundamental understanding of the molecular mechanisms of antigen presentation. This knowledge will also allow us to further investigate the communications between target cells and immune cells.

It is important to mention that the innate immune system is critical in initiating or silencing T-cell responses. For example, under noninflammatory environment, immature dendritic cells can modulate tolerant responses by induction of anergy, T-regulatory cells, or immunomodulatory cytokines; whereas mature dendritic cells stimulated by inflammatory signals, drugs, or drug-proteins adducts can efficiently prime naïve T cells and induce the development of T-effector cells [26,27]. A recent study has shown that amoxicillin can change the steady state of dendritic cells, from hypersensitivity patients but not controls, leading to a degree of maturation [28]. Although the exact mechanisms of triggering the dendritic cell maturation by drugs remain to be determined, activation of MAPK and NF- κ B signalling by amoxicillin is thought to be involved in dendritic cell maturation [29]. Further studies on drug–dendritic cell interactions will help establish the threshold of T-cell activation and tolerance.

The pharmacological interactions concept

The pharmacological interaction concept stands for pharmacological interaction with immune receptors and stipulates that a small molecular compound can interact with the TCR-major histocompatibility complex (MHC) complex by noncovalent interactions. If such interactions are strong enough, the stabilization of the complex leads to the activation of the T cell. For the pharmacological interaction concept, covalent modifications of proteins are not a prerequisite for the generation of the antidrug T-cell response. The pharmacological interaction concept arose around 20 years ago from the observation that drug-reacting T-cell clones (TCC) did not always follow the rules of T-cell activation by haptenated proteins [30]. Indeed, lidocaine or sulfamethoxazole reacting TCC, which were isolated from drug hypersensitive patients were activated only in the presence of the soluble drugs. Washing of antigen presenting cell (APC) preincubated with drugs abrogated the T-cell activation, showing that the antigen complex was not stable. Moreover, in the pharmacological interaction mechanism, the inhibition of either the endogenous or the hexogenic antigen presentation pathway did not influence the activation of drug reacting T cells in the opposite to

haptenated proteins. Indeed, the proteasome inhibition did not reduce the stimulation of CD8⁺ T-cell clones recognizing soluble flucloxacillin on HLA-B*57:01 molecules (Fig. 2). In contrast, when flucloxacillin was recognized by the hapten mechanism, proteasome inhibition abrogated the T-cell activation. In addition, it has been shown that even paraformaldehyde fixed APC were able to present drugs following the pharmacological interaction mechanism [31], showing the complete independence of pharmacological interaction mechanism toward the antigen processing pathways. Finally, the pharmacological interaction mechanism could be revealed by the activation kinetics of drug-reacting TCC. Whereas the presentation of haptenated proteins is time consuming (minutes to hours), intracellular calcium concentration increases immediately after the addition of the soluble drug into the cell culture and peaks within seconds [32].

The interaction partners in the pharmacological interaction concept

In drug-reacting T cells following the pharmacological interaction mechanism, all the interactions partners, that is, HLA molecule, the embedded peptide, the TCR and the drug are vital for T-cell activation, however, the drug plays a dominant role. Whereas the absence of any of the interactions, partner would abrogate the T-cell activation, only the addition of the drug triggers the reaction, as exemplified in calcium influx assay (see above). Drug reacting pharmacological interaction TCC were shown to be HLA restricted [30]. In the majority of the cases, CD4⁺ T cells are MHC class II restricted and CD8⁺ T cells are MHC class I restricted. Nevertheless, it is not uncommon to observe unconventional HLA restriction, a CD8⁺ TCC being MHC class II restricted for instance [33]. Although the majority of the TCC are also HLA allele restricted [34], it has been assessed that roughly one fourth of the drug-reacting T cells were not HLA allele restricted. However, this feature could not be observed in HLA-associated drug hypersensitivity reactions, such as abacavir and HLA-B*57:01, allopurinol and HLA-B*58:01 or carbamazepine and HLA-B*15:02. Here, tiny modifications of the restricting presenting HLA allele were shown to abolish the reactivity [35]. In the case of abacavir, crystals could be generated and revealed the drug-binding site within the peptide-binding groove of the HLA-B*57:01 [36–38]. Of note, the interactions between abacavir, the HLA molecule and the peptide were purely noncovalent, demonstrating the pharmacological interaction properties of this association. The van der Waals interactions

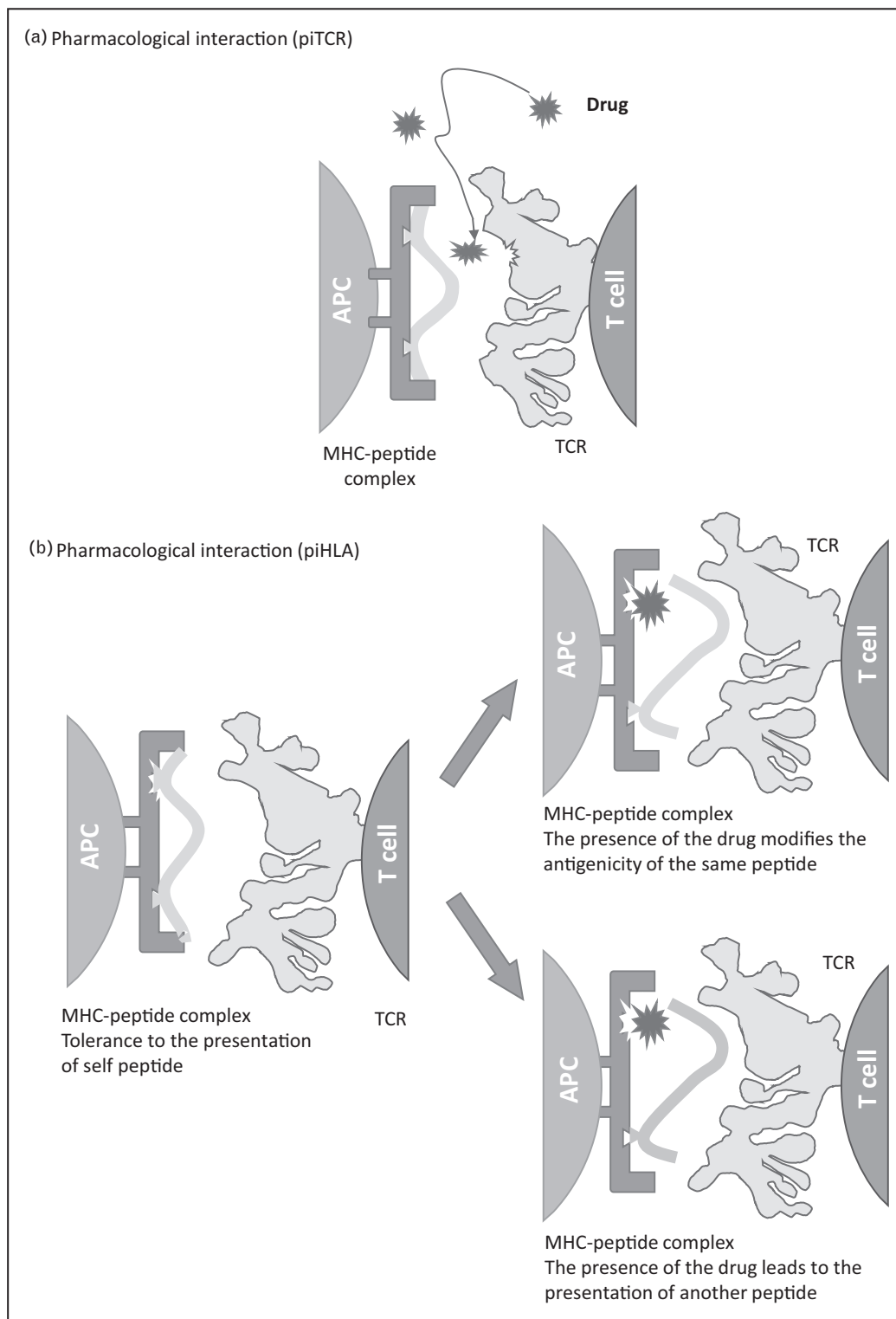


FIGURE 2. Possible pathways of presenting drug directly to T cells via the pharmacological interaction mechanism. (a) Drug interacts preferentially with the T-cell receptor; (b) drug interacts preferentially with the HLA leading to either altered antigenicity of the same peptide or binding of novel peptides. HLA, human leukocyte antigen.

between abacavir and the f-pocket are strong enough that it resists washing steps. This feature could not be reproduced for other drugs with similar HLA associations like flucloxacillin on HLA-B*57:01

or oxypurinol on HLA-B*58:01, for which interactions are very labile and do not sustain APC washings [4,32]. On MHC class I molecules, the C-terminal of the embedded peptide interacts with

the f-pocket to stabilize the peptide within the peptide groove, so that peptides with a Tyr or a Phe at their C-terminal are favoured in HLA-B*57:01 molecules. In the presence of abacavir, the space and properties of the f-pocket are modified so that smaller and aliphatic residues are preferred, like Leu or Ile. This leads to the presentation of a new set of endogenous peptides, which are not supposed to be presented by HLA-B*57:01. Of note this new set of presented peptides were never subjected to the thymic negative selection. To our knowledge, abacavir is the only known drug, which is able to modify the peptide-binding properties of HLA molecules. This feature could not be observed for other drugs such as flucloxacillin, oxypurinol or carbamazepine [39]. Thus, the presentation of another set of peptides cannot be generalized to other HLA-associated drug hypersensitivity reactions. Docking analysis suggest that oxypurinol binds within the f-pocket of HLA-B*58:01, although alterations in the peptide repertoire displayed by HLA-B*58:01 in the presence of oxypurinol have thus far not been observed. It is thought that oxypurinol can modify the shape of the presented peptide, altering its antigenic properties [32]. This proposition arose from the observation that oxypurinol TCC were immediately activated after the addition of oxypurinol in the cell culture, a process too rapid for a putative peptide exchange. For that matter, such a mechanism cannot be excluded for abacavir either, as similar activation kinetics can be observed in abacavir reacting TCC [40]. In the case of HLA-B*15:01-restricted carbamazepine-induced Stevens–Johnson syndrome, a particular TCR fragment of the TCR repertoire has also been detected [41]. Ko *et al.* could find in PBMC from affected patients that the vast majority of carbamazepine activated T cells were monoclonal or oligoclonal, carrying the V β -11 segment. In contrast, this particular clonotype could not be found in carbamazepine-tolerant patients, carrying the HLA-B*15:02 molecules. Such well defined associated clonotype could, however, not be detected in other HLA-associated drug hypersensitivity so far.

The consequences of the pharmacological interaction concept on the priming of a T-cell response

Explaining how interactions lead to TCR activation does not clarify how a T-cell response to drugs arises. According to textbook knowledge, two signals, that is, antigen and danger signals are necessary for the priming of an immune response. In many contact dermatitis models, the important role of dendritic cell maturation has been well studied and confirmed for haptens [42]. However, drug-reacting T cells following

the pharmacological interaction mechanism can be expanded in the absence of danger signals from the innate immune system. It has been shown that abacavir-reacting T cells could be primed *in vitro* without dendritic cell maturation in drug naïve individuals. It was even possible to generate such cells in the absence of dendritic cells or monocytes [43], arguing against the need of co-stimulatory signals in the primary response to drugs following the pharmacological interaction mechanism. An explanation for this feature could suggest that the reacting T cells originated from the memory compartment. It is hypothesized that pharmacological interaction reacting T cells could be cross-reactive with antigenic peptides from microorganisms representing the infectious history of the drug hypersensitive patients. Although a recent study tackled this hypothesis [38], a formal proof has not been discovered yet. Nevertheless, drug reacting cells can be generated from the memory as well as from the naïve pool of T cells in drug-naïve individuals.

EXPERT CONCLUSION AND FUTURE PERSPECTIVE

It is now absolutely clear that drugs and drug haptens activate human naïve and memory T cells. Future research should attempt to determine the contribution of the hapten and pharmacological interaction hypotheses in different forms of drug hypersensitivity. This will be complicated by the fact that the same patient often has T cells that are activated via both pathways. Furthermore, a single cloned T cell has been shown to be activated with the parent drug and a reactive metabolite via different pathway [20,21].

Prediction of the immunogenic potential of new chemical entities is incredibly difficult as any approach must include assessment of adduct formation and the display of haptenated peptides on HLA and direct drug HLA binding. Simple chemical reactivity screens have their usefulness, however, many drugs that form reactive metabolites are not associated with a high incidence of hypersensitivity. Moreover, the almost limitless number of drug hapten and drug-binding sites reduces the effectiveness of simple HLA-binding assays. The vision for the future should be a simplified T-cell assay capable of screening multiple compounds in a single assay. Incorporation of a drug hapten-generating system would be an important feature. In this respect, new culture plates where tissue and immune cells are separated, but exposed to the same medium offer some hope. The tissue cells will generate soluble metabolites and exosomes containing encapsulated protein adducts that will be transferred to antigen-presenting cells, prior to processing and presentation to T cells.

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Conflicts of interest

There are no conflicts of interest.

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- of outstanding interest

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