



The importance of hapten–protein complex formation in the development of drug allergy

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Purpose of review

Drug allergy is an adverse drug reaction that is immune-mediated. Immune activation can occur when drugs or haptens bind covalently to proteins and then act as antigens. The purpose of this review is to summarize the recent data on the formation of hapten–protein complexes and to assess the importance of these complexes in the generation of drug allergy.

Recent findings

The formation of hapten–protein complexes by drugs and their reactive metabolites has largely been investigated using model proteins such as human serum albumin. Precise identification of the structure of the hapten and the resulting modified residue(s) in the protein has been undertaken for a small number of drugs, such as *p*-phenylenediamine, nevirapine, carbamazepine, β -lactams and abacavir. Some progress has also been made in identifying hapten–protein complexes in the serum of patients with allergy.

Summary

Drug-specific T cells have been isolated from different patients with allergy. Formation of hapten–protein complexes, their processing and antigen presentation have been implicated in the development of drug allergy to *p*-phenylenediamine, sulfonamides and β -lactams. However, evidence also supports the π mechanism of immune activation wherein drugs interact directly with immune receptors. Thus, multiple mechanisms of immune activation may occur for the same drug.

Keywords

antigen, drug allergy, hapten, π concept, T cells

INTRODUCTION

The term drug allergy refers to an adverse drug reaction that is immune-mediated. This term is sometimes used interchangeably in the literature with drug hypersensitivity; however, the Nomenclature Review Committee of the World Allergy Organization defines hypersensitivity solely as the ‘symptoms resulting from drug exposure’ and allergy as ‘hypersensitivity initiated by specific immunological mechanisms’ [1]. In the majority of cases, the clinical symptoms of drug allergy involve skin reactions; however, fever and internal organ involvement may also occur. The variety of symptoms is diverse ranging from minor rashes to severe, potentially fatal reactions, such as Stevens–Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Any drug is potentially able to elicit hypersensitivity reactions; however, antibiotics, nonsteroidal anti-inflammatory drugs, contrast media and anticonvulsants are among the most prevalent. The risk of sensitization and the severity of symptoms depend on drug administration (dose, frequency of exposure, duration of exposure and

route of administration) and on the individual patient (age, sex, underlying disease, immune history and immunogenetic disposition).

IMMUNE ACTIVATION

There is some evidence that drug allergy is caused by a preexisting memory response to an infectious agent which then cross reacts with the drug [2]. In a study [3] of 38 patients with drug reaction with eosinophilia and systemic symptoms (DRESS), 76% of the patients showed oligoclonal proliferation of CD8⁺ T cells in response to viral antigens from

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Curr Opin Allergy Clin Immunol 2014, 14:293–300

DOI:10.1097/ACI.0000000000000078

KEY POINTS

- Drugs can become immunogenic by covalent binding to a protein forming a hapten–protein complex.
- Drug–protein complexes have been detected in patients with allergy and can activate T cells.
- Soluble drugs can directly interact with immune receptors to activate T cells.
- The HLA genotype of an individual can dictate the way in which a drug can cause allergy.

human herpes viruses, such as HHV-6, HHV-7 and Epstein–Barr virus (EBV). However, in most cases of allergy the drug initiates a de-novo immune response.

T cells are activated by antigen presenting cells (APCs) that acquire, process and present antigens on human leukocyte antigen (HLA) molecules to the T cell receptor. These antigens may be proteins that are already present within the APC and after processing are typically presented on HLA Class I molecules to CD8 T cells. Extracellular proteins may be taken up by the APC and after processing are typically presented on HLA Class II molecules to CD4 T cells. However, antigen presentation alone is not sufficient to activate T cells. Effective T cell activation requires additional costimulatory signals from the APC. These costimulatory or ‘danger’ signals are usually provided by infectious organisms, damaged cells and proinflammatory cytokines. The ways in which drugs or reactive metabolites provide danger signals are an active area of research [4–7].

Three mechanisms have been put forward to explain how drugs or chemicals (synthetic compounds, dyes, fragrances, plant resins or pharmaceutical drugs) initiate an immune response. First, the hapten hypothesis states that drugs or chemicals are too small to induce an immune response on their own and become immunogenic after coupling with a carrier protein to form a hapten–protein complex. The drug or chemical is referred to as the hapten and must bind irreversibly to proteins to induce the immune response. To act as an antigen, the hapten–protein complex requires antigen processing and presentation. Second, the pi concept proposes that drugs or chemicals interact noncovalently with immune receptors. In most cases, this is the HLA molecule but may also be the T cell receptor. Third, the altered self-hypothesis proposes that binding of the drug or chemical alters the normal repertoire of peptide antigens presented by the HLA molecules resulting in T cell activation.

In many cases of drug allergy, the mechanisms involved in immune activation are not fully understood. The three possible mechanisms of immune activation are not mutually exclusive and therefore multiple mechanisms may be responsible for initiating the allergic reaction.

HAPTEN–PROTEIN COMPLEX FORMATION

Formation of a hapten–protein complex requires that the hapten be protein reactive. This is not the case for many drugs (prohaptens), but protein-reactive intermediates may be generated during drug metabolism. An understanding of the role of hapten–protein complex formation in drug allergy requires information on drug bioactivation or the generation of reactive metabolites, how these reactive metabolites bind to proteins, which proteins are involved, whether these complexes are present in tolerant individuals and individuals with allergy and an understanding of the nature of the hapten–peptide antigen to which the T cell responds. In most allergic reactions, this information is incomplete. This review will summarize the recent data on the formation of hapten–protein complexes by the hair dye component p-phenylenediamine (PPD), sulfamethoxazole, nevirapine, carbamazepine, β -lactam antibiotics and abacavir and then assess the importance of these complexes in the generation of drug allergy.

HAIR DYE ALLERGY

The hair dye component, PPD, is associated with allergic contact dermatitis. PPD is not directly active but is oxidized into an unstable quinonediimine intermediate, which forms dimers, trimers, and ultimately a rearrangement product of the trimer, Bandrowski's base. Both the quinonediimine and the Bandrowski's base have been shown to bind selectively to cysteine residues in peptides and proteins, including glutathione S-transferase pi (GSTP), human serum albumin (HSA), haemoglobin and a synthetic 21-mer peptide containing a single cysteine residue [8–12]. Although investigation of PPD metabolism has been conducted in humans and experimental animals, information on hapten–protein complex formation *in vivo* is noticeably absent. PPD is known to penetrate the stratum corneum of the skin, persisting for as long as 3 days probably by binding to keratin, although this has not been confirmed [reviewed by [13]]. Lymphocytes from tolerant individuals were stimulated by Bandrowski's base but not PPD, suggesting that Bandrowski's base -specific T cell responses are not

responsible for PPD-mediated allergy. Lymphocytes from PPD-allergic patients were stimulated with PPD and PPD-modified HSA. However, analysis of T cell clones confirmed that although the majority were stimulated by PPD-modified HSA, a small proportion of clones (12%) responded to the parent drug [9–11,14]. To explore whether the protein carrier or the hapten itself represented the major antigenic determinant for T cell reactivity, responses to PPD and 2,5-dimethyl-1,4-benzoquinonediimine were compared. Both chemicals were able to bind covalently to the same cysteine residues in the 21-mer peptide and GSTP. A strong PPD-specific proliferative response was observed with lymphocytes from ten patients with allergy. In contrast, a weak proliferative response to 2,5-dimethyl-1,4-benzoquinonediimine was only detected in one patient with allergy. This shows that the structure of the hapten was critical to the lymphocyte response in PPD patients with allergy [10]. Collectively, these data suggest that both the hapten and the pi mechanisms of T cell activation are involved in allergic responses to PPD.

SULFAMETHOXAZOLE ALLERGY

Sulfamethoxazole is a sulphonamide with antimicrobial activity that can cause skin reactions. The drug is metabolized in liver, skin and immune cells to a hydroxylamine by oxidation of the terminal amine [15–20]. This metabolite undergoes spontaneous oxidation to liberate nitroso sulfamethoxazole [21,22], which binds extensively to cysteine residues on serum and cellular proteins [23–25]. The availability of synthetic hydroxylamine and nitroso metabolites has made it possible to explore pathways of drug-specific T cell activation. T cells isolated from the blood and the skin of patients with allergy were stimulated by the parent drug and the nitroso metabolite. Importantly, individual T cell clones can be activated specifically by sulfamethoxazole or nitroso sulfamethoxazole. The parent drug interacts directly with HLA or T cell receptors or both, whereas the nitroso metabolite acts as a hapten and activates T cells via covalent modification of HLA bound peptides and non-HLA associated proteins [26–29]. Recently, we have shown in cystic fibrosis patients with allergy to sulfamethoxazole that the antigen-specific T cell response is directed exclusively towards nitroso sulfamethoxazole [29]. The reason for this difference in patient groups is unclear.

NEVIRAPINE ALLERGY

Nevirapine hypersensitivity syndrome is characterized by fever, maculopapular rash, and in some instances SJS, TEN and hepatitis. Several possible

bioactivation pathways may contribute to nevirapine hapten–protein complex formation; direct oxidation of nevirapine could lead to the formation of reactive intermediates such as arene oxides or a quinone methide; whereas sulphation of 12-hydroxyl nevirapine followed by the dissociation of sulphate could also lead to the formation of the quinone methide. These reactive intermediates are electrophilic and have the potential to modify nucleophilic residues in proteins. Identification of two isomeric nevirapine mercapturates in the urine of patients and rats has provided the evidence that nevirapine undergoes bioactivation to arene oxide and quinone methide intermediates *in vivo* [30–32]. Hapten–protein complex formation has been studied in a number of in-vitro and in-vivo systems. Nevirapine has been shown to bind to liver tissue and plasma proteins in rats using ¹⁴C labeled drug [33]. Incubation of human and rat skin with the benzylic sulphate of nevirapine resulted in extensive conjugate formation with hapten–protein complexes of 25–60 kDa [34[■]]. However, the structures of these nevirapine hapten–protein complexes were not determined. Nevirapine haptenic structures have been identified by mass spectrometry when the 12-sulfoxyl-nevirapine metabolite was incubated *in vitro* with model proteins, such as HSA and GSTP [35[■]]. Modifications of three histidine residues (His146, His242 and His338) and one cysteine residue in HSA and a single-cysteine residue in GSTP were identified. Although knowledge of hapten–protein complex formation in model systems is helpful, identifying the formation of hapten–protein complexes in patients is critical. Isolation of HSA from serum samples of HIV-1 patients undergoing nevirapine therapy showed modification of a single-histidine residue (His146), the same residue identified by the in-vitro analysis [35[■]]. The N-terminal valine in haemoglobin was also modified in red blood cells from nevirapine-treated patients [36]. It should now be possible to investigate whether these hapten–protein complexes can act as T cell antigens in patients with nevirapine hypersensitivity syndrome.

CARBAMAZEPINE ALLERGY

Carbamazepine is a widely prescribed anti-convulsant which causes severe allergic reactions, such as hepatitis and SJS, in a small number of patients. Carbamazepine is extensively metabolized to over 30 metabolites. The most important pathway involves formation of carbamazepine 10,11-epoxide followed by hydroxylation to carbamazepine 10,11-trans-dihydroxy [37]. Several intermediates, including arene oxides, 10,11-epoxide, iminoquinone, carbamazepine-O-quinone and

9-acridine carboxaldehyde, have been proposed as reactive species responsible for covalent binding to proteins [38]. Hapten–protein complex formation has been studied in a number of in-vitro systems. In liver supersomes carbamazepine formed a hapten–protein complex with CYP3A4 [39]. Using lysozyme as a model protein, bioactivation of 3-hydroxycarbamazepine by myeloperoxidase led to covalent binding to at least one tyrosine [37]. Formation of hapten–protein complexes has also been detected with carbamazepine 2,4-epoxide and 3-hydroxycarbamazepine [37,40], carbamazepine-10,11-epoxide formed adducts when incubated with glutathione and human plasma [41,42] and 2-hydroxycarbamazepine and carbamazepine iminoquinone formed glutathione and *N*-acetylcysteine-conjugates [43]. As yet, no studies on hapten–protein complex formation have been carried out in patients with carbamazepine allergy.

A number of studies have characterized T cell responses in patients with carbamazepine allergy. Peripheral blood mononuclear cells (PBMCs) from patients with allergy proliferated upon stimulation with carbamazepine, whereas PBMCs from tolerant patients did not. Most carbamazepine-specific T cell clones were CD4, but CD8 and double positive CD4CD8 clones were also isolated. Antigen recognition was dependent on the presence of APCs for some clones but not necessary for others. Some clones showed limited cross-reactivity with closely related carbamazepine metabolites, but no cross-reactivity was seen with other anticonvulsants or structural analogues [44–46]. These data suggest that the pi mechanism is involved in T cell activation with carbamazepine, but do not rule out the possibility that hapten–protein complexes can also act as T cell antigens.

A strong HLA association between HLA-B*1502 and carbamazepine-induced SJS/TEN has been established in Asian populations. The development of allergy in these patients is critically dependent on the T cell receptor such that only patients with T cells carrying V β -11-ISGSY developed reactions [47]. T cell clones isolated from these patients were HLA-B*1502 restricted, cytotoxic CD8 cells. Metabolism of carbamazepine and intracellular antigen processing were not required for T cell activation. Carbamazepine and carbamazepine-10,11-epoxide could bind directly to the HLA-B*1502 molecule, showing that the pi mechanism was responsible for carbamazepine allergy in these patients [48,49].

β -LACTAM ANTIBIOTICS

β -Lactam antibiotics are widely prescribed for bacterial infections and are a frequent cause of allergic reactions. Formation of hapten–protein complexes

with various β -lactam antibiotics occurs through the nucleophilic opening of the β -lactam ring [50] and results in hapten addition to lysine residues.

Most research on hapten–protein complexes with β -lactams in humans and animals has identified albumin as the major target for β -lactams. Novel mass spectrometric methods have allowed us to define not only the precise haptenic structures, but also the binding profiles in which a number of different lysine residues were modified depending on the dose and length of incubation. At low concentrations, different β -lactams appeared to selectively target different lysine residues in HSA, with noncovalent interactions positioning the drugs in favourable orientations in the protein-binding pocket to facilitate covalent binding. Thus, the three-dimensional shape of the drug, as well as its inherent chemical reactivity, determines the selectivity of covalent binding. However, at high concentrations and with prolonged incubation, multiple residues were modified by the different β -lactams [51–53,54*].

Flucloxacillin was able to modify 10 lysine residues, with Lys190 and Lys212 modifications being the most reactive. Analysis of HSA isolated from the serum of eight flucloxacillin-tolerant patients also showed that modification of Lys190 and Lys212 occurred in all the patients, with up to nine other modified lysine residues being detected. Hapten–protein complex formation occurred with both flucloxacillin and its 5-hydroxymethyl metabolite [51]. Incubation of HSA with piperacillin resulted in the formation of two distinct haptens, cyclized piperacillin and a hydrolyzed form in which the dioxopiperazine ring had undergone hydrolysis [52]. Piperacillin modified up to 13 lysine residues (out of 59 lysine residues) at high concentrations, with Lys541 being the only residue reactive at lower concentration. Four of these (Lys190, Lys195, Lys432 and Lys541) were also detected in plasma from four piperacillin treated, tolerant patients. In addition to the hydrolyzed and cyclized haptens of piperacillin, a further haptenic structure was detected in patients' plasma, which was derived from the desethyl metabolite of piperacillin with a hydrolyzed piperazine ring. Compared with piperacillin, benzylpenicillin targeted different lysine residues at low concentrations: benzylpenicillin and its rearrangement product, benzylpenicillenic acid, modified Lys199 and Lys525, respectively, whereas exposure to higher concentrations resulted in up to 16 further lysine modifications. Fourteen different modified lysine residues were identified on HSA isolated from patients' serum [53]. A slightly different approach was taken by Ariza *et al.* [55] in which amoxicillin was incubated with human

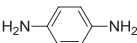
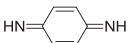
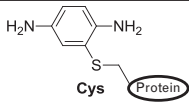
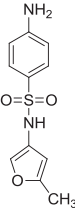
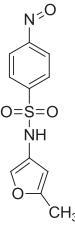
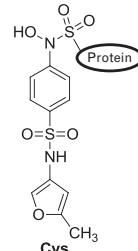
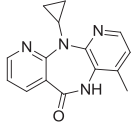
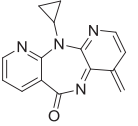
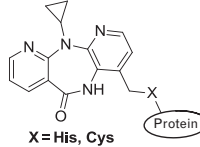
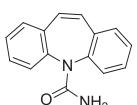
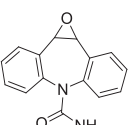
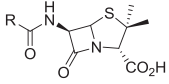
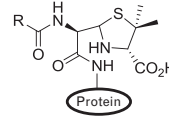
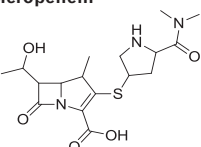
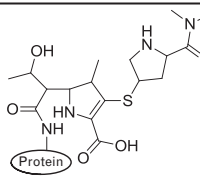
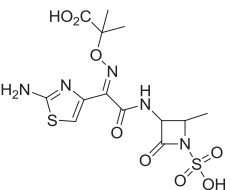
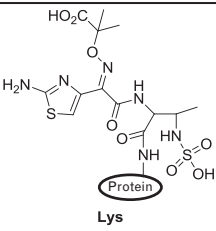
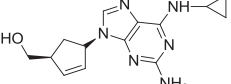
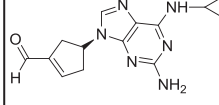
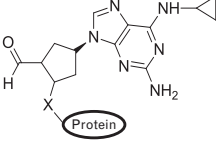
Drug	Reactive intermediates	Hapten-protein complex	References
PPD 		 Cys (Protein)	7–9
SMX 		 Cys (Protein)	22–24
Nevirapine* 		 X = His, Cys (Protein)	34**, 35
Carbamazepine* 		Unknown	
Penicillins 	-----	 Lys (Protein)	50–53*
Meropenem 	-----	 Lys (Protein)	56*
Aztreonam 	-----	 Lys (Protein)	53*
Abacavir* 		 X = Cys, His, Lys (Protein)	60

FIGURE 1. Hapten–protein complexes formed by drugs or reactive metabolites. *More than one reactive intermediate has been proposed responsible for covalent binding. PPD, p-phenylenediamine; SMX, sulfamethoxazole.

serum and then the modified protein identified as HSA, transferrin and immunoglobulin. The hapten was identified as amoxicilloyl on Lys190, Lys199 and Lys541 with higher concentrations eliciting more extensive modification.

Unlike the penicillin class of β -lactams, meropenem and aztreonam contain a β -lactam ring fused to another ring that is different from the thiazolidine rings present in penicillins. Thus, meropenem and aztreonam formed complex and structurally distinct haptenic structures with lysine residues on HSA. Each drug modified Lys190, with less common modifications at Lys12, Lys199 and Lys 351 for meropenem and Lys137, Lys432 and Lys541 for aztreonam [54[■]].

A number of different groups have detected antigen-specific T cell responses in patients with β -lactams allergy. A consistent feature of these responses is drug specificity; T cells with reactivity to one antibiotic do not generally react to other β -lactams [52,54[■]]. A hapten mechanism of T cell activation has been demonstrated for a number of β -lactams. PBMCs and T cell clones isolated from piperacillin patients with allergy were stimulated when piperacillin–albumin complexes were added to cultures [54[■]]. Similarly, PBMCs from patients allergic to benzylpenicillin were also stimulated by hapten–albumin complexes. Responses to the hapten benzylpenicillenic acid were greater than those to benzylpenicillin, showing the important role played by the precise chemical structure of the hapten [53]. The mechanism by which flucloxacillin activates T cells was dependent on the HLA restriction of the response. Flucloxacillin was stably presented on various HLA molecules, resistant to extensive washing and dependent on antigen processing, suggesting a hapten mechanism in certain individuals [56[■]]. However, in HLA-B*5701 positive individuals, T cell reactivity was also independent of antigen processing and could only occur in the presence of soluble drug, indicating a pi mechanism [57[■]]. T cell responses to benzylpenicillin–HSA can also be generated in healthy volunteers. CD4 T cells underwent multiple rounds of stimulation with hapten–HSA resulting in responses in 9 out of 10 volunteers. Experiments performed on naive (CD45RA+) and memory (CD45RO+) T cells showed that these benzylpenicillin-specific CD4 T cells belonged to the naive T cell subpopulation [58[■]].

An important point to note here is that hapten–protein complexes have been detected in drug-tolerant individuals [51]. This clearly shows that the presence of a potential antigen alone is not sufficient to cause allergy. The reasons why some individuals and not others develop allergy is a major unanswered question in the field.

ABACAVIR

Abacavir is a nucleoside-analogue reverse transcriptase inhibitor which is associated with severe allergic reactions in treated individuals carrying the HLA-B*5701 allele. Abacavir is extensively metabolized in the liver, resulting in inactive glucuronide and carboxylate metabolites. Formation of the carboxylate has been shown to proceed through a two-step oxidation process, via a reactive aldehyde intermediate. Hapten–protein complex formation has been studied using HSA, GSTP and haemoglobin, and abacavir–protein complexes have been identified in patients [59–61]. However, the role of these complexes in the development of drug allergy is unclear. In 2012, a novel mechanism of immune activation in drug allergy was proposed. This mechanism is the altered self hypothesis in which abacavir sits in the F pocket of HLA-B*5701 and alters the normal repertoire of peptide antigens bound by the molecule [49,62–64]. T cells activated by this altered peptide repertoire are thought to be responsible for the allergic reaction.

CONCLUSION

Immune activation resulting in drug allergy can occur in different ways. Most drugs act either by direct interaction with immune receptors, namely the pi mechanism, or by covalent binding to proteins, namely the hapten hypothesis. Most hapten–protein complex formation has involved analysis of model proteins *in vitro*, but information on patients' samples is available for some drugs. Furthermore, the precise structure of the hapten is known to be critical for some drugs (summarized in Fig. 1). Recent evidence suggests that the HLA genotype of the individual may play a critical role in determining which mechanism of immune activation occurs.

Acknowledgements

Lee Faulkner is funded by the MIP-DILI project which is supported by the European Community under the Innovative Medicines Initiative (IMI) Programme through Grant Agreement number 115336.

Conflicts of interest

There are no conflicts of interest.

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- of special interest
- of outstanding interest

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