**Immunological Mechanisms of Drug Hypersensitivity**

*Xiaoli Meng, Adriana Ariza, James Waddington, Kevin Park, Dean Naisbitt*

†MRC Centre for Drug Safety Science, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool L69 3GE, United Kingdom

Corresponding author: Dean Naisbitt ([dnes@liv.ac.uk](mailto:dnes@liv.ac.uk); 0044 151 7945346)

**Abstract**

Drug hypersensitivity reactions (DHRs) are adverse drug reactions that may be divided into several categories; namely pharmacologic intolerance, idiosyncratic reactions, pseudo-allergic reactions and allergic reactions. Drug allergic reactions are those DHRs that are mediated by either antibodies or drug-specific T cells. They vary in terms of severity, time-to-onset of clinical manifestations and target organ. Skin is most commonly implicated in drug hypersensitivity reactions; however, it is now apparent that reactions targeting internal organs fall under the definition of drug hypersensitivity. Multiple hypotheses have been proposed to explain the diverse immune mechanisms involved and the heterogeneous clinical presentation. The discovery of human leukocyte antigen (HLA) risk alleles for some DHRs has provided insights in the pathogenesis of these reactions. In this review we summarize immune cells involved in DHRs, discuss the possible immunological mechanisms of DHRs, with an emphasis on the IgE-mediated immediate reactions and T cell-dependent delayed type reactions.

1. **Introduction**

Drug hypersensitivity reactions (DHRs) represent a major problem for drug development and can lead to high global morbidity and mortality. It is therefore important to understand both patient- and drug-related risk factors and mechanisms involved in these reactions. A better understanding of DHRs will aid in the development of preclinical screening programs to enable safer, faster, and more cost-effective drug design.

DHRs are generally immune-mediated reactions and sometimes referred to as drug allergy. These reactions have traditionally been labelled as idiosyncratic or unpredictable hypersensitivity reactions, and are classified as Type B reactions. The most clinically relevant immune-mediated Type B reactions are the Type I or Type IV hypersensitivity reactions according to the Gell-Coombs system classification (1). Type I reactions are immediate, IgE-mediated reactions, usually occurring within 1 h after drug administration, and mainly cause urticaria, anaphylaxis, and/or bronchospasm. Penicillin allergy is an example of a Type I reaction commonly seen in clinical practice. Type IV reactions, in contrast, are delayed hypersensitivity reactions mediated by drug-reactive T lymphocytes [1]. The most important discovery in Type IV reactions has been the associations between certain DHRs and class I and/or II human leukocyte antigen (HLA) alleles. This association has led to insights into the immunological mechanisms of DHRs and to screening of HLA alleles for the prediction and prevention of DHRs. For examples, screening for HLA-B\*57:01 in clinical practice has effectively prevented abacavir hypersensitivity reaction [2].

**Drug-immune system interaction**

Most drugs associated with hypersensitivity reactions are low-molecular compounds (< 1000 Da) and their recognition by the immune system is most commonly explained through the hapten hypothesis [3]. This hypothesis states that drug molecules are too small to induce an immune response by themselves, and only they are able to induce them when they are covalently bound on macromolecules, such as proteins or polypeptides, in a process known as haptenation. The β-lactam antibiotics have been used as model to define this hypothesis because of its high reactivity and binding capacity to proteins [4]. Although the hapten hypothesis is the most accepted model, there are other hypotheses that attempt to explain the interaction of the drugs with the immune system without a covalent binding on macromolecules: the hypothesis of the altered peptide repertoire [5] and the hypothesis of the pharmacological interaction with immune receptors (p-i concept) [6]. The danger hypothesis refers to stress-related signalling that ultimately converts a dendritic cell from a toleragenic state into a potent antigen presenting cell.

**Clinical classification**

Sometimes, it is complex to associate each drug allergic with a correct immune mechanism. Thus, for diagnosis in the daily clinical practice, it is easier and useful to apply a classification based on the time interval between the drug administration and the development of the clinical manifestations. This classification was firstly proposed by Levine in 1966 [7] and divided the allergic reactions in three groups: immediate, accelerated and delayed reactions. This classification has had several modifications and nowadays a classification that divides the reactions into immediate (less than 1 h to 6 h after the last drug administration) and non-immediate (anytime from 1 h after the initial drug administration) has been proposed [8], with overlap between immediate and non-immediate reactions between 1 and 6 h [7].

In this review we focus on the understandings of immunological mechanisms of drug hypersensitivity, with an emphasis on the IgE-mediated immediate reactions and T cell-dependent delayed-type reactions.

1. **Immediate Reactions**

Immediate allergic reactions generally appear in the first hour after the drug administration. These reactions are mediated by specific IgE antibodies, although in some cases specific IgE is not detectable and the underlying mechanism remains unresolved. IgE and non-IgE-mediate immediate reactions may be clinically similar, with clinical manifestations that include local skin reactions, such as urticaria and/or angioedema, allergic rhinoconjunctivitis, bronchial asthma and severe systemic reactions such as anaphylactic shock.

*IgE-mediated drug allergy*

The development of IgE-mediated allergic reactions requires a previous phase of sensitization followed by a phase of elicitation.

*Sensitization phase:* The sensitization phase occurs after the first exposure to the drug. Inside the organism, drugs can bind covalently to autologous proteins, such as human serum albumin, and the resulting drug-proteins adducts can be captured and processed by the antigen presenting cells (APC) and presented on MHC class II molecules. On the other hand, there are drugs that can also modify directly MHC II-associated self-peptides [9]. Then, drug-modified immunogenic peptides are presented on MHC class II molecules to naïve CD4+ Th0 cells, and cytokines secreted by APCs (IL-4, IL-6) leads to the differentiation of Th0 cells to T helper 2 (Th2) cells. The mechanism of induction of an IgE antibody response needs a close cooperation of B cells with Th2 cells. In this process, cytokines secreted by Th2 cells (IL-4, IL-5, IL-10 and IL-13) and co-stimulatory surface signals (mainly Th2 cells – B cells interaction through CD154/CD40 interaction) lead to stimulation of IgE-producing B cells, promoting Ig class switching to IgE [10]. Finally, a large proportion of IgE antibodies released into the bloodstream bind reversibly on high affinity specific receptors (FcεRI) expressed on the surface of tissue mast cells and circulating basophils [11].

*Efector phase:* Following subsequent exposure, according to the hapten hypothesis, drug-carrier adducts (multivalent antigen) interact with at least two adjacent IgE molecules and induce mast cell and basophil activation and degranulation, with the release of preformed inflammatory mediators (histamine, tryptase, heparin or chemotactic factors), the synthesis and secretion of lipid mediators (leukotrienes and prostaglandins) [12] and cytokines (TNF, IL-1, IL-4, IL-5, IL-6, IL-13, MIP-1α, MIP-1β, IL-3, GM-CSF)that can contribute to allergic inflammation and may cause local and/or systemic symptoms. This effector phase may be divided into an early response, beginning within seconds or minutes, and a delayed response, occurring after 8–12 hours. Diverse chemokines, cytokines and interleukins from mast cells contribute to formation of the cellular infiltrate following the anaphylactic reaction as a type of delayed reaction. The infiltrate consists primarily of eosinophilic granulocytes and Th2 cells that contribute to the development of chronic illness if exposure to the drug persists. This leads to tissue damage and promotes the development of chronic inflammation. The re-appearance of symptoms upon re-encountering the drug, despite avoidance of exposure for a longer period of time, is due to memory cells.

**Drugs involved**

Immediate drug reactions have been reported for a great variety of drugs (Table 1). Although there are many drugs that can induce a hypersensitivity reaction with characteristics and clinical manifestations compatible with an immediate reaction, there is no in vitro methods available for detecting specific IgE antibodies to some of them, so the involvement of drug-specific IgE is questionable in some of these cases. Table I summarize immediate drug allergic reactions to different drugs reported, highlighting the studies in which specific IgE antibodies have been detected. A detailed review of in vitro tests for drug allergic reactions has been realized by ENDA/EAACI Drug Allergy Interest Group [13]. Drugs most frequently involved in IgE-mediated reactions are β-lactam antibiotics, with amoxicillin and most recently clavulanic acid as well as cephalosporins as the main elicitors [14] as well as anaesthetic–related drugs, particularly muscle relaxants. Although non-steroidal anti-inflammatory drugs (NSAIDs), induce in most of the cases non-immunological mediated reactions, up to 30% of cases can develop a selective IgE-mediated response [15]. Pyrazolones are the most commonly implicated NSAIDs in these reactions whilst for other NSAIDS, such as ibuprofen it is still not known if they can induce IgE-mediated responses. Non IgE-mediated reactions are elicited by an alternative activation pathway. Drugs most commonly involved in these reactions include some muscle relaxants, most radio-contrast media, platinum-containing chemotherapeutics or NSAIDS.

**Detection of specific IgE**

The detection and quantification of serum specific IgE can be performed by different immunoassay methods, which use a solid phase functionalized with drug-carrier adducts [16]. The most used commercial method is the fluoroimmunoassay (FEIA) (ImmunoCAP Thermo-Fisher, Uppsala, Sweden), which is only available for a limited number of drugs, some of them are betalactam antibiotics [17-27] (penicilloyl G, penicilloyl V, ampicilloyl, amoxicilloyl and cefaclor), chlorhexidine [28], suxamethodium [29-31], protamine, morphine [30, 32, 33], pholcodine, infliximab [34-36], cetoximab [37, 38]. Moreover, there are enzymoimmunoassay (e.g. ELISA) and in-house radioimmunoassay (RIA) that use different carrier molecules [39-42] and solid phases [43-45] for the detection of specific IgE antibodies to different drugs, such as betalactam antibiotics [17-27], neuromuscular blocking agents [29-33, 46, 47], fluoroquinolones [48, 49], pyrazolones, cetoximab [37, 38], aspirin [50] and propyphenazone [51]).

**Indirect detection of specific IgE through cellular tests**

In the diagnosis of immediate allergic reactions it is possible to use cellular tests focussed on the quantification of inflammatory mediators released by basophils, such as the histamine release test [52, 53], or on the percentage of activated basophils after the stimulation with the interest drug (reviewed in [54, 55]). However, immediate drug reactions with degranulation of mast cells and basophils do not always imply involvement of IgE antibodies with cross-linking of IgE/FcεRI. For that, a positive result obtained with these tests does not imply a positive IgE-mediated response. However, it is possible to identify an IgE mediated pathomechanism in an indirect way when drug-specific IgE cannot be evidenced by immunoassay. In the case of the basophil activation test, wortmannin (a strong inhibitor of phosphatidylinoitol 3-kinase (PI3K)), one of the most important kinases activated by the FcεRI and implied in the regulation of histamine release can be used [56]. This approach has been applied to confirm the existence of specific IgE antibodies to clavulanic acid and dipyrone, which until now they could be detected by immunoassay [57, 58].

Passive histamine release test is another indirect way to confirm that an immediate reaction is mediated by IgE antibodies [59]. This method has been used for immediate reactions to clavulanic acid [60].

**Evolution over time**

In IgE-mediated reactions, an increase in the level of IgE antibodies is seen when the reaction develops. However, it has been observed in certain cases that the high IgE levels persist for a short period of time [61]. This fact highlights that the time interval between the allergic episode and diagnostic evaluation is an important factor in the interpretation of diagnostic results. There are some studies that have followed results of specific IgE levels and skin tests and basophil activation test results over time in allergic patients to β-lactam antibiotics. An increased tendency to detect negative results was observed with time, which was more pronounced in patients with a selective reaction compared to cross-reactive patients [62-64].

1. **Delayed Type Reactions**

In contrast to immediate reactions, delayed type reactions (DTRs) present with much more complex clinical phenotypes ranging from mild skin reactions such as eczema and maculopapular eruption to life-threatening reactions including severe cutaneous adverse reactions (SCARs) and drug induced liver injury (DILI). DTRs can be further divided into four types according to the clinical presentation and different types of drug-responsive T-cells involved [65]. Type IVa reactions involve Th1 cells that secrete IFN-γ leading to macrophage activation. Type IVb reactions are characterised by the secretion of IL-4, IL-13 and IL-5 by Th2 cells, leading to eosinophilic inflammatory responses; Type IVc reactions are cytotoxic reactions that are mediated by CD4+ and CD8+ cells. The effector cells in such reactions are cytotoxic T cells that can kill tissue cells in a granulysin, perforin, granzyme B and FasL-dependent manner. Type IVd reactions involve T cells secreting high levels of CXCL8/GM-CSF, leading to recruitment and activation of neutrophils [66]. The following section discusses different components of the immune system and possible mechanisms involved in DTRs. It should be noted that this classification was established over 10 years ago and has not been updated. The discovery of new populations of T-cell (Th9, 17, 22…) means that there is now an urgent need for mechanistic studies that shed new light on the immune response that develops in patients with different forms of DTRs.

* 1. **Key immune cells in the development of DTRs**

It is now appreciated that a variety of immune cells are involved and orchestra the DTRs. In particular, dentritic cells and T lymphocytes have been shown to play crucial roles in the development of DTRs.

*Dendritic Cells*

Dendritic cells (DCs), act as a link between the innate and adaptive immune systems, are essential for initiating cell-mediated adaptive immune responses [67]. DCs take up and process complex antigens into short peptides which can be presented as peptide – HLA complexes on the surface of DCs for T-cell recognition. The interactions of T-cell receptors (TCR) with peptide–HLA complexes determine the specificity of the response. However, various additional receptor–ligand interactions including those accessory molecules such as CD80 and CD54, and secretion of soluble mediators are needed to modulate the outcome of T-cell activation. Activation of naive T cells by DCs results in their clonal expansion and differentiation into effector and memory T cells [68]. DCs exert different functions depending on the state of maturation: immature DCs (imDCs) are specialized for antigen capture by endocytosis but exhibit limited capacity for antigen proteolysis; during maturation, DCs lose their abilities to internalize antigens, but facilitate efficient formation of peptide-HLA complex by enhanced lysosomal acidification and antigen proteolysis [69].

It is now well known that a variety of signals including pro-inflammatory cytokines, viruses, LPS, allergens, and haptenic chemicals can drive DC maturation [70-72]. The maturation is mediated by different transcription factors including nuclear factor-κB (NF-κB), p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK), and extra-cellular signal-regulated protein kinase (ERK1/2) [73-75]. For example, haptenic chemicals such as dinitrochlorobenzene (DNCB) can activate DCs via multiple pathways, including activation of toll like receptors via degradation of hyaluronic acid, formation of reactive oxygen species, and/or direct modification of cysteine-containing proteins [76]. Amoxicillin has been shown to cause DC maturation only in allergic patients via activation of both the MAPK family and NF-κB pathways [77]. Moreover, an elegant study by Schmidt et al has demonstrated that Ni2+ can trigger an inflammatory response by directly activating human Toll-like receptor 4 (TLR4). Structural analysis revealed that binding of Ni2+ to the non-conserved histidines 456 and 458 of human TLR4 was essential for the activation of DC [78].

*T Lymphocytes*

The involvement of T lymphocytes in DTRs has been firmly established. Immunohistochemistry analysis of effector cells in tissues revealed infiltration of drug-specific T cells in the local infection sites, including acute skin lesions from patients with cutaneous reactions [79], liver biopsy from patients with DILI [80], and kidney biopsy from patients with interstitial nephritis [81]. Moreover, drug-specific T cells have been detected in peripheral blood from hypersensitive patients [82-85]. Upon stimulation, naive T cells can be differentiated into several distinct subsets of effector T helper cells including Th1, Th2, Th9, Th17, and Th22 on the basis of their cytokine production pattern [86, 87]. Each T-cell subset can promote different types of inflammatory responses but also be able to counter-regulate each other [88]. For example, IL-17 producing Th17 cells infiltrating skin of patients with allergic contact dermatitis (ACD) have been shown to enhance the immune responses to antigens by promoting non-antigen specific Th1 cells to kill keratinocytes [89]. On the other hand, antigen-specific regulatory T cells (Treg cells) play vital roles in controlling the development and maintenance of DHRs. For example, Treg cells have been shown to supress both the sensitization and effector phases in allergic reactions via both cell interactions and secretion of cytokines [90-93].

It is now well established that CD8+ cytotoxic T cells represent the primary effector cells in most forms of drug hypersensitivity; whereas CD4 + T cells produce cytokines, helping to regulate the action of other immune cells like macrophages, B cells, and CD8 +T cells. CD4+ cells are predominately involved in moderate cutaneous reactions such as MPE and AGEP; these cells can either cause local inflammation by secretion of cytokines or execute direct toxicity, and ultimately cause keratinocyte damage. Most likely cytotoxicity and cytokine mediated inflammation can occur simultaneously [94]. On other hand, cytotoxic CD8+ cells are mainly involved in more severe cutaneous reactions such as SJS/TEN. The fully activated CD8+ cells, natural killer (NK) cells, and NK like T (NKT) cells can produce massive amount of granulysin that causes keratinocytes death and tissue damage in SJS/TEN [95, 96].

*B Lymphocytes*

B cells play a central role in humoral immunity by secreting antibodies, but they also function as APCs and regulate T cells [97]. Different antibody titers secreted by B cells have been shown to exert crucial roles in initiating DTRs and subsequent recruiting drug specific T cells. Early studies have demonstrated that activated B-1 B cells were able to mediate early phase of DTRs via production of IgM antibodies. The antigen specific IgM antibodies were found to bind to antigens to generate antibody-antigen complex that can activate complement and recruit the effector T cells into the tissue to mediate the late phase of DTRs [98]. IgG4 antibodies are now well known to play a central role in immune regulation after grass pollen immunotherapy. High-affinity allergen-specific IgG4 antibodies resulted from immunotherapy were shown to block IgE-mediated allergen presentation and basophil activation [99, 100]. Despite this, the role of B-cells or more specifically IgG4 antibodies in regulating drug hypersensitivity has not been investigated.

In addition, antigen-specific B cells also function as APCs that can effectively present antigenic peptides to specific T cells [101]. The high-affinity BCR enables B cells to efficiently capture specific antigens via membrane-associated antibodies and concentrate the antigens prior to processing. Exogenous proteins are processed by B cells mainly through receptor-mediated mechanisms such as BCR-mediated endocytosis [102]. Subsequently, activated B cells may prime CD4+ cells through the interactions of peptide-MHC II complex with TCRs, together with various other ligand pairs, including B7:CD28, CD40:CD40L, ICAM-1:LFA-1, and CD48:CD2 [103]. However, the contribution of B cells as APCs for CD4+ T cells is still controversial as they are able to induce both priming and anergy in T cells. It is now known that the lack of B cell activation and the low level of peptide determinants associated with the presentation of nonspecific antigens could lead to T cell tolerance. Over the past decade, much progress has been made to characterise a population of B cells that can induce T cell tolerance. B cells can suppress the differentiation of T helper cells such as Th17, Th1 and cytotoxic CD8+ T cells via the secretion of IL10, TGF-β, and IL35 [97, 104]. In addition, B cells can also induce the differentiation of immunosuppressive T cells such as Foxp3+ T cells and T regulatory 1 (Tr1) cells [105].

*Natural Killer Cells*

NK cells play an important role in both innate and adaptive immune responses [106]. They can kill infected cells by releasing large quantities of granulysin and other cytotoxic molecules upon activation. In severe skin reactions, activated NK cells may initiate apoptosis of epidermal cells through the release of cytotoxic molecules such as perforin and granzymes A and B [106, 107]. Recently, amoxicillin has been shown to activate NK-producing cytotoxic markers (perforin and granzyme B) mainly in the CD56dim subpopulation and a Th1 cytokine (IFNγ) in CD56bright cells. Furthermore, amoxicillin can increase the NK cytotoxic effect only in allergic patients [108]. It is important to note that NK cells not only exert cytotoxic activity but also can regulate other immune cells. NK cells can alter T cell responses through secretion of cytokine or chemokine and direct cellular contact [109]. For example, IL-10-secreting NK cells were shown to suppress antigen-specific T cells [110].

* 1. **Mechanisms of DTR**

Multiple mechanisms have been proposed to explain how drugs can induce an immune response. These can be categorized into two major groups on the basis of the interaction between antigens and immune cells including APCs and T cells. The first group involves covalent interactions between antigens and immune cells, for example, the hapten hypothesis. For the second group, antigens interact directly with immune cells (non-covalent interactions); this includes direct binding of antigen to T cell receptors and/or MHC molecules on the surface of APCs, for example, pharmacological interactions (PI hypothesis) and altered self-peptide repertoire hypothesis (Figure 2). It must be stressed that DTRs are generally heterogeneous; therefore it is very likely multiple mechanisms are simultaneously be operative in the hypersensitive reactions induced by single drug. Moreover, it is sometimes difficult to rationalize the findings if in vitro assays that might use milliM concentrations of the drug to therapeutic exposure in patients at the time of T-cell priming.

***Hapten Hypothesis (Covalent Interactions)***

The hapten hypothesis states that drugs or chemicals (haptens) are too small to induce an immune response on their own and can become immunogenic after covalent binding to a macromolecule to form a hapten–protein conjugate. Some haptens such as β-lactam antibiotics are intrinsically reactive and can from hapten-protein conjugates simultaneously; whereas most haptens are chemically inert and require metabolic activation in order to bind to proteins. Advances in mass spectrometry based proteomics have now allowed characterisation of haptenic structures in great details to unravel the precise structure and the exact location on proteins [111-115]. Several *in vitro* studies have identified many protein targets for certain drugs or reactive metabolites, including Keratin 14 in the skin [116-118], circulating proteins such as Hb (hemoglobin)and serum albumin [119, 120], P450 and GSTP in the liver [121, 122]. Moreover, hapten-protein conjugates have been detected in cell culture medium [82, 123] as well as macrophages, monocytic THP-1, and B lymphoma cells [124]. Most importantly, haptenated proteins have been detected in patients taking therapeutic drugs, reaffirming the significant clinical relevance of hapten-protein conjugate formation in DTRs [82, 114, 125, 126].

The involvement of haptenated proteins in DTRs is exemplified by hypersensitive reactions associated with β-lactam antibiotics. Penicillin-modified proteins have been shown to induce both IgE mediated and T-cell mediated responses. Early studies with penicillins have demonstrated that human IgE antibodies are able to recognize penicillin-modified proteins and trigger immune responses. Both the haptenic structure and protein carrier are important for IgE recognition [1]. Moreover, several studies have demonstrated that the formation of hapten-protein conjugates is an important step in activation of drug-specific T cells in β-lactam hypersensitivity [84, 123, 127-129]. Stimulation of drug specific CD4+ and CD8+ clones was found to be highly specific in terms of drug structure and dependent on the formation of protein adducts. The immunogenicity of hapten-protein conjugates was further evidenced by stimulation of drug-specific T cell clones using a synthetic piperacillin-albumin conjugate as a close analog to that seen in patients. To act as an antigen, the hapten-protein conjugates must be processed to form haptenated peptides that can be presented on the surface of APCs in the context of HLA molecules for T cell recognition. Indeed, the T-cell response to β-lactam albumin conjugates is inhibited when antigen processing is blocked, indicating the antigen processing was a prerequisite for the activation of T-cells. Since β-lactam antibiotics are intrinsically reactive, it is possible that direct modification of HLA and/or HLA peptides could occur, resulting in stimulation of drug responsive clones via an antigen processing independent pathway. Under these circumstances, a large polyclonal repertoire of effector T cells may be generated, leading to stimulation of a heterogeneous immune response. Although much progress has been made in characterisation of hapten-protein conjugates, the bon fide haptenated peptides that presented on the surface of APCs remain undefined. Future research to identify these natural processed peptides will certainly provide affirmative evidence for the hapten hypothesis.

Unlike β-lactam antibiotics, for many drugs, bioactivation to form reactive metabolites is mandatory for protein conjugation. Therefore the sites of bioactivation may have a great impact on the pathogenesis of DTRs. Liver is the major organ for drug metabolism, therefore haptic proteins may be the targets for many drugs. These drug-protein conjugates may play an important role in certain forms of drug-induced liver injury where drug-specific T-cells have recently been characterized in patients [83, 130]. On the other hand, the cutaneous reactions are more likely associated with haptenated skin proteins. An example that reactive drug metabolites are involved in DTRs is nevirapine (NVP) hypersensitivity. NVP treatment has been associated with severe skin and liver injury in exposed patients. An immune-mediated mechanism involving the drug-specific activation of patient T-cells has been proposed for NVP-induced tissue injury. NVP undergoes extensive metabolism in humans, forming a number of reactive metabolites that bind covalently to proteins, and thus have the potential to function as haptens, antigens and immunogens. Studies have demonstrated that NVP forms protein adducts in the skin, liver, and plasma in a number of in vitro and in vivo systems [126, 131-133]. Furthermore, the incidence of NVP-induced skin rash has been demonstrated in an animal model, in which skin rash has been shown to be dependent on drug-specific activation of CD4+ T-cells and associated with a NVP reactive metabolite (12-OH NVP sulphate) [134, 135]. The incidence of skin rash declines as the extent of metabolism and covalent binding decrease. However, as opposed to skin rash, there was no clear association between the outcome of liver injury and bioactivation even though NVP binds extensively to haptic proteins [136]. A more recent study using a mouse model has shown that NVP can produce significant liver injury when PD1 was blocked, indicating immune tolerance plays an important role in NVP induced liver injury [137].

It is therefore important to appreciate that the overall quality of immune response to drugs is only partly driven by haptenated proteins; other factors such as antigen dose and genetics, may also play a crucial role in the severity of the hypersensitive phenotype [138, 139] and in determining the characteristics of the responding T-cell repertoire [140, 141]. In particular, danger signals derived from inflammation, disease, and oxidative stress have been shown to initiate and/or enhance immune responses [5, 142]. Moreover, extensive protein modification, both in terms of the epitope density and the number of sites on proteins resulted in the activation of a larger pool of T lymphocytes, leading to a broader repertoire of TCRs [143]. It is therefore important to perform quantitative analyses to define the quantitative relationship between haptenation and T-cell activation.

***Pharmacological Interactions (Non Covalent Interactions)***

The observation that certain drugs induce immune responses without the requirement of bioactivation has led to the pharmacological interactions (p-i) hypothesis. It postulates that drugs may bind non-covalently to immune receptors such as TCR, HLA, or HLA peptides [144, 145]. These interactions are spontaneous and could result in T cell activation within seconds of the drug exposure [80, 146]. The p-i concept derived from several experimental findings. First, non-reactive drugs were found to stimulate T cells via their T-cell receptor in an MHC restricted manner (e.g. carbamazepine-induced SJS/TEN); second, APC that are fixed with glutaraldehyde, can still activate drug-specific T cell clones (TCCs) in the presence of native drugs (19); and finally, the removal of soluble drug through repeated washing of drug-treated APCs prevented T-cell activation.

Non-covalent interaction of drug with immune receptors can occur in several pathways. First, a drug can bind directly to TCR (p-i TCR), resulting in altered conformation of TCR and increased TCR binding affinity. Recent in vitro studies have identified two types of sulfamethoxazole (SMX) reacting TCCs that can be stimulated by SMX binding to CDR2 region of TCR-Vβ20-1 or CDR3 of the α-chain of the TCR [147, 148]. The stimulation was found to be exclusively dependent on SMX without the need for peptide and HLA recognition. Second, non-covalent interaction of a drug with the peptide presented in the binding groove of HLA may change the conformation of peptide-HLA complex that may be seen as a neo-antigen by TCRs; finally, a drug can bind directly to the binding groove of HLA (p-i HLA), resulting in formation of a foreign drug-peptide-HLA complex referred as allo-HLA [149, 150].

In addition, direct binding of drug to HLA can lead to alteration of the normal repertoire of peptides presented by the HLA molecules. This is also referred as the altered self-peptides repertoire hypothesis [151-153]. Recent studies of abacavir hypersensitivity revealed that abacavir bound to the F-pocket of the protein encoded by the risk allele HLA-B \* 57: 01 with great affinity. In the presence of abacavir, peptides with a small aliphatic amino acid such as valine, isoleucine and leucine at the anchor residue are favoured instead of the normal HLA-B\*57:01peptides with tryptophan at this position. These peptides may act as neo-antigens that induce an autoimmune reaction. [32]. It is important to emphasize that currently the altered peptide repertoire hypothesis remains exclusively for abacavir; it has not been proved for any other drugs. Furthermore, several abacavir-responsive clones are activated by a p-I HLA pathway.

* 1. **HLA association in DTRs**

The discovery of associations between certain drug hypersensitivity reactions and specific HLA alleles has greatly improved our understanding of the immunological mechanisms of T cell medicated drug hypersensitivity. A growing number of risk HLA alleles are being identified (Table 2), with the strength of association ranging from weak to strong. The strongest HLA-associated DTRs are between HLA-B\*57:01 and abacavir hypersensitivity in the Caucasian population [2], HLA-B\*15:02 and carbamazepine-induced SJS/TEN in Asian populations, and HLA-B\*58:01 in patients with allopurinol hypersensitivity syndrome and SJS/TEN [154]. The strong association between abacavir hypersensitivity and HLA-B\*57:01 has led to screening for HLA alleles in clinical practice to prevent abacavir DHRs [2]. Interestingly, only 50% of patients positive for HLA-B\*57:01 develop DHRs, suggesting other factors, in addition to drug exposure and genetic factors, may also contribute to the development of DHRs. For example, disease phenotype may also impact on susceptibility to these reactions. Asian individuals carrying HLA-B\*15:02 are at high risk for carbamazepine induced SJS/TEN; whereas HLA-A\*31:01 positive patients are more likely to develop carbamazepine-induced hypersensitivity syndrome or maculopapular exanthema [155, 156].

It must be emphasized that the drug-derived antigen interacts with numerous HLA molecules, thereby leading to much more heterogeneous immune responses. Indeed, both HLA-class I restricted CD8+ T cells and HLA-class II restricted CD4+ T cells have been detected in patients with most forms of DTRs. For example, flucloxacillin specific CD8+ T cells detected in patients with liver injury were activated in an HLA B\*57:01 restricted manner. However, HLA-class II associated CD4+ cells were also detectable in these patients [83]. The similar observation has been found with carbamazepine hypersensitivity in several studies [157-159]. Moreover, drug-responsive T cells can be activated in both HLA restricted and unrestricted fashion. For example, heterogeneous T cells detected in patients with SMX hypersensitivity are responsive to both SMX and its metabolite, nitroso SMX (SMX-NO) [160]. The SMX and SMX-NO responsive T cells can be activated in an HLA-DR allele unrestricted manner, probably due to the direct interactions of SMX with TCR [147] or multiple antigenic determinants derived from extensive haptenation by SMX-NO. However, Ogese et al have recently demonstrated that the activation of some SMX-NO specific CD4+T cells was HLA-DQ allele-restricted [161]. Thus, the relationship between the expression of the HLA risk allele, activation of HLA-restricted T cells and the disease is complex and additional research is needed to explore pathways of drug-specific activation that are active in patients.

Recently, the association of HLA class II alleles with DILI has also been identified with several drugs, for example, ximelagatran [162], amoxicillin-clavulanate [163], lapatinib[164], and lumiracoxib [165], indicating that drug-specific CD4+ T-cell may be involved in the disease pathogenesis. This is supported by the detection of amoxicillin and clavulanic acid specific T cells in patients with DILI [84]. Both CD4+and CD8+ clones were responsive to amoxicillin and clavulanic acid in HLA class II and I restricted manner, respectively, with the drug antigen being presented to CD4+ clones in the context of HLA-DR molecules. However, for the majority of HLA-restricted DILI, the nature of the T-cell response and the MHC restriction has not been fully defined.

* 1. **Viruses/disease association in DTRs**

It is now well known that viruses and disease play an important role in drug hypersensitivity. For example, high rates of DTRs to β-lactam have been reported in patients with cystic fibrosis who are normally exposed to multiple courses of the drug (e.g. approximate 30% for piperacillin). A higher incidence of DTRs has also been reported in HIV-infected patients, for example, up to 60% HIV positive patients have been reported to experience hypersensitivity associated with exposure to co-trimoxazole, whereas these reactions only occur in 5% HIV negative patients [166]. A growing number of viruses including Epstein–Barr virus (EBV), herpes simplex virus (HSV), human herpesvirus 6 (HHV-6), cytomegalovirus (CMV), and varicella-zoster virus (VZV) have been shown to enhance the progression of specific forms of DHR[167]. However, the exact role of infection in the disease pathogenesis remains unknown.

There is some evidence that DHRs are initiated by a pre-existing memory response to an infectious agent which then cross-reacts with the drug. After viral infection, tissue-resident memory T cells (TRM) may persist in the skin for over 6 months, providing long-lived protective T cell immunity against re-infection [168]. These CD8+ TRM cells utilized a very limited range of TCR, it is therefore very likely that they recognise limited antigens presented by APCs, leading to cross reactivity with drug-antigen [169].

1. **Conclusion**

Both the innate and adaptive immune systems appear to participate in drug hypersensitivity. Drug-specific antibodies and drug antigen-responsive T cells control the effector and regulatory processes that determine the nature of the clinical response. The interaction of a drug antigen and immune receptors has been extensively investigated and several hypotheses (the hapten, p-i, and altered peptide) have been proposed to explain how drugs induce an immune response. It is important to appreciate that these hypotheses are not mutually exclusive, but sometimes overlap and each will contribute to the observed DHRs. The challenge moving forward is to explain how each model contributes to the pathogenic immune response in patients.

Advance technologies and methodologies in bioanalysis have greatly improved our fundamental understanding of the chemical basis of T-cell antigenicity and immunogenicity. However, little is known about the nature of protein targets in local tissue and the antigenic determinants that can drive immune responses. The discovery of association between HLA molecules and drug hypersensitivity has allowed clinicians to predict susceptible patient groups and restrict drug use for a limited number of reactions. However, for the majority cases, the HLA association is too weak to be used in clinical practice for diagnosis and prediction of DHRs. Additionally, the rapid progression in characterisation of the phenotype of immune cells involved in DHRs has shed further light on pathogenesis of DHRs. Future investigations employing new proteomic technologies to identify the bon fide antigenic determinants and define thresholds (drug, metabolite, and drug-protein conjugate) that can drive immune responses will certainly set an important precedent for the discovery of better diagnostic tools for DHRs. Characterisation of the immune components and understanding their roles in DHRs will assist the development of new medicines that will pose a lower allergy risk in the clinic [170].

**Table 1.** Drugs elicitors of reported immediate drug reactions

|  |  |  |  |
| --- | --- | --- | --- |
| **Drugs** | | **IgE detection method** | **References** |
| Anesthetics / Muscle relaxants | Alcuronium | RIA, Sepharose-RIA | [31, 33, 46] |
| Atracurium | Not performed | [171] |
| Morphine | CAP-FEIA, RIA | [30, 32, 33] |
| Pancuronium | Sepharose-RIA, RIA | [31] |
| Rocuronium | CAP-FEIA, RIA | [30, 47] |
| Thiopentone |  | [172, 173] |
| Tubocurarine | RIA | [46] |
| Suxamethonium | CAP-FEIA, RIA | [29-31] |
| Vecuronium | Sepharose-RIA, RIA | [31] |
| Antiseptic | Chlorhexidine | CAP-FEIA | [28] |
| Betalactam antibiotics | Penicillins | ELISA, CAP-FEIA, RIA | [17-23] |
| Cephalosporins | RIA, CAP-FEIA, Sepharose-RIA | [24-27] |
| Clavulanic acid | Indirect methods:  BAT inhibition with WTM  Passive HRT | [58, 60] |
| Corticosteroids | | Not performed | [174], [175], [176] |
| Fluoroquinolones | | Sepharose-RIA | [48, 49] |
| Heparins | | Not performed | [177] |
| Monoclonal antibodies | Cetoximab | ELISA, CAP-FEIA | [37, 38] |
| Infliximab | CAP-FEIA | [34-36] |
| Non-steroidal antinflammatory drugs | Aspirin | RIA | [50] |
| Arilpropionics, Ibuprofen | Not performed | [178] |
| Metamizole | Indirect methods:  BAT inhibition with WTM | [57] |
| Propyphenazone | ELISA | [51] |
| Other antibiotics | Trimethoprim | Immunoassay | [24, 179] |
| Pristanamicyn | Not performed | [180] |
| Vancomycin | Not performed | [181] |
| Protamine | | Not performed | [182] |
| Proton Pump Inhibitor | | Not performed | [183] |
| Streptokinase | | Not performed | [184] |
| Sulfamethoxazole | | Not performed | [185, 186] |

**Table 2.** Drugs elicitors of reported delayed-type drug reactions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Reaction type** | **Drug** | **Known HLA association(s)** | | **References** | |
| MPE  *(Maculopapular eruption)* | Amoxicillin | - | | [187] | [188, 189] |
| Piperacillin | - | | [189] |
| DRESS  *(Drug reaction with eosinophilia and systemic symptoms)* | Phenobarbital | - | | [190] | [191] |
| Dapsone | HLA-B\*13:01 | | [192] |
| Vancomycin | - | | [191, 193] |
| Carbamazepine | HLA-A\*31:01 | | [194, 195] |
| Allopurinol | HLA-B\*58:01 | | [196] |
| SJS/TEN  *(Stevens Johnson syndrome/toxic epidermal necrolysis)* | Lamotrigine | HLA-B\*15:02 | | [194, 197] | [187, 198] |
| Carbamazepine | HLA-B\*15:02 | | [195] |
| Phenobarbital | HLA-A\*02:07  HLA-B\*51:01 | | [190, 199] |
| Co-trimoxizole | HLA-B\*15:02  HLA-C\*06:02  HLA-C\*08:01 | | [200] |
| Allopurinol | HLA-B\*58:01 | | [196, 201] |
| Telaprevir | - | | [202] |
| Vanomycin | - | | [203] |
| Nevirapine | HLA–B\*35:05  HLA-DRB1\*0101  HLA–B\*1402 | | [198] |
| Methazolamide | HLA-B\*59:01 | | [204] |
| AGEP  *(Acute generalized exanthematous pustulosis)* | *Sulfonamides* | - | HLA-DQ3 HLA-DR11  HLA-B\*51 | [187, 194] | [205, 206] |
| Ampicillin | - | [207-209] |
| Amoxicillin | - | [207, 209] |
| Terbinafine | - | [209, 210] |
| *Corticosteroids* | - | [211] |
| *NSAIDs*  (e.g. Nimesulide & Valdecoxib) | - | [212, 213] |
| Pristinamycin | - | [209] |
| DILI  *(Drug induced liver injury)* | Flucloxacillin | HLA-B\*57:01 | | [214, 215] | [216] |
| Amoxy-clavulanic acid | HLA-DRB1\*15:01-  HLA-DRB5\*01:01-  HLA-DQB1\*06:02 haplotype | | [214, 215, 217] |
| Isoniazid | - | | [214] |
| Lapatinib | HLA-DRB1\*07:01  HLA-DQA1\*02:01 | | [164, 218] |

**Abbreviations**

DHRs - Drug hypersensitivity reactions

HLA - Human leukocyte antigen

APC - Antigen presenting cells

MHC - Major histocompatibility complex

IL - Interleukin

Th - T helper

FcεRI - Fc epsilon region of immunoglobulin

TNF - Tumour necrosis factor

MIP - Macrophage inflammatory proteins

GM-CSF - Granulocyte macrophage colony-stimulating factor

ENDA - European Network for Drug Allergy

EAACI - European Academy of Allergy and Clinical Immunology

NSAIDs - Non-steroidal anti-inflammatory drugs

FEIA - Fluoroimmunoassay

ELISA - Enzyme linked immunosorbent assay

RIA - Radioimmunoassay

PI3K - Phosphatidylinoitol 3-kinase

DTRs - Delayed type reactions

SCARs - Severe cutaneous adverse reactions

DILI - Drug induced liver injury

IFN-γ - Interferon gamma

FasL - Fas ligand

CXCL - Chemokine (C-X-C motif) ligand

DCs - Dendritic cells

TCR - T-cell receptors

LPS - Lipopolysaccharide

NF-κB - Nuclear factor-κB

MAPK - Mitogen-activated protein kinase

JNK - c-Jun N-terminal kinase

ERK - Extra-cellular signal-regulated protein kinase

DNCB – Dinitrochlorobenzene

TLR - Toll-like receptor

ACD - Allergic contact dermatitis

Treg cells/Tr - Regulatory T cells

MPE - Maculopapular eruption

AGEP - Acute generalized exanthematous pustulosis

SJS/TEN - Stevens Johnson syndrome/toxic epidermal necrolysis

NK - Natural killer

NKT - NK like T cells

BCR - B cell receptor

Foxp3 - Forkhead box P3

P-i - Pharmacological interactions

Hb - Hemoglobin

P450 - Cytochrome P450

GSTP - Glutathione S-transferase Pi

NVP - Nevirapine

PD1 - Programmed cell death protein 1

TCCs - T cell clones

SMX - Sulfamethoxazole

HIV - Human immunodeficiency virus

EBV - Epstein–Barr virus

HSV - Herpes simplex virus

HHV-6 - Human herpesvirus 6

CMV - Cytomegalovirus

VZV - Varicella-zoster virus

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