Check for updates

DR. PATRICIA ILLING (Orcid ID : 0000-0002-3870-0796)

Article type : Review

ARTICLE: Review

TITLE: The complexity of T cell-mediated penicillin hypersensitivity reactions

AUTHORS: Shawn J. R. Goh[1], Johanna E. E. Tuomisto[1], Anthony W. Purcell[1*], Nicole A. Mifsud[1*],Patricia T. Illing[1*]

*These authors contributed equally to this work.

AFFILIATION:

[1]Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton, 3800, Victoria, Australia.

CORRESPONDING AUTHORS: Dr Patricia Illing and Dr Nicole Mifsud, Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton 3800, Victoria, Australia; Email: patricia.illing@monash.edu or nicole.mifsud@monash.edu; Telephone: (613) 9902 9373 or (613) 9902 9311.

ORCIDs: 0000-0001-5371-6641 (S.J.R.G.); 0000-0002-9286-8186 (J.E.E.T.); 0000-0003-0532-8331 (A.W.P.); 0000-0001-8647-2102 (N.A.M.); 0000-0002-3870-0796 (P.T.I.)

Short title: T cell-mediated penicillin hypersensitivity

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ALL.14355

This article is protected by copyright. All rights reserved

Accepted Article

Word Count: 4394

Total statement of contribution: S.J.R.G., N.A.M. and P.T.I. designed the manuscript. S.J.R.G, J.E.E.T., N.A.M. and P.T.I. wrote the first draft. S.J.R.G., A.W.P., N.A.M. and P.T.I. revised the manuscript and are responsible for the final version. All authors revised and approved the submitted version of the manuscript.

Accepted Article

This article is protected by copyright. All rights reserved

ABSTRACT:

Penicillin refers to a group of beta-lactam antibiotics that are the first-line treatment for a range of infections. However, they also possess the ability to form novel antigens, or neoantigens, through haptenation of proteins and can stimulate a range of immune-mediated adverse reactions – collectively known as drug hypersensitivity reactions (DHRs). IgE-mediated reactions towards these neoantigens are well studied, however, IgE-independent reactions are less well understood. These reactions usually manifest in a delayed manner as different forms of cutaneous eruptions or liver injury consistent with priming of an immune response. *Ex vivo* studies have confirmed the infiltration of T cells into the site of inflammation, and the subsets of T cells involved appear dependent on the nature of the reaction. Here, we review the evidence that has led to our current understanding of these immune-mediated reactions, discussing the nature of the lesional T cells, the characterisation of drug-responsive T cells isolated from patient blood, and the potential mechanisms by which penicillins enter the antigen processing and presentation pathway to stimulate these deleterious responses. Thus, we highlight the need for a more comprehensive understanding of the underlying genetic and molecular basis of penicillin-induced DHRs.

Keywords: penicillin, beta-lactam, drug allergy, hypersensitivity, T cell, HLA

INTRODUCTION

Penicillins are one of the most widely used classes of essential antimicrobial drugs (1) and are a first-line treatment against a broad array of bacteria, including commonly encountered *Streptococcus pneumoniae* and *Staphylococcus aureus*. Penicillins (also termed penams), such as Amoxicillin (AX) and Flucloxacillin (Flux), are a sub-group of beta-lactam (β-lactam) antibiotics, which are defined by the core penam structure consisting of a five-membered ring fused to the four-membered β-lactam ring (Figure 1a) (2). This structure, which resembles the D-Ala-D-Ala

This article is protected by copyright. All rights reserved

Accepted Article

motif of cell wall precursors (Figures 1b), facilitates competitive binding within the active site of bacterial peptidases (penicillin-binding proteins) involved in peptidoglycan cell wall synthesis (2). The irreversible binding is mediated by a conserved active site serine, which performs a nucleophilic attack on the carbonyl of the β-lactam ring (3).

The discovery of Benzylpenicillin (BP) revolutionised the treatment of bacterial diseases and provided a foundation for the synthesis of numerous antimicrobial agents used in modern medicine. Yet, the tremendous benefit experienced by most individuals prescribed these antibiotics is contrasted by a range of clinically diverse adverse drug reactions (ADRs) in a subset of recipients (8-11.5%)(4-6).Adverse reactions to penicillins include 1) non-immune mediated reactions such as vascular hyperpermeability (7), and 2) immune-mediated ADR, or drug hypersensitivity reactions (DHRs)(8). DHRs are categorised based on their underlying immune-related mediators (*e.g.* IgE antibody production, proinflammatory cytokines [interferon-γ (IFNγ), interleukin (IL)-4] and chemokine [IL-8] release) and resulting clinical presentation of symptoms (*e.g.* anaphylaxis and skin rash severity/extent) (9,10) (Table 1).

Whilst the manifestations of immediate DHRs due to penicillin exposure are clinically and mechanistically well-defined (such as thedegranulation of IgE-primed mast cells [reviewed in (11)]), delayed DHRs are less well characterised.

Here, we review the current understanding of delayed β-lactam-induced DHRs and the role of T cells in their aetiology, focusing on the involvement of specific T cell subtypes that drive immunopathology and the underlying molecular events that stimulate their activation.

CLASSIFICATION OF ADVERSE DRUG REACTIONS

An ADR is defined as a "noxious and unintended" response to a therapeutic dose of a drug (12) and can be categorised based on predictability, the time of onset, and the underlying mechanisms driving pathology.

Predictability

ADRs are categorised into two types of reactions based on predictability. Type A represents the vast majority of ADRs, which involve predictable side effects associated with drug pharmacology and are often described as an exaggeration of a drug's intended effect. However, Type B reactions

This article is protected by copyright. All rights reserved

are considered unpredictable, are less well understood, and are often immune-mediated DHRs (13).

Onset time

Type B reactions are segregated into immediate (typically minutes to <1 hour) or delayed (>1 hour) reactions based on onset time following drug administration (14). Regarding penicillin-induced DHRs, immediate reactions that result in anaphylaxisare usually mediated by drug-specific IgE (11) and typically occur within an hour of exposure (15,16). In contrast, penicillin-induced delayed reactions, known as delayed-type hypersensitivity (DTH) reactions, can manifest anywhere between 1 hour to a few days after drug administration (17,18).

Pathological mechanisms

DHRs are further delineated based on their underlying pathological mechanisms. B cell-mediated reactions constitute type I, II and III reactions, whilst T cell-mediated delayed reactions are classified as type IVa-d reactions (Table 1) (9) based on distinct pathological mediators; IVa – macrophage/monocyte activation, IVb – eosinophil activation, IVc – cytotoxic T cell activation, IVd – neutrophil activation (10). Delayed penicillin hypersensitivity most commonly manifests as maculopapular exanthema (MPE), which can be classified as IVa, IVb or IVc (19,20).

T CELLS AND ADVERSE REACTIONS TO PENICILLINS

αβT cells can be divided into two main subsets, $CD4^+$ and $CD8^+$ T cells, and are activated through their highly specific T cell receptors (TCRs). T cell-mediated immunity can be invoked upon recognition of foreign or non-self peptides (*e.g.* viral) presented by the Human Leukocyte Antigen (HLA) molecules of an antigen presenting cell (APC).

Activation of T cells by penicillins in DHRs is thought to occur due to the ability of penicillins to covalently modify proteins to form neoantigens, which have not been previously encountered by the immune system. This may give rise to novel self-derived peptides either directly through the covalent modification of a residue within a displayed peptide, or via alteration of the expression or processing of the source protein.

Detection of penicillin-specific T cells

This article is protected by copyright. All rights reserved

T cells undergo a process known as clonal expansion upon activation and they release cytokines for downstream immune activation. Proliferation of lymphocytes in response to penicillin was observed as early as the 1960s (21,22). Lymphocyte transformation into mitotically active cells, was reported to occur spontaneously in *in vitro* cultures from patients during the acute phase of reaction, but not in healthy controls or non-allergic or recovered patients. Transformation was also observed on stimulation with penicillin at a higher rate in samples from allergic patients than in healthy subjects (22). Modern-day lymphocyte transformation tests (LTTs) generally detect proliferation by the measurement of ^{3}H-thymidine uptake of dividing cells or dilution of Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE) staining by flow cytometry (23,24). As for other drugs, sensitivity and specificity of LTT for diagnosis of penicillin DHRs varies across studies, and can be influenced by clinical stage and nature of the DHR (25,26). For example, across 3 studies, LTT for DHR to BP and AX showed sensitivity and specificity ranges of 62.5%-75% and 85-100% respectively (27-29).

More recently, IFNγ ELISpot has been established as one of the key diagnostic tests to show drug-specific T cell involvement in suspected DHRs (18,30). Rozieres *et al.*compared the capability of both LTT and IFNγ ELISpot to identify circulating AX-specific lymphocytes in peripheral blood mononuclear cells (PBMCs) of AX-DTH patients (n=22). They showed that the IFNγ ELISpot assay exhibited a higher sensitivity, detecting AX-specific lymphocytes in 91% of AX-DTH patients, as compared to 68% via LTT (29). Alternatively, Granzyme B ELISpot assays have been used to evaluate the presence of cytotoxic cells (31,32). Of note, it is recommended by Mayorga *et al.*, as part of theENDA/EAACI Drug Allergy Interest Group, that a combination of LTT and ELISpot assays should be conducted for a robust evaluation (23).

Whilst the ELISpot assay is highly sensitive for the detection of drug-responsive T cells, flow cytometry techniques have been critical in the characterisation of T cell phenotypes in both *in vitro* expanded T cells and directly *ex vivo* (33). Furthermore, obtaining biopsies for direct immunohistochemical investigations has provided information about the immune cell populations infiltrating the area of inflammation (34,35). Combinations of *in vitro* and *in vivo* techniques are now routinely used to detect, isolate and characterise the T cells involved (Table 2) in different clinical phenotypes of penicillin-induced DHRs (36). Through characterisation of cell types, migration behaviour, and cytokine signature, researchers have highlighted potential roles for various cytokines (*e.g.* C-X-C chemokine ligand [CXCL] 10 and IL-4) and receptors (*e.g.* C-C

This article is protected by copyright. All rights reserved

chemokine receptor [CCR] 4 and CCR9) in the pathology of penicillin-induced DHRs (37-39) (Table 3).Indeed, more broadly across DHR *in vitro* investigations have not only revealed cytokine signatures and roles for T regulatory cells and dendritic cells in disease pathology (40-43), but led to recommendation of their manipulation for enhanced diagnostic readouts (23).

Penicillin-induced cutaneous adverse drug reactions

Cutaneous adverse drug reactions (cADRs) range from mild, non-life-threatening reactions presenting as a rash, to potentially fatal conditions such as AGEP, DRESS, and Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) (39,44-48). As summarised in Table 3, the majority of T cell studies have included patients experiencing MPE that clinically presents as widespread, raised skin lesions occurring 24-48 hours after drug administration (49).

BP, AX, Flux and piperacillin-reactive T cell lines and clones isolated from MPE patients are predominantly from the $CD4^+$ T cell compartment (55-100% of T cell clones identified using various strategies [1-10 patients per study]; Table 3) (30,50-54). Further phenotyping of $CD4^+$ T cell clones using immunoassays including flow cytometry, ELISpot or commercially available cytokine-detection kits (*e.g.* Bio-Plex Pro™ (55)) revealed a heterogeneous immunoprofile, mostly composed of Th2 cytokines (IL-4, IL-5 and IL-13) (30,51,52,54-57), and to a lesser extent Th1 cytokines (IFNγ, IL-2) (30,54). The mix of both Th1 and Th2 cytokine profiles and the detection of eotaxins fits well with the understanding that these are vital regulators of eosinophil maturation and recruitment, consistent with MPE pathology (57,58). Interestingly, during an acute phase reaction, a high prevalence of blood-derived $CD8^+$T cells (~55-75% of total T cells) were detected in patient PBMCs who experienced AX- or AX in combination with clavulanic acid (AC)-induced angioedema and generalised erythema (59). Furthermore, these cells were releasing proinflammatory cytokines and cytotoxic molecules (*i.e.* IFNγ, tumour necrosis factor (TNF), perforin, granzyme B) that have been shown elsewhere to directly contribute to keratinocyte apoptosis and epidermal lesions (60).

Immunohistochemical examination of inflamed skin from patients experiencing penicillin-induced MPE and acute generalized exanthematous pustulosis (AGEP) showed perivascular infiltration of mainly $CD4^+$ T cells (60-70% total T cells), where no blistering of the skin was present (35,39,50,59,61). A distinct feature in AGEP however, is the release of IL-8 that might play a role in neutrophil recruitment to the site of lesion (39). Infiltrating $CD4^+$ T cells have also been shown

This article is protected by copyright. All rights reserved

to express upregulated levels of HLA-DR (late activation marker) in 80-100% of the total $CD4^+$ T cell population (Yawalkar *et al.*: 9 controls, 3 penicillin-hypersensitive patients; Britschgi *et al.*: 4 controls, 2 penicillin-hypersensitive patients) (39,57). Moreover, enhanced expression of skin-homing markers such as cutaneous lymphocyte-associated antigen (CLA), CCR10, and C-X-C motif chemokine receptor (CXCR) 3 on infiltrating T cells are commonly detected across the spectrum of penicillin-cADRs (59,62,63). These receptors are fundamental in the recruitment of T cells to the dermis where the corresponding ligands are produced, including CXCL9 for CXCR3 and C-C chemokine ligand (CCL)27 for CCR10 (38,59,64).

In contrast, infiltrating T cells in bullous skin lesions (*i.e.* blistering skin) caused by AX and BP are predominantly from the $CD8^+$ T cell compartment (74-90% of $CD3^+$ T cells) (34). Supporting $CD8^+$ T cell responses in bullous reactions (*i.e.* SJS and TEN), T cell clones from the same patients released elevated levels of proinflammatory cytokines IL-2 and IFNγ, which promote the release of perforin, granzyme B and FasL that cause keratinocyte apoptosis (65,66).

Thus, despite the overlap of the cytokines underlying these different pathologies, phenotypic differences appear to align to the dominant T cell subsets involved – $CD4^+$ T cells in MPE, and $CD8^+$ T cells in SJS/TEN. Why biases towards either $CD4^+$ or $CD8^+$ T cells evolve, leading to these distinct pathologies, remains poorly understood. Further investigation into site-specific and resident immune cells and APCs (*e.g.* Langerhans versus dermal dendritic cells) may aid delineation.

Drug-induced liver injury

Penicillins can also cause drug-induced liver injury (DILI), an umbrella term used to describe toxic hepatitis or hepatotoxicity associated with drugs. The pattern of DILI can be biochemically determined though the alteration of alanine aminotransferase or alkaline phosphatase levels in patient serum (67). Penicillin- DILI typically manifests within 1-3 weeks post-drug administration and usually presents as cholestatic, or mixed cholestatic and hepatocellular injury (75% of total DILI patients), where there is both impaired function of hepatocytes and damaged liver cells (68,69). The most commonly reported penicillins that induce DILI are AC (AC-DILI) (69,70) and Flux (Flux-DILI) (71-75).

The first report of T cell involvement in DILI was published by Bauer and Bircher, where drug-specific T cells against BP and AX were detected from DILI-patient PBMCs (76). The

This article is protected by copyright. All rights reserved

immunoprofile of DILI-associated T cells was further characterised by the Naisbitt group following outgrowth of Flux-specific $CD4^+$ and $CD8^+$ T cell clones (n=4 and n=35, respectively) from PBMCs of Flux-DILI patients (37). Moreover, DILI-associated T cell clones have been reported to express high levels of the liver-homing receptors, CCR2 and CCR9 (77,78). The $CCR9^+$ T cells were also shown to migrate towards CCL25 (CCR9 ligand) in transwell migration assays (2- to 4-fold change migration against no ligand control) (37,77). This effect was also observed in a follow-up study by the same group focused on AC-DILI patients. Here, AX-specific $CD4^+$ and $CD8^+$ T cell clones (n=40 and n=65, respectively) also demonstrated upregulation of CCR9 (79). Finally, immunohistopathological interrogation of liver biopsy from a Flux-DILI patient displayed infiltration by $CD3^+$ $CD8^+$ T cells, corresponding to the proposed involvement of $CD8^+$ T cells in Flux-DILI pathology (80).

ACTIVATION OF T CELLS BY SMALL MOLECULE DRUGS

The prevailing hypothesis as to how small molecule drugs can stimulate T cell responses is that the drug (or a metabolite) associates with self-peptide-HLA (pHLA) complexes to generate structurally novel complexes that can be recognised by T cells. Three main models for how drugs might be incorporated have been proposed; 1) the pharmacological interaction with immune receptors (p.i) concept, 2)the hapten/prohapten concept and 3) the altered peptide repertoire concept.To date, no evidence for large-scale changes in peptide repertoire (altered repertoire) as described for the anti-retroviral drug abacavir (81) has been published for penicillins. Therefore, we focus on evidence for penicillin presentation via the p.i and the hapten/prohapten concepts.

Pharmacological interaction with immune receptors concept

The p.i concept stipulates that a drug (*e.g.* carbamazepine, allopurinol and sulfamethoxazole) interacts with an immune receptor (such as the pHLA) in a non-covalent, labile manner to induce a T cell response (82-84). *In vitro* investigations demonstrated that activation of drug-specific T cells was abrogated when pulsed APCs (incubation of APC with the drug before washing at physiological pH) were used for stimulation suggesting that the interaction between drug and pHLA is labile (82). Consequently, no *de novo* antigen processing is required, and response to the drug-pHLA complex is immediate (Figure 2, pathway 4) (85).Alternatively, it has also been proposed that drugs (*e.g.* sulfamethoxazole) can interact with the TCR itself, altering recognition

This article is protected by copyright. All rights reserved

Accepted Article

of the pHLAcomplex (86,87). Interestingly, Fluxhas been proposed to employ a p.i. mechanism of presentation by HLA instead of peptide ligand haptenation (88-90), as discussed in detail below (Influence of HLA polymorphisms).

Hapten/Prohapten concept

Unlike the p.i concept, haptens form a covalent bond with the carrier protein, and physiological washing steps do not abrogate T cell activation (Figure 2) (51,91). Penicillins are known to preferentially bind to free amines, including lysine residues of proteins, and can generate multiple novel antigenic determinants (56,90,92). However, these stable neoantigens can only be recognised by T cells after antigen processing and presentation of the haptenated peptide by the HLA, unless haptenation occurs directly at the cell surface (Figure 2, pathways 1 and 2) (54,93). In contrast, other small molecules, such as the antimicrobial agent Sulfamethoxazole, are inert in their native forms and gain hapten-like properties via metabolism. These molecules are termed 'prohaptens'.

Penicillin Haptens

The reactivity of penicillins is engendered by the spatially constrained β-lactam ring, which can be the target of nucleophilic attack by free amine groups, leading to the opening of the four-membered ring and formation of thepenicilloyl adduct on lysine residues and free amine moieties (94). Spontaneous conversion of penicillin into penicillenic and penicilloic acid may enable modifications at cysteine residues, but these adducts are considered minor determinants (95-97). The highly abundant serum protein, human serum albumin (HSA), is a long-studied target of penicillin modification. In early studies, high-performance liquid chromatography (HPLC) coupled with protein sequencing via Edman degradation of tryptic peptides defined three lysine residues within HSA that were subject to modification by BP (98,99). More recent investigations have capitalised on tandem mass spectrometry to identify individual peptide species within a complex mixture to define sites of modification in proteins such as HSA. This approach has enabled the identification of lysine modification by diverse penicillins both *ex vivo* (HSA isolated from patient blood) or *in vitro* (Table 4) (56,90,92,100-102). Alternatively, drug-specific antibodies have been utilised to detect penicillin haptenation of proteins (usually coupled with 2D gel electrophoresis), although this approach lacks the ability to define the precise site of haptenation within the protein sequence (103-105). Crucially, the isolation of haptenated HSA from the blood of patients undergoing antibiotic therapy confirms that haptenation of proteins by

This article is protected by copyright. All rights reserved

penicillins occurs under physiological conditions (52,56), thus haptenated proteins may be a source of drug-modified neoepitopes for T cells.

T CELL ACTIVATION BY PENICILLIN

Early studies by Padovan *et al.* provided direct evidence for the involvement of covalently modified penicilloyl neopeptides in the activation of BP-specific T cells *in vitro*(106,107). Utilising $CD4^+$ BP-specific T cell clones expanded from the PBMCs of four penicillin hypersensitive patients, and immortalised autologous Epstein–Barr virus (EBV)-transformed B lymphoblastoid cell lines, they showed that metabolically active APCs were required to induce activation (measured by proliferation) of some, but not all, T cell clones (106). Furthermore, the ability of HSA-BP conjugates to invoke $CD4^+$ T cell activation suggests a requirement for antigen processing competent APCs and an ability to present penicillin modified peptide(s) originating from proteins of the extracellular milieu (Figure 2, pathway 2) (52,93,108). Compellingly, synthetic penicilloyl peptides, designed to bind the expressed HLA class II molecules, also induced $CD4^+$ T cell proliferation, confirming that responses can be stimulated by covalently modified peptides (93,107).

To date, the majority of studies have focused on penicillin responses from the $CD4^+$ T cell compartment as these cells commonly dominate the drug-expanded T cell population in studies of penicillin hypersensitivity (51,54,61,93,108). However, drug-responsive $CD8^+$ T cell clones have also been observed (34,59,109), suggesting that penicilloyl peptides may be presented via both HLA class I and II pathways. Haptenation of proteins in the intracellular compartment is supported by the identification of modified proteins in lysates of cells cultured in protein-free medium containing a biotinylated AX-analogue (AX-B) (Figure 2, pathway 1) (110). Interestingly, AX-B, and more recently native AX and Flux, were shown to not only haptenate intracellular proteins but toalso be exported to target cells in exosomes or carrier vesicles for peptide presentation (Figure 2, pathway 3) (105,111). Consistent with the provision of antigen from intracellular sources, $CD8^+$ T cell responses can be significantly inhibited when the HLA class I processing and presentation pathway is impeded through treatment with a proteasome inhibitor (MG132) (112).

This article is protected by copyright. All rights reserved

Accepted Article

RISK FACTORS

Influence of HLA polymorphism

Whilst variants of co-stimulatory molecules CD40 and CD40L have been implicated as risk factors in penicillin DHRs(113), the impact of HLA polymorphism has received a high level of scrutiny in the broader field of DHR research revealing numerous associations between DHRs and specific HLA alleles (114-117). These associations suggest that in some instances the ability of the causative drug to interact with the HLA is allotype specific, or at least augmented. HLA molecules are highly polymorphic with around 17,000 HLA class I and 7,000 HLA class II alleles identified to date (118). Polymorphisms often map to the anchor pockets of the binding cleft that dictate peptide-binding specificity (A, B, C, D, E, F pockets for HLA class I, and P1, P4, P6 and P9 pockets for HLA class II) (119). In the case of HLA-associated DHR, it is thought that these polymorphisms also dictate the ability of, or increase the potential for, the causative drug to interact with the HLA, as has been shown for abacavir and HLA-B*57:01 (81).

Identification of specific HLA alleles as genetic risk factors for DHRs has involved both targeted sequencing of the HLA and genome-wide association studies (GWAS), frequently prompting *in vitro* investigations. Although associations between penicillin DHRs and specific HLA are less apparent than those for drugs such as carbamazepine (HLA-B*15:02) (120,121) and abacavir (HLA-B*57:01) (122,123), associations between DILI induced by certain penicillins and a range of HLA have been observed (116) (Table 5). The strongest of these associations was identified in a genotyping and GWAS study that implicated HLA-B*57:01 in Flux-DILI (odds ratio (OR) of 80.6 and negative predictive value of 99.99%) (124). *In vitro* relevance was reported by Monshi *et al.* where the majority of T cell clones obtained from Flux-DILI HLA-B*57:01$^+$ patient PBMCs were CD8$^+$ (n=35/38)(37). Notably, these DILI-patient-derived T cell clones also expressed liver-homing receptors CCR4 and CCR9 that aid migration to the site of inflammation (77,125).

The immunomechanism utilised by Flux is under debate. Multiple studies have demonstrated T cell responses towards Flux-pulsed APCs in PBMCs derived from Flux-DILI HLA-B*57:01$^+$ patients (37,88), Flux-naïve HLA-B*57:01$^+$donors(37) and Flux-naïve HLA-B*57:01$^-$donors(89). Furthermore, like other penicillins, Flux has been shown to form stable haptenic structures *in vitro* and *in vivo*(30,90), suggesting that haptenated peptides form the antigenic determinants in Flux-DILI. However, it has been observed that most Flux-specific T cell lines generated from Flux-

This article is protected by copyright. All rights reserved

naïve HLA-B*57:01$^+$ healthy donors did not recognise Flux-pulsed APCs, implying that the antigenic determinant recognised by some T cells was labile (*i.e.* p.i concept) (88,89). Furthermore, T cell clones generated from Flux-naïve HLA-B*57:01$^+$donors could be activated immediately after Flux addition (<5 mins) (89). Together, these findings suggest heterogeneity in the mode of interaction of Flux and HLA molecules that necessitates further study into: 1) the possible role of initial non-covalent interactions between Flux and its target that catalyses haptenation (126) and 2) pH of the target microenvironment (*i.e.* lysine residues) that might influence this reaction (127), in order to understand the molecular basis of the association of Flux-DILI with HLA-B*57:01.

To date, GWAS and genotyping reports have identified several different HLA allotypes as risk factors for AX-DILI. The two most prominent allotypes, HLA-A*02:01 and HLA-DRB1*15:01, were speculated as potential risk factors in American, European, Spanish, and Scottish populations (summarised in Table 5, with associated single nucleotide polymorphism [SNP]) (128-131). Mechanistic studies by Kim *et al.* showed that AX could induce proliferation of AX-specific CD8$^+$ and CD4$^+$ T cells in DILI patients who were HLA-A*02:01$^+$ (n=4/7) and HLA-DRB1*15:01$^+$ (n=1/7). Activation of both AX-specific CD8$^+$ and CD4$^+$ T cell clones could be inhibited when APCs were treated with pan HLA class I or II antibodies, respectively (79). In contrast, another GWAS study postulated that the HLA-A*33:01 allele (SNPs rs114577328 and rs72631567) might predispose patients to AX-DILI (OR 2.7 and 1, $p=2.4x10^{-8}$ and $9.8x10^{-9}$, respectively) (132). In a separate genotyping study, HLA-A*30:02 was significantly higher in the AX-DILI cohort (OR 6.7, $p=5x10^{-8}$) (133). The less compelling associations between penicillin ADR and specific HLA allotypes may reflect the vast range of potential modification sites of human proteins (~6% lysine) contributing modified peptide ligands to diverse HLA molecules (134).

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder caused by a mutation in the CF transmembrane conductance regulator (CFTR) gene (135). The mutation consequently leads to protein misfolding impairing an individual's mucociliary clearance ability. Build-up of viscous mucus forms an extremely conducive environment for bacteria to thrive, therefore CF patients are often chronically infected with *Staphylococcus aureus*and *Pseudomonas aeruginosa*(136). As a result, penicillin therapy, comprised of piperacillinin combination with Tazobactam, has become the cornerstone of CF treatment (137). Unfortunately, penicillin-hypersensitivity is three to six

This article is protected by copyright. All rights reserved

times more prevalent in CF patients when compared to patients receiving microbial treatment for other ailments (138,139). Amongst these reactions, piperacillin-induced DTH (30-50% of ADRs in CF patients) usually manifests as MPE, AGEP and DRESS (140-142).

In a study of 28 piperacillin-hypersensitive CF patients, excluding patients on oral steroids, Whitaker *et al.* demonstrated expansion of piperacillin-responsive T cells from PBMCs in 76% of patients (56). Further investigations conducted by El-Ghaiesh *et al.* showed that piperacillin-specific T cells clones (n=414) from five CF patients were mainly of the $CD4^+$ T cell phenotype ($CD4^+$: 55-100%, $CD8^+$: 0-45%, $CD4^+CD8^+$ double positives: 0-12%) (51). This prompted a series of studies, which reported a consistent dominance of $CD4^+$ T cell expansion (79-92% of all clones) (30,52,62), the characterisation of which revealed a dominant Th2 immunoprofile, secreting significantly higher levels of IL-4, IL-5 and IL-13 when compared to controls (30,51,52,56,62). Interestingly, Sullivan *et al.* presented novel evidence showing that piperacillin-specific Th22 T cell clones (producing IL-22 and not IL-17), can be expanded from piperacillin-hypersensitive CF patient PBMCs (62). This finding is particularly interesting as Th22 cells express CCR4 and CCR10, both skin-homing receptors that might be key regulators of the MPE pathology (143). The cutaneous nature of many penicillin-induced DTHs, in conjunction with the role of IL-22 in other cutaneous diseases (*i.e.* atopic dermatitis and psoriasis) (144-146), suggests that Th22 T cells warrant further investigation in penicillin-induced cADR progression.

Desensitisation of piperacillin-hypersensitive patients has shown success rates of 100% and 55% in those who experience immediate and non-immediate reactions, respectively (147,148). However, the mechanisms of desensitisation have not been fully elucidated in this context and need to be explored in detail.

COMMENTS AND CONCLUSION

Understanding penicillin hypersensitivity has proven to be extremely complex, involving both humoral and cellular immune components. Whilst testing for penicillin-specific IgE in patients is relatively straightforward; evaluating if an individual might experience a T cell-mediated response is complicated and requires more detailed investigation. Although the dominant T cell subsets in various penicillin-induced DHRs can be defined, the underlying causes of these reactions and the molecular targets are yet to be fully explained. Furthermore, not all of these reactions are

This article is protected by copyright. All rights reserved

persistent, and patients can be de-labelled as allergic to penicillin (149). Hence prediction of who may experience a penicillin-induced DHR, and if so, in what form, remains clinically obscure. As the formation of penicillin-haptenated proteins can occur either intracellularly or extracellularly, there is potential to invoke activation of different T cell subsets that paint distinct clinical pictures. Whilst the breadth of potential haptenation sites in the human proteome may generate a diverse range of neopeptides enabling these reactions across HLA backgrounds. Thus, the question may be – "why don't more people experience these reactions?". Whether dampening of responses by regulatory T cells (40,150) or biases towards particular TCR sequences (151,152) shape reaction susceptibility, as suggested in adverse responses to other drugs, is worthy of further investigation. Understanding these factors and defining the precise targets of penicillin-responsive T cells, represent the next hurdles to unravelling the mechanisms of these complex reactions.

ACKNOWLEDGEMENTS

AWP is supported by a National Health and Medical Research Council of Australia (NHMRC) Principal Research Fellowship. We acknowledge funding support from a NHMRC Project grant 1122099 (to AWP).

CONFLICT OF INTEREST STATEMENT

Dr. Goh has nothing to disclose.

Dr. Tuomisto has nothing to disclose.

Dr. Mifsud reports grants from National Health & Medical Research Council , during the conduct of the study.

Dr. Purcell reports grants from National Health and Medical Research Council, during the conduct of the study.

Dr. Illing reports grants from National Health and Medical Research Council, during the conduct of the study.

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

REFERENCES

1. Organization WH. *World Health Organization model list of essential medicines: 21st list 2019.* World Health Organization;2019.
2. Dalhoff A, Janjic N, Echols R. Redefining penems. *Biochemical pharmacology.* 2006;71(7):1085-1095.
3. Zapun A, Contreras-Martel C, Vernet T. Penicillin-binding proteins and β-lactam resistance. *FEMS microbiology reviews.* 2008;32(2):361-385.
4. Albin S, Agarwal S. Prevalence and characteristics of reported penicillin allergy in an urban outpatient adult population. Paper presented at: Allergy and asthma proceedings2014.
5. Macy E. Penicillin and beta-lactam allergy: epidemiology and diagnosis. *Current allergy and asthma reports.* 2014;14(11):476.
6. Macy E, Contreras R. Health care use and serious infection prevalence associated with penicillin "allergy" in hospitalized patients: a cohort study. *Journal of Allergy and Clinical Immunology.* 2014;133(3):790-796.
7. Han J, Yi Y, Li C, et al. Involvement of histamine and RhoA/ROCK in penicillin immediate hypersensitivity reactions. *Scientific reports.* 2016;6:33192.
8. Pichler WJ, Hausmann O. Classification of drug hypersensitivity into allergic, pi, and pseudo-allergic forms. *International archives of allergy and immunology.* 2016;171(3-4):166-179.
9. Gell PGH, Coombs RRA. Clinical aspects of immunology. *Clinical Aspects of Immunology.* 1963.
10. Pichler WJ. Delayed drug hypersensitivity reactions. *Annals of internal medicine.* 2003;139(8):683-693.
11. Doña I, Bogas G, Pérez-Sánchez N, Fernández T, Moreno E, Torres M. Penicillin and Cephalosporin-Induced Anaphylaxis: an Update. *Current Treatment Options in Allergy.* 2018;5(2):188-203.
12. Organization WH. *International drug monitoring: the role of national centres, report of a WHO meeting [held in Geneva from 20 to 25 September 1971].* World Health Organization; 1972.
13. Zhang X, Liu F, Chen X, Zhu X, Uetrecht J. Involvement of the immune system in idiosyncratic drug reactions. *Drug metabolism and pharmacokinetics.* 2010:1011100116-1011100116.
14. Demoly P, Adkinson NF, Brockow K, et al. International Con sensus on drug allergy. *Allergy.* 2014;69(4):420-437.
15. Macy E, Ngor EW. Safely diagnosing clinically significant penicillin allergy using only penicilloyl-poly-lysine, penicillin, and oral amoxicillin. *The Journal of Allergy and Clinical Immunology: In Practice.* 2013;1(3):258-263.

This article is protected by copyright. All rights reserved

16. Gonzalez-Estrada A, Radojicic C. Penicillin allergy: a practical guide for clinicians. *Cleve Clin J Med.* 2015;82(5):295-300.
17. Park B, Laverty H, Srivastava A, Antoine D, Naisbitt D, Williams D. Drug bioactivation and protein adduct formation in the pathogenesis of drug-induced toxicity. *Chemico-biological interactions.* 2011;192(1-2):30-36.
18. Romano A, Blanca M, Torres M, et al. Diagnosis of nonimmediate reactions to β-lactam antibiotics. *Allergy.* 2004;59(11):1153-1160.
19. Fernández TD, Canto G, Blanca M. Molecular mechanisms of maculopapular exanthema. *Current opinion in infectious diseases.* 2009;22(3):272-278.
20. Mirakian R, Leech S, Krishna M, et al. Management of allergy to penicillins and other beta-lactams. *Clinical & Experimental Allergy.* 2015;45(2):300-327.
21. Ripps C, Fellner M, Hirschhorn K. Response of lymphocytes to tetracycline. *Lancet (London, England).* 1965;2(7419):951.
22. Gill FA. The association of increased spontaneous lymphocyte transformation in vitro with clinical manifestations of drug hypersensitivity. *The Journal of Immunology.* 1967;98(4):778-785.
23. Mayorga C, Celik G, Rouzaire P, et al. In vitro tests for drug hypersensitivity reactions: an ENDA/EAACI Drug Allergy Interest Group position paper. *Allergy.* 2016;71(8):1103-1134.
24. Pichler Wa, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy.* 2004;59(8):809-820.
25. Kano Y, Hirahara K, Mitsuyama Y, Takahashi R, Shiohara T. Utility of the lymphocyte transformation test in the diagnosis of drug sensitivity: dependence on its timing and the type of drug eruption. *Allergy.* 2007;62(12):1439-1444.
26. Polak M, Belgi G, McGuire C, et al. In vitro diagnostic assays are effective during the acute phase of delayed-type drug hypersensitivity reactions. *British Journal of Dermatology.* 2013;168(3):539-549.
27. Schnyder B, Pichler W. Skin and laboratory tests in amoxicillin-and penicillin-induced morbilliform skin eruption. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology.* 2000;30(4):590-595.
28. Luque I, Leyva L, Jose Torres M, et al. In vitro T-cell responses to β-lactam drugs in immediate and nonimmediate allergic reactions. *Allergy.* 2001;56(7):611-618.
29. Rozières A, Hennino A, Rodet K, et al. Detection and quantification of drug-specific T cells in penicillin allergy. *Allergy.* 2009;64(4):534-542.

Accepted Article

This article is protected by copyright. All rights reserved

30. Jenkins RE, Yaseen FS, Monshi MM, et al. β-Lactam antibiotics form distinct haptenic structures on albumin and activate drug-specific T-lymphocyte responses in multiallergic patients with cystic fibrosis. *Chemical research in toxicology.* 2013;26(6):963-975.
31. Zawodniak A, Lochmatter P, Yerly D, et al. In vitro detection of cytotoxic T and NK cells in peripheral blood of patients with various drug-induced skin diseases. *Allergy.* 2010;65(3):376-384.
32. Porebski G, Pecaric-Petkovic T, Groux-Keller M, Bosak M, Kawabata T, Pichler WJ. In vitro drug causality assessment in Stevens–Johnson syndrome–alternatives for lymphocyte transformation test. *Clinical & Experimental Allergy.* 2013;43(9):1027-1037.
33. Karlsson AC, Martin JN, Younger SR, et al. Comparison of the ELISPOT and cytokine flow cytometry assays for the enumeration of antigen-specific T cells. *Journal of immunological methods.* 2003;283(1-2):141-153.
34. Hertl M, Bohlen H, Jugert F, Boecker C, Knaup R, Merk HF. Predominance of epidermal CD8+ T lymphocytes in bullous cutaneous reactions caused by β-lactam antibiotics. *Journal of investigative dermatology.* 1993;101(6):794-799.
35. Warrington RJ, Silviu-Dan F, Magro C. Accelerated cell-mediated immune reactions in penicillin allergy. *Journal of Allergy and Clinical Immunology.* 1993;92(4):626-628.
36. Torres MJ, Celik GE, Whitaker P, et al. A EAACI drug allergy interest group survey on how European allergy specialists deal with β-lactam allergy. *Allergy.* 2019;74(6):1052-1062.
37. Monshi MM, Faulkner L, Gibson A, et al. Human leukocyte antigen (HLA)-B* 57: 01-restricted activation of drug-specific T cells provides the immunological basis for flucloxacillin-induced liver injury. *Hepatology.* 2013;57(2):727-739.
38. Fernandez T, Mayorga C, Torres M, et al. Cytokine and chemokine expression in the skin from patients with maculopapular exanthema to drugs. *Allergy.* 2008;63(6):712-719.
39. Britschgi M, Steiner UC, Schmid S, et al. T-cell involvement in drug-induced acute generalized exanthematous pustulosis. *The Journal of clinical investigation.* 2001;107(11):1433-1441.
40. Takahashi R, Kano Y, Yamazaki Y, Kimishima M, Mizukawa Y, Shiohara T. Defective regulatory T cells in patients with severe drug eruptions: timing of the dysfunction is associated with the pathological phenotype and outcome. *The Journal of Immunology.* 2009;182(12):8071-8079.
41. Srinoulprasert Y, Pichler WJ. Enhancement of drug-specific lymphocyte proliferation using CD25hi-depleted CD3+ effector cells. *International archives of allergy and immunology.* 2014;163(3):198-205.

This article is protected by copyright. All rights reserved

42. Rodriguez-Pena R, Lopez S, Mayorga C, et al. Potential involvement of dendritic cells in delayed-type hypersensitivity reactions to β-lactams. *Journal of Allergy and Clinical Immunology.* 2006;118(4):949-956.
43. Sanchez-Quintero MJ, Torres MJ, Blazquez AB, et al. Synergistic effect between amoxicillin and TLR ligands on dendritic cells from amoxicillin-delayed allergic patients. *PloS one.* 2013;8(9).
44. Romano A, Valluzzi RL, Caruso C, Maggioletti M, Gaeta F. Non-immediate cutaneous reactions to beta-lactams: approach to diagnosis. *Current allergy and asthma reports.* 2017;17(4):23.
45. Hjortlund J, Mortz C, Skov P, Bindslev-Jensen C. Diagnosis of penicillin allergy revisited: the value of case history, skin testing, specific IgE and prolonged challenge. *Allergy.* 2013;68(8):1057-1064.
46. Mori F, Cianferoni A, Barni S, Pucci N, Rossi ME, Novembre E. Amoxicillin allergy in children: five-day drug provocation test in the diagnosis of nonimmediate reactions. *The Journal of Allergy and Clinical Immunology: In Practice.* 2015;3(3):375-380. e371.
47. Atanaskovic-Markovic M, Gaeta F, Medjo B, et al. Non-immediate hypersensitivity reactions to beta-lactam antibiotics in children–our 10-year experience in allergy work-up. *Pediatric Allergy and Immunology.* 2016;27(5):533-538.
48. Cabanas R, Calderon O, Ramirez E, et al. Piperacillin-induced DRESS: distinguishing features observed in a clinical and allergy study of 8 patients. *J Investig Allergol Clin Immunol.* 2014;24(6):425-430.
49. Torres M, Mayorga C, Blanca M. 1 Nonimmediate Allergic Reactions Induced by Drugs: Pathogenesis and Diagnostic Tests. *Journal of investigational allergology & clinical immunology.* 2009;19(2):80.
50. Yawalkar N, Hari Y, Frutig K, et al. T cells isolated from positive epicutaneous test reactions to amoxicillin and ceftriaxone are drug specific and cytotoxic. *Journal of investigative dermatology.* 2000;115(4):647-652.
51. El-Ghaiesh S, Monshi MM, Whitaker P, et al. Characterization of the antigen specificity of T-cell clones from piperacillin-hypersensitive patients with cystic fibrosis. *Journal of Pharmacology and Experimental Therapeutics.* 2012;341(3):597-610.
52. Meng X, Al-Attar Z, Yaseen FS, et al. Definition of the nature and hapten threshold of the β-lactam antigen required for T cell activation in vitro and in patients. *The Journal of Immunology.* 2017;198(11):4217-4227.
53. Koponen M, Pichler WJ, de Weck AL. T cell reactivity to penicillin: phenotypic analysis of in vitro activated cell subsets. *Journal of Allergy and Clinical Immunology.* 1986;78(4):645-652.

This article is protected by copyright. All rights reserved

54. Brander C, Mauri-Hellweg D, Bettens F, Rolli H, Goldman M, Pichler WJ. Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals. *The Journal of Immunology.* 1995;155(5):2670-2678.
55. Lochmatter P, Beeler A, Kawabata T, Gerber B, Pichler WJ. Drug-specific in vitro release of IL-2, IL-5, IL-13 and IFN-γ in patients with delayed-type drug hypersensitivity. *Allergy.* 2009;64(9):1269-1278.
56. Whitaker P, Meng X, Lavergne SN, et al. Mass spectrometric characterization of circulating and functional antigens derived from piperacillin in patients with cystic fibrosis. *The Journal of Immunology.* 2011;187(1):200-211.
57. Yawalkar N, Shrikhande M, Hari Y, Nievergelt H, Braathen LR, Pichler WJ. Evidence for a role for IL-5 and eotaxin in activating and recruiting eosinophils in drug-induced cutaneous eruptions. *Journal of Allergy and Clinical Immunology.* 2000;106(6):1171-1176.
58. Gleich GJ. Mechanisms of eosinophil-associated inflammation. *Journal of Allergy and Clinical Immunology.* 2000;105(4):651-663.
59. Gómez E, Blanca-Lopez N, Salas M, et al. Induction of accelerated reactions to amoxicillin by T-cell effector mechanisms. *Annals of Allergy, Asthma & Immunology.* 2013;110(4):267-273.
60. Posadas SJ, Padial A, Torres MJ, et al. Delayed reactions to drugs show levels of perforin, granzyme B, and Fas-L to be related to disease severity. *Journal of Allergy and Clinical Immunology.* 2002;109(1):155-161.
61. Yawalkar N, Egli F, Hari Y, Nievergelt H, Braathen L, Pichler W. Infiltration of cytotoxic T cells in drug-induced cutaneous eruptions. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology.* 2000;30(6):847-855.
62. Sullivan A, Wang E, Farrell J, et al. β-lactam hypersensitivity involves expansion of circulating and skin-resident Th22 cells. *Journal of Allergy and Clinical Immunology.* 2018;141(1):235-249. e238.
63. Blanca M, Torres M, Leyva L, et al. Expression of the skin-homing receptor in peripheral blood lymphocytes from subjects with nonimmediate cutaneousallergic drug reactions. *Allergy.* 2000;55(11):998-1004.
64. Reiss Y, Proudfoot AE, Power CA, Campbell JJ, Butcher EC. CC chemokine receptor (CCR) 4 and the CCR10 ligand cutaneous T cell–attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. *Journal of Experimental Medicine.* 2001;194(10):1541-1547.
65. Kohanim S, Palioura S, Saeed HN, et al. Stevens-Johnson syndrome/toxic epidermal necrolysis–a comprehensive review and guide to therapy. I. Systemic disease. *The ocular surface.* 2016;14(1):2-19.

This article is protected by copyright. All rights reserved

66. Paul C, Wolkenstein P, Adle H, et al. Apoptosis as a mechanism of keratinocyte death in toxic epidermal necrolysis. *British Journal of Dermatology.* 1996;134(4):710-714.
67. Andrade RJ, Aithal GP, Björnsson ES, et al. EASL clinical practice guidelines: drug-induced liver injury. *Journal of hepatology.* 2019;70(6):1222-1261.
68. Björnsson ES. Drug-induced liver injury due to antibiotics. *Scandinavian journal of gastroenterology.* 2017;52(6-7):617-623.
69. Delemos AS, Ghabril M, Rockey DC, et al. Amoxicillin–clavulanate-induced liver injury. *Digestive diseases and sciences.* 2016;61(8):2406-2416.
70. Moreno L, Sánchez-Delgado J, Vergara M, Casas M, Miquel M, Dalmau B. Recurrent drug-induced liver injury (DILI) with ciprofloxacin and amoxicillin/clavulanic. *Revista Española de Enfermedades Digestivas.* 2015;107(12):767-768.
71. Lucena MI, Andrade RJ, Fernández MC, et al. Determinants of the clinical expression of amoxicillin-clavulanate hepatotoxicity: a prospective series from Spain. *Hepatology.* 2006;44(4):850-856.
72. Björnsson E. Hepatotoxicity by drugs: the most common implicated agents. *International journal of molecular sciences.* 2016;17(2):224.
73. Devereaux B, Crawford D, Purcell P, Powell L, Roeser H. Flucloxacillin associated cholestatic hepatitis. An Australian and Swedish epidemic? *European journal of clinical pharmacology.* 1995;49(1-2):81-85.
74. Wing K, Bhaskaran K, Pealing L, et al. Quantification of the risk of liver injury associated with flucloxacillin: a UK population-based cohort study. *Journal of Antimicrobial Chemotherapy.* 2017;72(9):2636-2646.
75. Teschke R. Top-ranking drugs out of 3312 drug-induced liver injury cases evaluated by the Roussel Uclaf Causality Assessment Method. *Expert Opinion on Drug Metabolism & Toxicology.* 2018;14(11):1169-1187.
76. Bauer TM, Bircher AJ. Drug-induced hepatocellular liver injury due to benzylpenicillin with evidence of lymphocyte sensitization. *Journal of hepatology.* 1997;26(2):429-432.
77. Eksteen B, Grant AJ, Miles A, et al. Hepatic endothelial CCL25 mediates the recruitment of CCR9+ gut-homing lymphocytes to the liver in primary sclerosing cholangitis. *Journal of Experimental Medicine.* 2004;200(11):1511-1517.
78. Sahin H, Trautwein C, Wasmuth HE. Functional role of chemokines in liver disease models. *Nature Reviews Gastroenterology & Hepatology.* 2010;7(12):682.

This article is protected by copyright. All rights reserved

Accepted Article

79. Kim SH, Saide K, Farrell J, et al. Characterization of amoxicillin-and clavulanic acid-specific T cells in patients with amoxicillin-clavulanate–induced liver injury. *Hepatology.* 2015;62(3):887-899.
80. Wuillemin N, Terracciano L, Beltraminelli H, et al. T cells infiltrate the liver and kill hepatocytes in HLA-B∗ 57: 01-associated floxacillin-induced liver injury. *The American journal of pathology.* 2014;184(6):1677-1682.
81. Illing PT, Vivian JP, Dudek NL, et al. Immune self-reactivity triggered by drug-modified HLA-peptide repertoire. *Nature.* 2012;486(7404):554-558.
82. Wei C-Y, Chung W-H, Huang H-W, Chen Y-T, Hung S-I. Direct interaction between HLA-B and carbamazepine activates T cells in patients with Stevens-Johnson syndrome. *Journal of Allergy and Clinical Immunology.* 2012;129(6):1562-1569. e1565.
83. Zanni MP, von Greyerz S, Schnyder B, et al. HLA-restricted, processing-and metabolism-independent pathway of drug recognition by human alpha beta T lymphocytes. *The Journal of clinical investigation.* 1998;102(8):1591-1598.
84. Lin C-H, Chen J-K, Ko T-M, et al. Immunologic basis for allopurinol-induced severe cutaneous adverse reactions: HLA-B* 58: 01-restricted activation of drug-specific T cells and molecular interaction. *Journal of Allergy and Clinical Immunology.* 2015;135(4):1063-1065. e1065.
85. Pichler WJ. The pi concept: pharmacological interaction of drugs with immune receptors. *World Allergy Organization Journal.* 2008;1(6):96.
86. Watkins S, Pichler WJ. Sulfamethoxazole induces a switch mechanism in T cell receptors containing TCRVβ20-1, altering pHLA recognition. *PloS one.* 2013;8(10):e76211.
87. Zhou P, Zhang S, Wang Y, Yang C, Huang J. Structural modeling of HLA-B* 1502/peptide/carbamazepine/T-cell receptor complex architecture: implication for the molecular mechanism of carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis. *Journal of Biomolecular Structure and Dynamics.* 2016;34(8):1806-1817.
88. Yaseen FS, Saide K, Kim S-H, et al. Promiscuous T-cell responses to drugs and drug-haptens. *Journal of Allergy and Clinical Immunology.* 2015;136(2):474-476. e478.
89. Wuillemin N, Adam J, Fontana S, Krähenbühl S, Pichler WJ, Yerly D. HLA haplotype determines hapten or pi T cell reactivity to flucloxacillin. *The Journal of Immunology.* 2013;190(10):4956-4964.
90. Jenkins RE, Meng X, Elliott VL, Kitteringham NR, Pirmohamed M, Park BK. Characterisation of flucloxacillin and 5-hydroxymethyl flucloxacillin haptenated HSA in vitro and in vivo. *PROTEOMICS–Clinical Applications.* 2009;3(6):720-729.
91. Illing PT, Vivian JP, Purcell AW, Rossjohn J, McCluskey J. Human leukocyte antigen-associated drug hypersensitivity. *Current opinion in immunology.* 2013;25(1):81-89.

This article is protected by copyright. All rights reserved

92. Meng X, Jenkins RE, Berry NG, et al. Direct evidence for the formation of diastereoisomeric benzylpenicilloyl haptens from benzylpenicillin and benzylpenicillenic acid in patients. *Journal of Pharmacology and Experimental Therapeutics.* 2011;338(3):841-849.
93. Azoury M, Filì L, Bechara R, et al. Identification of T-cell epitopes from benzylpenicillin conjugated to human serum albumin and implication in penicillin allergy. *Allergy.* 2018;73(8):1662-1672.
94. Batchelor F, Dewdney JM, Gazzard D. Penicillin allergy: the formation of the penicilloyl determinant. *Nature.* 1965;206:362-364.
95. Levine BB. Formation of D-penicillamine-cysteine mixed disulphide by reaction of D-benzylpenicilloic acid with cystine. *Nature.* 1960;187(4741):940-941.
96. Levine B, Redmond A. Minor haptenic determinant-specific reagins of penicillin hypersensitivity in man. *International archives of allergy and immunology.* 1969;35(5):445-455.
97. Park BK, Sanderson JP, Naisbitt DJ. Drugs as haptens, antigens, and immunogens. In: *Drug Hypersensitivity.* Karger Publishers; 2007:55-65.
98. Yvon M, Anglade P, Wal J-M. Identification of the binding sites of benzyl penicilloyl, the allergenic metabolite of penicillin, on the serum albumin molecule. *FEBS letters.* 1990;263(2):237-240.
99. Yvon M, Wal J-M. Identification of lysine residue 199 of human serum albumin as a binding site for benzylpenicilloyl groups. *FEBS letters.* 1988;239(2):237-240.
100. Garzon D, Ariza A, Regazzoni L, et al. Mass spectrometric strategies for the identification and characterization of human serum albumin covalently adducted by amoxicillin: ex vivo studies. *Chemical research in toxicology.* 2014;27(9):1566-1574.
101. Meng X, Earnshaw CJ, Tailor A, et al. Amoxicillin and clavulanate form chemically and immunologically distinct multiple haptenic structures in patients. *Chemical research in toxicology.* 2016;29(10):1762-1772.
102. Ariza A, Garzon D, Abánades DR, et al. Protein haptenation by amoxicillin: high resolution mass spectrometry analysis and identification of target proteins in serum. *Journal of proteomics.* 2012;77:504-520.
103. De Haan P, De Jonge A, Verbrugge T, Boorsma D. Three epitope-specific monoclonal antibodies against the hapten penicillin. *International archives of allergy and immunology.* 1985;76(1):42-46.
104. Magi B, Marzocchi B, Bini L, Cellesi C, Rossolini A, Pallini V. Two-dimensional electrophoresis of human serum proteins modified by ampicillin during therapeutic treatment. *Electrophoresis.* 1995;16(1):1190-1192.
105. Sánchez-Gómez FJ, González-Morena JM, Vida Y, et al. Amoxicillin haptenates intracellular proteins that can be transported in exosomes to target cells. *Allergy.* 2017;72(3):385-396.

This article is protected by copyright. All rights reserved

106. Padovan E, Mauri-Hellweg D, Pichler WJ, Weltzien HU. T cell recognition of penicillin G: structural features determining antigenic specificity. *European journal of immunology.* 1996;26(1):42-48.
107. Padovan E, Bauer T, Tongio MM, Kalbacher H, Weltzien HU. Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy. *European journal of immunology.* 1997;27(6):1303-1307.
108. Nhim C, Delluc S, Halgand F, et al. Identification and frequency of circulating CD 4+ T lymphocytes specific to B enzylpenicillin in healthy donors. *Allergy.* 2013;68(7):899-905.
109. Mori F, Fili L, Barni S, et al. T-cell activation in two cases of Stevens-Johnson syndrome after receiving amoxicillin-clavulanic acid. *Pediatric Allergy and Immunology.* 2017;28(6):602-606.
110. Ariza A, Collado D, Vida Y, et al. Study of protein haptenation by amoxicillin through the use of a biotinylated antibiotic. *PloS one.* 2014;9(3):e90891.
111. Ogese MO, Jenkins RE, Adair K, et al. Exosomal transport of hepatocyte-derived drug-modified proteins to the immune system. *Hepatology.* 2019.
112. Bechara R, Maillere B, Joseph D, Weaver RJ, Pallardy M. Identification and characterization of a naïve CD 8+ T cell repertoire for benzylpenicillin. *Clinical & Experimental Allergy.* 2019;49(5):636-643.
113. Kim SH, Lee JE, Kim SH, et al. Allelic variants of CD40 and CD40L genes interact to promote antibiotic-induced cutaneous allergic reactions. *Clinical & Experimental Allergy.* 2009;39(12):1852-1856.
114. Mullan KA, Anderson A, Illing PT, Kwan P, Purcell AW, Mifsud NA. HLA-associated antiepileptic drug-induced cutaneous adverse reactions. *Hla.* 2019;93(6):417-435.
115. Fan W-L, Shiao M-S, Hui RC-Y, et al. HLA association with drug-induced adverse reactions. *Journal of immunology research.* 2017;2017.
116. Illing PT, Purcell AW, McCluskey J. The role of HLA genes in pharmacogenomics: unravelling HLA associated adverse drug reactions. *Immunogenetics.* 2017;69(8-9):617-630.
117. Konvinse KC, Trubiano JA, Pavlos R, et al. HLA-A* 32: 01 is strongly associated with vancomycin-induced drug reaction with eosinophilia and systemic symptoms. *Journal of Allergy and Clinical Immunology.* 2019.
118. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The IPD and IMGT/HLA database: allele variant databases. *Nucleic acids research.* 2014;43(D1):D423-D431.
119. Illing PT, Pymm P, Croft NP, et al. HLA-B57 micropolymorphism defines the sequence and conformational breadth of the immunopeptidome. *Nature communications.* 2018;9(1):4693.

This article is protected by copyright. All rights reserved

120. Ferrell PB, McLeod HL. Carbamazepine, HLA-B* 1502 and risk of Stevens–Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. 2008.
121. Chen P, Lin J-J, Lu C-S, et al. Carbamazepine-induced toxic effects and HLA-B* 1502 screening in Taiwan. *New England Journal of Medicine.* 2011;364(12):1126-1133.
122. Hetherington S, Hughes AR, Mosteller M, et al. Genetic variations in HLA-B region and hypersensitivity reactions to abacavir. *The Lancet.* 2002;359(9312):1121-1122.
123. Mallal S, Nolan D, Witt C, et al. Association between presence of HLA-B* 5701, HLA-DR7, and HLA-DQ3 and hypersensitivity to HIV-1 reverse-transcriptase inhibitor abacavir. *The Lancet.* 2002;359(9308):727-732.
124. Daly AK, Donaldson PT, Bhatnagar P, et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet.* 2009;41(7):816--819.
125. Oo YH, Weston CJ, Lalor PF, et al. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. *The Journal of Immunology.* 2010;184(6):2886-2898.
126. Singh J, Petter RC, Baillie TA, Whitty A. The resurgence of covalent drugs. *Nature reviews Drug discovery.* 2011;10(4):307-317.
127. Divkovic M, Pease CK, Gerberick GF, Basketter DA. Hapten–protein binding: from theory to practical application in the in vitro prediction of skin sensitization. *Contact dermatitis.* 2005;53(4):189-200.
128. Lucena MI, Molokhia M, Shen Y, et al. Susceptibility to amoxicillin-clavulanate-induced liver injury is influenced by multiple HLA class I and II alleles. *Gastroenterology.* 2011;141(1):338-347.
129. Donaldson PT, Daly AK, Henderson J, et al. Human leucocyte antigen class II genotype in susceptibility and resistance to co-amoxiclav-induced liver injury. *Journal of hepatology.* 2010;53(6):1049-1053.
130. O'donohue J, Oien K, Donaldson P, et al. Co-amoxiclav jaundice: clinical and histological features and HLA class II association. *Gut.* 2000;47(5):717-720.
131. Andrade RJ, Lucena MI, Alonso A, et al. HLA class II genotype influences the type of liver injury in drug-induced idiosyncratic liver disease. *Hepatology.* 2004;39(6):1603-1612.
132. Nicoletti P, Aithal GP, Bjornsson ES, et al. Association of liver injury from specific drugs, or groups of drugs, with polymorphisms in HLA and other genes in a genome-wide association study. *Gastroenterology.* 2017;152(5):1078-1089.
133. Stephens C, López-Nevot M-Á, Ruiz-Cabello F, et al. HLA alleles influence the clinical signature of amoxicillin-clavulanate hepatotoxicity. *PloS one.* 2013;8(7):e68111.

This article is protected by copyright. All rights reserved

134. Tekaia F, Yeramian E, Dujon B. Amino acid composition of genomes, lifestyles of organisms, and evolutionary trends: a global picture with correspondence analysis. *Gene.* 2002;297(1-2):51-60.
135. Lukacs GL, Chang X-B, Bear C, et al. The delta F508 mutation decreases the stability of cystic fibrosis transmembrane conductance regulator in the plasma membrane. Determination of functional half-lives on transfected cells. *Journal of Biological Chemistry.* 1993;268(29):21592-21598.
136. Oliver A, Cantón R, Campo P, Baquero F, Blázquez J. High frequency of hypermutable Pseudomonas aeruginosa in cystic fibrosis lung infection. *Science.* 2000;288(5469):1251-1253.
137. Kuti JL, Pettit RS, Neu N, et al. Microbiological activity of ceftolozane/tazobactam, ceftazidime, meropenem, and piperacillin/tazobactam against Pseudomonas aeruginosa isolated from children with cystic fibrosis. *Diagnostic microbiology and infectious disease.* 2015;83(1):53-55.
138. Roehmel JF, Schwarz C, Mehl A, Stock P, Staab D. Hypersensitivity to antibiotics in patients with cystic fibrosis. *Journal of Cystic Fibrosis.* 2014;13(2):205-211.
139. Burrows JA, Nissen LM, Kirkpatrick CM, Bell SC. Beta-lactam allergy in adults with cystic fibrosis. *Journal of Cystic Fibrosis.* 2007;6(4):297-303.
140. Kim TI, Jeong KH, Shin MK, Kim NI. Piperacillin/tazobactam-associated hypersensitivity syndrome with overlapping features of acute generalized exanthematous pustulosis and drug-related rash with eosinophilia and systemic symptoms syndrome. *Annals of dermatology.* 2016;28(1):98-101.
141. Mathews N, Zayed S, Winters N, et al. Evaluation of the safety of piperacillin/tazobactam use in admitted pediatric patients with cystic fibrosis. *Official Journal of the Association of Medical Microbiology and Infectious Disease Canada.* 2018;3(2):93-99.
142. Parmar J, Nasser S. Antibiotic allergy in cystic fibrosis. *Thorax.* 2005;60(6):517-520.
143. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T H-17, T H 1 and T H 2 cells. *Nature immunology.* 2009;10(8):864.
144. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nature immunology.* 2011;12(5):383.
145. Cavani A, Pennino D, Eyerich K. Th17 and Th22 in skin allergy. In: *New Trends in Allergy and Atopic Eczema.* Vol 96. Karger Publishers; 2012:39-44.
146. Zheng Y, Danilenko DM, Valdez P, et al. Interleukin-22, a TH 17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature.* 2007;445(7128):648-651.

Accepted Article

This article is protected by copyright. All rights reserved

147. Legere III HJ, Palis RI, Bouza TR, Uluer AZ, Castells MC. A safe protocol for rapid desensitization in patients with cystic fibrosis and antibiotic hypersensitivity. *Journal of Cystic Fibrosis.* 2009;8(6):418-424.
148. Whitaker P, Shaw N, Gooi J, Etherington C, Conway S, Peckham D. Rapid desensitization for non-immediate reactions in patients with cystic fibrosis. *Journal of Cystic Fibrosis.* 2011;10(4):282-285.
149. Stone Jr CA, Trubiano J, Coleman DT, Rukasin CR, Phillips EJ. The challenge of de-labeling penicillin allergy. *Allergy.* 2019.
150. Gibson A, Faulkner L, Lichtenfels M, et al. The effect of inhibitory signals on the priming of drug hapten–specific T cells that express distinct Vβ receptors. *The Journal of Immunology.* 2017;199(4):1223-1237.
151. Pan R-Y, Chu M-T, Wang C-W, et al. Identification of drug-specific public TCR driving severe cutaneous adverse reactions. *Nature communications.* 2019;10(1):1-13.
152. Ko T-M, Chung W-H, Wei C-Y, et al. Shared and restricted T-cell receptor use is crucial for carbamazepine-induced Stevens-Johnson syndrome. *Journal of Allergy and Clinical Immunology.* 2011;128(6):1266-1276. e1211.
153. Rozieres A, Hennino A, Rodet K, et al. Detection and quantification of drug-specific T cells in penicillin allergy. *Allergy.* 2009;64(4):534-542.
154. Renn CN, Straff W, Dorfmüller A, Al-Masaoudi T, Merk H, Sachs B. Amoxicillin-induced exanthema in young adults with infectious mononucleosis: demonstration of drug-specific lymphocyte reactivity. *British Journal of Dermatology.* 2002;147(6):1166-1170.
155. Mauri-Hellweg D, Zanni M, Frei E, et al. Cross-reactivity of T cell lines and clones to beta-lactam antibiotics. *The Journal of Immunology.* 1996;157(3):1071-1079.
156. Bell S, Pichler W. Penicillin-allergic patients react to penicillin-modified “self”. *Allergy.* 1989;44(3):199-203.
157. Yoshimura T, Hamaguchi E, Usami E, et al. Increased in vitro release of interferon-gamma from ampicillin-stimulated peripheral blood mononuclear cells in Stevens–Johnson syndrome. *Biological and Pharmaceutical Bulletin.* 2004;27(6):929-931.
158. Nicoletti P, Aithal GP, Chamberlain TC, et al. Drug-Induced Liver Injury due to Flucloxacillin: Relevance of Multiple Human Leukocyte Antigen Alleles. *Clinical Pharmacology & Therapeutics.* 2019;106(1):245-253.
159. Guéant J-L, Romano A, Cornejo-Garcia J-A, et al. HLA-DRA variants predict penicillin allergy in genome-wide fine-mapping genotyping. *Journal of Allergy and Clinical Immunology.* 2015;135(1):253-259. e210.

This article is protected by copyright. All rights reserved

160. Hautekeete ML, Horsmans Y, van Waeyenberge C, et al. HLA association of amoxicillin-clavulanate–induced hepatitis. *Gastroenterology.* 1999;117(5):1181-1186.
161. Horton H, Weston S, Hewitt C. Allergy to antibiotics: T-cell recognition of amoxicillin is HLA-DR restricted and does not require antigen processing. *Allergy.* 1998;53(1):83-88.

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

TABLES

Table 1. Hypersensitivity reaction manifestations and immune mediators. (Adapted from Pichler 2003 (10)).

Table 2. Workflow for detection and characterisation of penicillin-specific T cells.

Table 3. Immunoprofiling of penicillin-specific T cells.

Table 4. Penicillin modification sites identified through mass spectrometry analysis of trypsin digested HSA after *in vitro* incubation or *ex vivo* from patient serum.

Table 5. Reported associations between penicillin-induced ADRs and HLA genes.

FIGURE LEGENDS

Figure 1: Molecular structures of penicillins. a) The core structure of penicillin, also known as a penam, consists of a β-lactam ring fused with a thiazolidine ring that resembles b) D-Ala-D-Ala, the natural substrate of penicillin-binding protein. c) The penam forms the core of various penicillins extracted from the list of essential medicine curated by the World Health Organisation (WHO) (1).

Figure 2: Schematic representation of the generation of HLA-peptide complexes. Whilst penicillins, such as benzylpenicillin (pictured), can haptenate preformed HLA class I (1a) or class II (2a)-peptide complexes directly on the cell surface to induce T cell activation, intracellular (1b) and extracellular (2b) haptenation of proteins can also occur. Intracellular proteins that undergo proteasomal degradation (1c) typically furnish HLA class I ligands, which are transferred into the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP) (1d) for loading onto HLA class I molecules. Novel peptides presented by HLA class I can invoke $CD8^+$ T cell activation (1e). On the other hand, proteins haptenated within the extracellular milieu can be phagocytosed and degraded in the endosomal compartment (2c) for HLA class II loading. Novel peptides traversing this pathway can invoke $CD4^+$ T cell activation. More recently, it has been shown that intracellularly haptenated proteins (3a) can be exported in exosomes (3b) to target cells providing a novel source of haptenated peptides. Furthermore, flucloxacillin is also proposed to interact with pHLA in a labile fashion (p.i. concept) (4).

This article is protected by copyright. All rights reserved

Table 1. Hypersensitivity reaction manifestations and immune mediators. (Adapted from Pichler 2003 (10)).

Reaction		Symptom/Diagnosis	Immune Mediators	Cell Type
Type I		Urticaria, anaphylaxis	IgE	B cells
Type II		Blood cell dyscrasia	IgG and Fc receptor	
Type III		Vasculitis	IgG, complement proteins or Fc Receptor	
Type IV	a	Eczema, dermatitis, MPE	IFNγ, TNF	T cells
	b	DRESS, MPE	IL-4, IL-5, IL-13, eotaxins	
	c	DILI, SJS/TEN, MPE	Granzyme B, perforin, Fas ligand	
	d	AGEP	GM-CSF, IL-8 (CXCL8)	

AGEP, acute generalized exanthematous pustulosis; DRESS, drug reaction with eosinophilia and systemic symptoms; DILI, drug-induced liver injury; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFNγ, interferon-γ; Ig, immunoglobulin; IL, interleukin; MPE, maculopapular exanthema; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis; TNF, tumour necrosis factor.

This article is protected by copyright. All rights reserved

Table 2. Workflow for detection and characterisation of penicillin-specific T cells.

Assay type	Description	References
Diagnostic evaluation of drug-specific T cells	LTT	(24,27,28,30,34,37,39,50-57,59,62,76,79,89,92,93,106-109,153-156)
	IFNγ ELISpot	(30,37,51,56,62,79,89,93,108,112,153)
In vivo tests	Drug provocation test, Intradermal test, Patch test, Skin prick test, Skin test	(36)
Generation of T cell lines or clones	Lines: Stimulation of PBMCs with drug	(30,34,37,39,50-52,54,56,62,79,89,93,106-108,112,155)
	Clones: Serial dilution of penicillin-responsive T cells	(30,34,37,39,50-52,54,56,62,79,89,106,107,155)
Characterisation	Mechanism for penicillin-specific T cell activation • labile/covalent interaction • antigen processing requirements • HLA restriction	 (52,54,89,112) (54,93,108,112) (79,106)
	Immunophenotyping techniques • Flow cytometry • ELISA • ELISpot	 (30,34,59,79) (39,53,155) (39,51,112)

This article is protected by copyright. All rights reserved

	TCR repertoire • Vβ staining	(50,52,54,106,155)

ELISA, Enzyme-linked immunosorbent assay; ELISpot, Enzyme-linked immune absorbent spot; IFNγ, interferon-γ; LTT, Lymphocyte transformation test; PBMC, Peripheral blood mononuclear cell; TCR, T cell receptor

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Table 3. Immunoprofiling of penicillin-specific T cells.

Drug(s)	DHR	Cases	Onset time	Immunoassays	T cell subtypes	Immunoprofile	Reference
Amp	SJS	1	25 days	LTT with MTT		IFNγ	(157)
Amp, BP	Severe erythema multiforme, fever, malaise, blistering	2 delayed	5-7 days	Immunohistochemistry, FC analysis of lesional epidermis, LTT, proliferation assay, ELISA	$CD8^+ > CD4^+$	IL-2, IFNγ (CD8+ T cell clones, n=7). IL-4 (CD4+ T cell clone, n=1), HLA-DR	(34)
Amp, Piperacillin	MPE	2	1 day	Immunohistochemistry	$CD4^+ > CD8^+$	HLA-DR	(35)
Amp, AX, AC, BP	SJS	2	2-3 days	LTT	$CD4^+$		(109)
Amp, BP, Penicillin V	BP-hypersensitivity	4		LTT, proliferation assay, FC	$CD4^+ > CD8^+$		(106)
Amp, AX, BP, Flux	Exanthema, fever, oedema, urticaria	7		LTT, ELISA, FC	$CD4^+$ (62-90%), $CD8^+$ (10-38%)		(155)
AX	MPE, Lyell's syndrome	23 delayed (positive skin patch test)	6-48 hours	LTT, IFNγ ELISpot		IFNγ	(29)
	MPE	11 controls (negative skin patch test)					

This article is protected by copyright. All rights reserved

Accepted Article

	Anaphylaxis	15 immediate	<30 minutes				
AC	MPE	1	3 days	Scratch-patch, immunohistochemistry, LTT, ELISA, FC	$CD4^+/CD45RO^+$ (2/3), $CD8^+/CD45RO^+$ (1/3)	HLA-DR, CD45, perforin, IL-5, TNFα	(50)
AX, AC	MPE, angioedema	3	9-54 hours	Drug-provocation, immunohistochemistry, LTT, FC	$CD8^+$ (dominant during reaction), $CD4^+$	HLA-DR, CLA, granzyme B, CXCL9, CCL27, CXCR3, CCR10	(59)
AX	MPE	4	7-8 days	LTT			(154)
		10 healthy controls					
		5 tolerant controls					
AX, AC	AGEP,	2	4-36 hours	Immunohistochemistry, LTT, ELISA, ELISpot	$CD4^+$ (60-70%), $CD8^+$ (30-40%)	HLA-DR, CD25, IL-4, IL-5, IFNγ, RANTES (CCL5), GM-CSF, IL-8, CXCR1	(39)
	Non-AGEP (drug not specified)	4 controls					
AC	MPE, pneumonia, skin infection, arthritis	3	3-10 days	Immunohistochemistry, LTT		IL-5, eotaxin, RANTES (CCL5), MCP-3	(57)

This article is protected by copyright. All rights reserved

Accepted Article

		9 controls					
AX	4 MPE, 1 erythema exsudativum multiforme	5		LTT, 17- plex bead- based immunoassay (BioRad)		Baseline secretion: CCL2, CCL4, IL- 6 ($p<0.05$) PBMC stimulation: IL- 5, IL- 13, IFNγ, TNFα and GM- CSF	(55)
		5 controls					
AX, AC	MPE	13		Quantitative real-time PCR, immunohistochemistry	$CD4^+$, $CD8^+$	TNF, IFNγ, CCR6, CCL20, and CCL27; Skin: CXCR2, CXCL9 and CXCL10; Blood: CXCR3 Peripheral blood: CCR10 Skin: CCL20, CXCL9, CXCL10, CCL27	(38)
	MPE induced by non-penicillins	14					
		26 drug tolerant controls					

This article is protected by copyright. All rights reserved

Accepted Article

						Both: TNFα, IFNγ, perforin, granzyme B, CXCR3, CCR6	
AX, BP	DILI	1	4 weeks	LTT			(76)
AX, BP	Morbilliform exanthema	12	<2 weeks	LTT, proliferation assay			(27)
		6 controls					
AX, AC, Cloxacillin, Penicillin V	Exanthema	4	>6 hours	FC		CLA (median=20.4 vs 9.4) ($p<0.001$). HLA-DR	(63)
		10 healthy controls					
		8 tolerant controls					
AC	DILI	7	2-39 days	LTT, IFNγ ELISpot, FC	$CD4^+ > CD8^+ > CD4^+/CD8^+$	IFNγ, perforin, granzyme B, FasL, IL-17, IL-22, CCR4,CCR9, CXCR3	(79)
BP	Urticaria, angioedema, MPE, diarrhoea, fever, shortness of breath,	10		LTT, ELISA, FC	$CD4^+$ (16 clones) > $CD8^+$ (3 clones)	BP: IL-4, IL5 > IFNγ; BP-HSA: IFNγ > IL-4, IL-5; both: IL-2, IFNγ, TNFα	(54)

This article is protected by copyright. All rights reserved

Accepted Article

	generalised pruritus						
BP	Immediate facial swelling, erythematory rash	2	Immediate	LTT			(92)
		8 controls					
BP	Healthy	10		LTT, IFNγ ELISpot	$CD4^+/CD45RA^+$	IFNγ	(108)
BP	Anaphylaxis, exanthema, angioedema, Urticaria, dyspnoea, generalised pruritus, drug fever	5 immediate		LTT, IFNγ ELISpot	$CD4^+/CD45RA^+$	IFNγ	(93)
	Urticaria, drug fever, exanthema	2 delayed					
		33 controls					
BP, AX	Urticaria (2), angioedema, anaphylaxis (29)	31 immediate	<60 minutes	LTT			(28)
	urticaria (12), MPE (7)	19 delayed	9-48 hours				
		28 controls					

This article is protected by copyright. All rights reserved

Accepted Article

BP, Amp	MPE, immediate asthmatic reaction	4		LTT, proliferation assay ELISA, FC	$CD4^+$ (85-95%) > $CD8^+$ (5%-15%)	L-selectin	(53)
		10 controls					
BP, Amp	BP-hypersensitive	2		LTT, proliferation assay, ELISA, FC	$CD4^+$ (2 clones)	IL-4	(107)
BP	Exanthema, urticaria, anaphylaxis	6		LTT	$CD4^+$	L-selectin	(156)
		6 healthy controls					
BP		7 healthy		IFNγ ELISpot, IFNγ/granzyme- B fluorospot assay	$CD8^+$	IFNγ, granzyme B	(112)
Flux, AX, BP, Piperacillin	Flux-DILI	6 (4/6 HLA-B*57:01+)	3-4 weeks	LTT, IFNγ ELISpot, FC, chemotaxis assay	Patient: $CD8^+$ (35 clones) > $CD4^+$ (10 clones) Drug naïve: $CD8^+$	IFNγ, FasL, perforin, granzyme B, CCR2, CCR4, CCR9, CXCR3	(37)
		6 tolerant controls					
		3 HLA-B*57:01+ drug naïve					
Flux		9 Healthy, Flux-naïve, HLA-		LTT, IFNγ ELISpot, FC	$CD8^+$ > $CD4^+$	IFNγ, CD107a	(89)

This article is protected by copyright. All rights reserved

Accepted Article

		B*57:01+					
		8 Healthy, Flux-naïve, HLA-B*57:01- controls					
Piperacillin	PIP-hypersensitive	4		LTT, IFNγ ELISpot, FC, chemotaxis assay	Blood: 84.25% $CD4^+$, 15.75% $CD8^+$ Inflamed skin: 85.5% $CD4^+$, 14.5% $CD8^+$	IFNγ, IL-13, IL-22, granzyme B, CCR4, CCR5, CCR8, CCR10, CXCR3, CXCR6	(62)
		4 controls					
Piperacillin	MPE, fever, urticarial eruptions, flu-like symptoms	28	2-11 days	Immunohistochemistry, LTT, IFNγ ELISpot	$CD4^+ > CD8^+$	IFNγ, IL-13, gramzyme B, IL-1β, IL-6, TNF, MIP-1α	(56)
		9 tolerant controls					

AGEP, acute generalized exanthematous pustulosis; Amp, Ampicillin; AC, Amoxicillin and Clavulanic Acid; AX, Amoxicillin; BP, Benzylpenicillin; CCL, C-C chemokine ligand; CCR, C-C chemokine receptor; CLA, Cutaneous lymphocyte-associated antigen; CXCL, C-X-C chemokine ligand; CXCR, C-X-C chemokine receptor; DILI, drug-induced liver injury; ELISA, Enzyme-linked immunosorbent assay; ELISpot, Enzyme-linked immune absorbent spot; FasL, Fas ligand; FC, Flow cytometry; Flux, Flucloxacillin; GM-CSF, granulocyte-macrophage colony-

This article is protected by copyright. All rights reserved

Accepted Article

stimulating factor; HSA, human serum albumin; IFNγ, interferon-γ; IL, interleukin; LTT, lymphocyte transformation test; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory proteins; MPE, Maculopapular exanthema; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBMC, peripheral blood mononuclear cell; SJS, Stevens–Johnson syndrome; TNF, tumour necrosis factor

This article is protected by copyright. All rights reserved

Accepted Article

Table 4. Penicillin modification sites identified through mass spectrometry analysis of trypsin digested HSA after *in vitro* incubation or *ex vivo* from patient serum.

This article is protected by copyright. All rights reserved

Accepted Article

		Amoxicillin		Benzylpenicillin		Flucloxacillin		Piperacillin	
Lysine Position	**Peptide**	**Patient serum**	***In vitro***	**Patient serum**	***In vitro***	**Patient serum**	***In vitro***	**Patient serum**	***In vitro***
4	DAHK*SEVAHR								f
12	FK*DLGEENFK		b	d	d				f
137	K*YLYEIAR	b	b	d	d	e			f
159	RHPYFYAPELLFFAK*R			d	d				
162	YK*AAFTECCQAADK		b		d		e		f
190	LDELRDEGK*ASSAK	a,b	a,b, c	d	d	e	e	b, f	f
195	ASSAK*QR			d	d	e	e	b, f	f
199	LK*CASLQK	b	a, b, c	d	d	e	e		f
212	AFK*AWAVAR		b,	d	d	e	e		f
351	LAK*TYETTLEK	b	a, b, c	d	d	e	e		f
378	VFDEFK*PLVEEPQNLIK				d				
432	NLGK*VGSK	b	a, b, c	d	d	e	e	b, f	f
436	VGSK*CCK		b		d				
475	VTK*CCTESLVNR		b		d				
525	K*QTALVELVK	b	b	d	d	e	e		f
541	ATK*EQLK	b	a, b, c	d	d	e	e	b, f	f
545	EQLK*AVMDDFAAFVEK		a, c	d	d		e		f

This article is protected by copyright. All rights reserved

Accepted Article

This article is protected by copyright. All rights reserved

Accepted Article

Table 5. Reported associations between penicillin-induced ADRs and HLA genes.

Drug	ADR	Type of study - (Subclass)	Gene association		OR	P-value	PPV (%)	NPV (%)	Reference
			HLA	SNP					
Flux	DILI	Patient HLA genotyping	B*57:01		80.6	9.0×10^{-19}	0.12	99.99	(124)
		GWAS	B*57:01	rs2395029	45	8.7×10^{-33}			
Flux	DILI	Patient HLA genotyping	B*57:01		36.62	$2.67\text{x}10^{-97}$			(158)
			B*57:03		19.77	0.001			
		GWAS	B*57:01	rs2395029	35.48	$5.7\text{x}10^{-97}$			
Flux	DILI	Mechanistic	B*57:01/B*58:01			8.97×10^{19}			(37)
BP, AX	Anaphylaxis	GWAS - Spanish	DRA/DRB5	rs7754768	0.64	1.6×10^{-6}			(159)
				rs9268832	0.64	4.9×10^{-6}			
				rs7192	0.65	7.4×10^{-6}			
				rs2227139	0.65	7.7×10^{-6}			
				rs2213586	0.66	1.2×10^{-5}			

This article is protected by copyright. All rights reserved

Accepted Article

				rs2213585	0.66	1.4×10^{-5}			
			DRA	rs7195	0.66	1.7×10^{-5}			
				rs8084	0.68	1.7×10^{-5}			
AC	DILI	Patient HLA genotyping - Belgian	DRB1*15:01-DRB5*01:01-DQB1*06:02			<0.0005			(160)
			DRB1*15:01			<0.003			
			DRB5*01:01			<0.003			
			DQB1*06:02			<0.0005			
AC	DILI	GWAS - Combined	DRB1*15:01-DQB1*06:02	rs9274407	3.1	4.8×10^{-14}			(128)
				rs9267992	3.1	6.8×10^{-13}			
				rs3135388	2.8	3.5×10^{-11}			
			A*02:01	rs2523822	2.3	1.8×10^{-10}			
		Patient genotyping - Combined	DQB1*06:02		3.3	1.4×10^{-6}			
			A*02:01		2.2	2×10^{-6}			

This article is protected by copyright. All rights reserved

Accepted Article

AC	DILI	Patient HLA genotyping - Spanish	A*30:02		6.7	5×10^{-5}			(133)
			B*18:01		2.9	0.22			
			DRB1*15:01		2.6	0.06			
			DQB1*06:02		2.8	0.03			
			A*30:02-B*18:01		5.3	0.026			
			DRB1*15:01-DQB1*06:02		3	0.014			
AC	DILI	Mechanistic - European	A*02:01						(79)
			DRB1*15:01-DQB1*06:02						
AC	DILI	Patient HLA genotyping - European	DRB1*15		2.29	0.002			(129)
			DRB1*07 (protective)		0.18	0.0011			
AC	DILI	Patient HLA genotyping - Scottish	DRB1*15:01		9.25	2.5×10^{-6}			(130)
			DRB1*15:01-DRB5*01:01-DQA1*01:02-		35.54	$< 10^{-8}$			

This article is protected by copyright. All rights reserved

Accepted Article

			DQB1*06:02						
AC	DILI	Patient HLA genotyping - DILI - Spanish	DRB1*15			0.025			(131)
			DQB1*06			0.035			
			DRB1*07 (protective)			0.005			
			DQB1*02 (protective)			0.01			
		Patient HLA genotyping - Hepatocellular injury – Spanish	DRB1*15		2.31	0.002			
			DQB1*06		2.32	0.001			
			DRB1*07 (protective)		0.37	0.003			
			DQB1*02 (protective)		0.39	0.003			
		Patient HLA genotyping - Jaundice - Spanish	DRB1*15			0.009			
			DQB1*06			0.009			
			DRB1*07 (protective)			0.006			
			DQB1*02 (protective)			0.01			
AX	Urticaria	Mechanistic - European,	DRB1*03:01						(161)
			DRB1*15:01/15:02						

This article is protected by copyright. All rights reserved

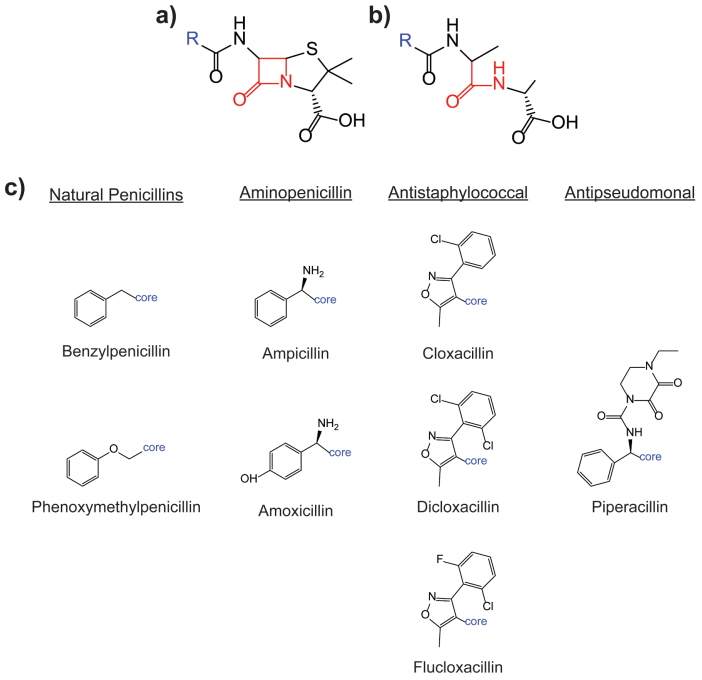
Accepted Article

		Leicester							
AX, Flux	DILI	GWAS - European	A*33:01	rs114577328	2.7	2.4×10^{-8}			(132)
			A*33:01	rs72631567	1	9.7×10^{-9}			

AX-Clav, Amoxicillin and clavulanic acid; AX, Amoxicillin; BP, Benzylpenicillin; DILI, Drug-induced liver injury; Flux, Flucloxacillin; GWAS, genome-wide association study; NPV, negative predictive value; OR, Odds ratio; PPV, positive predictive value; SNP, single nucleotide polymorphism. Note: SNPs are single nucleotide variants in an individual's genome then can be used as genetic markers when they are in linkage disequilibrium.

This article is protected by copyright. All rights reserved

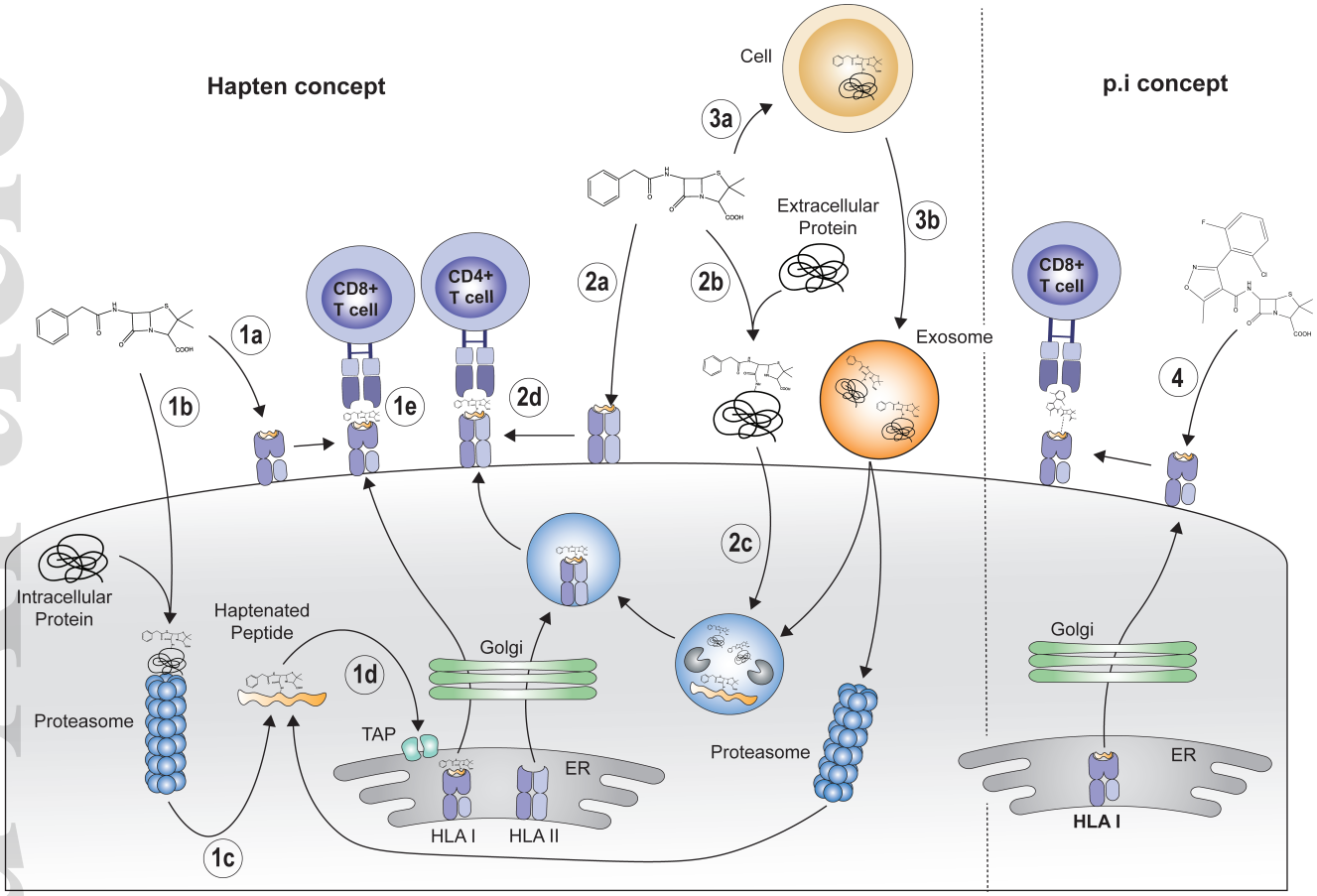
Accepted Article



all_14355_f1.tif

This article is protected by copyright. All rights reserved

Accepted Article



all_14355_f2.tif

This article is protected by copyright. All rights reserved