

Immunological aspects of nonimmediate reactions to β -lactam antibiotics

Expert Rev. Clin. Immunol. 6(5), 789–800 (2010)

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β -lactam antibiotics are the agents most frequently implied in immune drug adverse reactions. These can be classified as immediate or nonimmediate according to the time interval between the last drug administration and their onset. Mechanisms of immediate IgE-mediated reactions are widely studied and are therefore better understood. Nonimmediate reactions include a broad number of clinical entities like mild maculopapular exanthemas, the most common, and other less frequent but more severe reactions such as Stevens–Johnson syndrome, toxic epidermal necrolysis, acute exanthematic pustulosis or cytopenias. These nonimmediate reactions are mainly mediated by T cells but the precise underlying mechanisms are not well elucidated. This fact complicates the allergological evaluation of patients with this type of reaction and available tests have demonstrated poor sensitivity and specificity.

KEYWORDS: acute generalized exanthematous pustulosis • aminopenicillins • β -lactam antibiotics • cephalosporins • contact dermatitis • DRESS • fixed drug eruption • maculopapular exanthema • nonimmediate reactions • penicillins • Stevens–Johnson syndrome • T cell • toxic epidermal necrolysis

Drug adverse reactions constitute a public health problem because of their frequent occurrence, occasional severity and their impact on the use of medications [1]. The most frequent drug adverse reactions result from mechanisms that do not involve an immunological process. Despite this, allergy reactions account for a third of all adverse drug reactions [2] and include some of the more severe and potentially fatal occurrences. β -lactam antibiotics are the most widely prescribed antibiotics around the world and are also the most frequently involved drugs in immune adverse drug reactions.

According to Coombs and Gell [3], allergic reactions can be classified into four types: type I immediate or IgE-mediated hypersensitivity reactions; type II or cytotoxic reactions; type III or immune complex-mediated reactions; and type IV delayed-type hypersensitivity reactions or reactions mediated by drug-specific T lymphocytes. In 1966, Levine classified allergic reactions to β -lactam antibiotics into immediate, accelerated and delayed reactions [4]. Immediate reactions are those reactions that occur within 60 min after drug administration and are mainly IgE-mediated. Accelerated reactions develop between 1 and 24 h after drug intake and delayed reactions

appear after 24 h post-drug intake. This classification has since been simplified into immediate or IgE-mediated reactions and nonimmediate reactions. These reactions are mainly mediated by T cells [5] and this group covers a broad number of clinical entities. Among them, the most common are maculopapular exanthemas (MPEs), in addition to urticaria and other less common but more severe entities such as drug rash with eosinophilia and systemic symptoms (DRESS), and Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) or acute generalized exanthematous pustulosis (AGEP) [6]. Fixed drug eruption (FDE) is a unique clinical entity characterized by recurrent well-defined lesions that appear in the same location each time the responsible drug is taken. This entity can sometimes be greatly extended into the skin and become a serious disease [7]. Drug-induced contact dermatitis is another type of delayed reaction characterized by eczematous lesions due to contact with the responsible drug. β -lactam antibiotics induce contact dermatitis in an occupational setting that affects exposed persons working in the pharmaceutical industry or healthcare workers [8]. Finally, β -lactam antibiotics can cause blood alterations and other organ-specific

reactions like interstitial nephritis, pneumonitis and hepatitis. Serum sickness-like reactions have also been reported, mostly due to cefaclor [9].

Maculopapular exanthema is the most common of all the aforementioned entities. Although any β -lactam antibiotic can produce MPEs, aminopenicillins are the more frequently involved agents [10–14]. The real prevalence of these reactions is unknown, with differences between countries due probably to the great variation in antibiotic consumption between different countries [15].

The mechanisms of the immediate reactions have been most intensively studied and are therefore better known. In this article, we will not discuss the mechanisms of immediate hypersensitivity; instead, we will focus on delayed or nonimmediate reactions.

Immunogenicity of β -lactam antibiotics

Most drugs have a molecular mass of less than 1000 Da and are considered incapable of inducing an immune response in their native state. For these agents to become effective immunogens, they must bind covalently to high-molecular-weight proteins, thereby forming a hapten–carrier complex, which then undergoes processing and presentation. Penicillins are chemically reactive drugs as their molecular structure allows spontaneous covalent binding to proteins. T cells recognize a hapten peptide fragment that is generated by cellular uptake and processing of the hapten–carrier complex and ulterior presentation to T cells in the presence of major histocompatibility complex (MHC) [16]. Alternatively, the hapten can bind directly to the immunogenic peptide presented by the MHC molecule itself or alter the MHC molecule directly without requiring processing [16,17].

In nonimmediate reactions, both the core structure of β -lactam antibiotics and the whole molecule are recognized by T cells [18,19]. *In vitro* analysis of β -lactam reactivity of T cells from different donors showed two patterns: one group of patients that had a restricted specificity, as their T-cell lines proliferated only when incubated with penicillin G, but not with other β -lactam antibiotics including cephalosporins, even if the side chain was identical. The second group included patients with more broadly reactive T cells. These proliferated when incubated with penicillin but also with amoxicillin or ampicillin. These findings imply that some T cells recognize or tend to recognize the whole molecule, and not a part of it, while other T cells mainly recognize the core structure and possibly part of the side chain; exclusive side chain reactivity seems to be rare [20]. This is in contrast to IgE-mediated reactions, as IgE might react quite exclusively with the side chain [21]. Nevertheless, it is noteworthy that *in vivo* the aminobenzyl group plays a predominant role, because an important percentage of patients display a selective response to aminopenicillins in intradermal and/or patch tests with good tolerance to both benzylpenicillin [22] and cephalosporins [23]. *In vitro*, it has been observed that the same side chain presented in a cephalosporin (e.g., cephadroxil) is not recognized by T cells, which could be due to the fact that T cells must recognize the whole molecule or the core with part of the side chain in order to proliferate [19]. Thus, cross-reactivity between cephalosporins and penicillins seems to be rare for T-cell-mediated reactions.

Classification & immunopathological mechanisms involved in nonimmediate reactions

Most nonimmediate reactions correspond to T-cell-mediated type IV reactions of the classical Gell and Coombs classification. This classification has been revised and T-cell-mediated type IV reactions have been further subdivided into IVa–IVd reactions as proposed by Pichler (FIGURE 1) [24]. This classification of type IV reactions is based on the effector cells involved (monocytes, eosinophils, T cells and neutrophils). However, in most reactions, different type IV reactions may occur together; for example, cytotoxic T cells and monocyte/macrophage activation contribute to the bullous exanthema.

According to this subclassification, type IV reactions include:

- Type IVa reactions that correspond to Th1 immune reactions. Th1-type T cells activate macrophages by secreting large amounts of IFN- γ , drive the production of complement-fixing antibody isotypes (IgG1 and IgG3), and are costimulatory for proinflammatory responses (TNF and IL-12) and CD8⁺ T-cell responses. An example of this type of reaction would be skin test to tuberculin or granuloma in sarcoidosis;
- Type IVb reactions that correspond to Th2-type immune response. Th2 cells secrete IL-4, IL-13 and IL-5 cytokines, which induce B cells to produce antibodies, mast cell response and eosinophilic inflammation, which is characteristic of eosinophil MPE or DRESS;
- Type IVc reactions, which occur in most drug-induced nonimmediate hypersensitivity reactions, mostly together with other type IV reactions. Type IVc reactions appear to be predominant in bullous skin reactions, such as SJS and TEN, where activated CD8⁺ T cells kill keratinocytes. Drug-specific CD8⁺ cytotoxic T cells utilizing perforin/granzyme B trigger keratinocyte apoptosis. Subsequently, there may be an expansion of apoptosis involving the interaction of Fas and Fas ligand (FasL);
- Type IVd reactions, in which T cells lead to the recruitment and activation of neutrophils, whose products initiate tissue damage. A typical example is AGEP. In this disease, CXCL8- and GM-CSF-producing T cells recruit neutrophilic leukocytes via CXCL8 release and prevent their apoptosis via GM-CSF release.

Pathophysiology

Most nonimmediate reactions to β -lactam antibiotics are MPEs with a mild clinical course. However, although infrequent, this class of antibiotics can produce more severe reactions like DRESS or SJS/TEN. β -lactam antibiotics are also the most frequent agents implied in the rare entity AGEP. In all forms of nonimmediate drug reactions, cytotoxic mechanisms seem to play an important role. The clinical picture is determined by the strength of cytotoxicity, the generation of cytotoxic CD8⁺ versus CD4⁺ cells, and the type of associated effector mechanism and cell type (monocyte/macrophage, eosinophils or neutrophils). Functional analysis of T-cell clones from peripheral blood and from affected tissues shows different types of T-cell reactions that lead to different forms of drug-induced hypersensitivity [25,26].

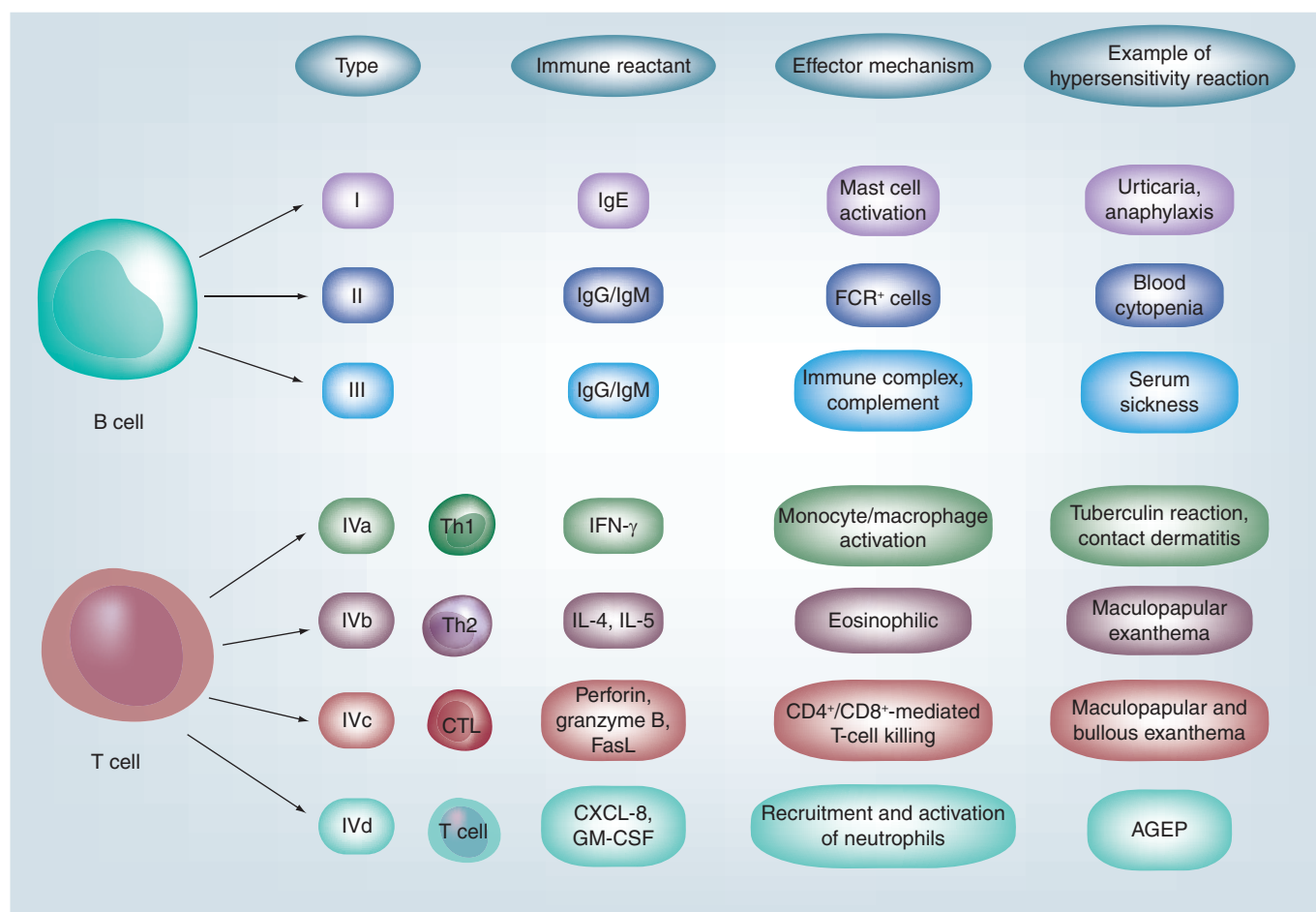


Figure 1. Revised Gell and Coombs classification of drug reactions.

AGEP: Acute generalized exanthematous pustulosis; CTL: Cytotoxic T lymphocyte; CXCL: Chemokine chemoattractant IL-8; FasL: Fas ligand; FCR: Fc receptor.

Adapted from [24].

Maculopapular exanthema

As mentioned previously, maculopapular reactions are the most frequent nonimmediate reactions caused by β -lactam antibiotics, particularly during treatment with amoxicillin [13,27,28]. The eruption usually occurs between 4 and 14 days after initiation of therapy and clinical manifestations are variable, with a range from macules to widespread erythematous and maculopapular lesions, which usually begin on the trunk, neck and upper extremities. The picture resembles viral or bacterial exanthemas and can adopt a morbilliform, rubelliform or scarlatiniform aspect.

Maculopapular exanthema is now classified as a subtype of type IV cell-mediated hypersensitivity reactions [24].

The T-cell subset responsible for MPE is still a matter of debate. Immunohistological studies of skin lesions as well as studies based on peripheral blood T cells indicate that drug-specific cytotoxic T cells are the dominant effector cells in these drug eruptions. Hertl *et al.* found that T-cell clones derived from peripheral blood lymphocytes of allergic patients with MPEs were CD3⁺CD8⁺, producing the Th1 cytokines IL-2 and IFN- γ [17]. Cutaneous T cells were identified mainly as CD8⁺ and, when they were expanded *in vitro*, they expressed cytolytic activity against

epidermal keratinocytes and autologous B cells, indicating that the skin was the target organ for CD8⁺ cells [25]. Recent studies confirmed that CD8⁺ T cells are a major player in MPE since drug-specific CD8⁺ cytotoxic T cells were found in the blood of most patients who had developed a MPE induced by β -lactam antibiotics [26]. Others authors have found that the majority of specific T lymphocytes isolated from peripheral blood and skin lesions show a CD4⁺ phenotype [29–33]. These CD4⁺ T cells are mainly located in the perivascular dermis. Together with some CD8⁺ T cells, these CD4⁺ T cells are also found in the epidermis and along the dermoepidermal junction zone [30]. These infiltrating T cells appear highly activated, and express CD25, HLA-DR and adhesion molecules like L-selectin. Up to 20% of the infiltrating T cells in drug-induced MPE express perforin and granzyme B [30]. In MPEs, perforin and granzyme B are observed in both CD4⁺ and CD8⁺ T cells, but not FasL [30,34]. Although cytotoxic CD8⁺ T cells may also collaborate in inducing keratinocyte cell damage, their preferential activation may lead to more severe reactions like SJS or TEN [25]. In addition to T cells, other cells including dendritic cells, macrophages and eosinophils are also observed in the cell infiltrate. Studies with dendritic cells from patients suffering

from nonimmediate reactions to amoxicillin have shown that these cells were able to present the drug to autologous T lymphocytes, inducing a specific response [35].

T cells and other infiltrating cells may also contribute to cutaneous inflammation through both the release and the induction of production of different cytokines and chemokines. Studies of cytokine expression have shown heterogeneous results, with some authors reporting results indicating a Th1 pattern (e.g., IFN- γ and TNF- α) [30,36–39] whereas others found a Th2 pattern (e.g., IL-5) [40]. An increased expression of chemokines (e.g., CXCL9, CXCL10, CCL20 and CCL27) and chemokine receptors (e.g., CXCR3, CCR6 and CCR10) has also been found in this type of reaction [30,39,41]. CCL27–CCR10 interactions have been reported to play a role in the recruitment of CD4⁺ and CD8⁺ T cells in MPE and particularly in SJS and TEN [41]. IL-5 together with chemokines including eotaxin can be involved in the recruitment, growth and differentiation of eosinophils.

Viral infections are well-recognized cofactors that participate in the elicitation of MPEs due to β -lactam antibiotics. Between 70 and 100% of patients receiving aminopenicillins during an Epstein–Barr virus (EBV) infection develop a MPE. This phenomenon may also occur with herpes simplex virus, cytomegalovirus, HIV and probably many other viral infections. Mechanisms suspected in the pathogenesis of antibiotic-induced viral eruptions include breakdown of immune tolerance, enhancement of the immune reaction and/or altered drug metabolism [42–45]. In many cases, EBV-associated amoxicillin exanthema is reversible and patients tolerate the antibiotic after they have recovered from the reaction. However, in other patients, re-exposure can induce the reaction again [46].

Acute generalized exanthematous pustulosis

The term AGEF was first introduced in 1980 to describe aseptic generalized pustular eruptions in the setting of infections or following administration of medications [47]. AGEF typically presents as a pustular rash with a diffuse, often acute, edematous erythema that arises predominantly in intertriginous areas or on the face. AGEF primarily needs to be differentiated from pustular psoriasis. Because the pustules are clinically indistinguishable, histopathologic analysis can be helpful.

Antibiotics, principally aminopenicillins [48–51], but also ceftriaxone [52], cefotaxime [53], piperacillin [54], macrolides [50], clindamycin [55] and levofloxacin [56], are the most common causative agents. In the recent EuroSCAR study, pristinamycin and amoxicillin were the most frequently implicated drugs [57].

A role for drug-specific T cells in the pathogenesis of AGEF has recently been proposed based on the finding that these cells produce high amounts of the neutrophil-attracting chemokine IL-8, suggesting that they constitute a subgroup of T cells that differs from the usual Th1 and Th2 subgroups [58]. As well as other delayed drug reactions, drug-specific cytotoxic CD4⁺ and CD8⁺ T cells play an important role in mediating keratinocyte killing by perforin/granzyme B and the Fas/FasL-mediated process that leads to vesicle formation. They also produce large amounts of CXCL8, which stimulates activation and recruitment

of polymorphonuclear neutrophils, which fill the vesicles and turn them into pustules [59,60].

Drug-induced hypersensitivity syndrome or DRESS

Drug reaction with eosinophilia and systemic symptoms is another entity in which a latent herpesviridae infection (e.g., human herpes virus 6 [HHV-6]) is suspected to play a role in the pathophysiology. It is a life-threatening multi-organ reaction characterized by rash, fever, lymphadenopathy, hepatitis and leukocytosis with eosinophilia. The main causative drugs are anticonvulsants, allopurinol, minocycline, sulfonamides and the antiretroviral abacavir. β -lactam antibiotics are not frequently implicated agents and only a few reports of DRESS due to β -lactam derivatives have been published [61–65].

Patients with this syndrome have a massive immune stimulation with many activated T cells in the circulation. T cells can secrete large amounts of IL-5 or IFN- γ [66,67]. A peculiar feature of this syndrome is its long-lasting clinical course despite withdrawal of the causative drug. There may also be persistent intolerance to other chemically distinct drugs [63]. Reactivation of HHV-6, cytomegalovirus and EBV, most often detected serologically, has been implicated in its pathogenesis [68]. A viral reactivation can be found during the third or fourth week of the disease [69,70]. The drug-induced massive immune stimulation may somehow reactivate these herpes viruses, which subsequently replicate and possibly contribute to the chronic course and persistent drug intolerance. The virus-stimulated T cells show substantial cross-reactivity with certain drugs, which continues even after cessation of drug exposure [68].

The involvement of genetic factors in the pathogenesis of hypersensitivity syndrome has been demonstrated in abacavir hypersensitivity reactions [71–73]. A strong association between abacavir hypersensitivity syndrome and HLA-B*5701 alleles has been found in Caucasians but not in African populations [72,73]. Randomized controlled trials have demonstrated the effectiveness of prospective screening of the predisposing genetic marker in the prevention of hypersensitivity reactions to abacavir [74,75].

SJS & TEN

Stevens–Johnson syndrome and TEN form a spectrum of rare, but severe and potentially fatal, mucocutaneous diseases, characterized by widespread epidermal necrosis and mucosal involvement secondary to keratinocyte apoptosis. SJS and TEN are considered to be opposite spectrums of essentially the same disease, and are classified according to the extent of detached and detachable epidermis (FIGURE 2). In SJS, epidermal loss affects less than 10% of total body surface area, whereas TEN involves more than 30% of the body surface area. The majority of cases of SJS and TEN involve a specific response to drugs, constituting a form of delayed or nonimmediate hypersensitivity. More than 100 drugs have been associated with SJS and TEN, but those most frequently implicated are sulfonamides, anticonvulsants, NSAIDs, allopurinol and nevirapine [76–77]. Although β -lactam antibiotics are not frequently implicated drugs, several cases of SJS and TEN have been described with amoxicillin and other β -lactam antibiotics [78–82].

Although drug-specific T cells are implicated in these processes, our understanding of the immunopathology is not complete. It has been well established that the epidermal damage in these diseases is due to keratinocyte apoptosis. SJS and TEN fit with the type IVc mechanism of hypersensitivity. Skin is infiltrated by CD8⁺ T cells that predominate within the epidermis with an activated cytotoxic cell phenotype [83]. Over the course of the drug reaction, the T-cell expression of cutaneous lymphocyte-associated antigen rises and falls in parallel with disease severity, facilitating lymphocyte traffic to the skin. There is also a corresponding increase in E-selectin expression (the ligand of cutaneous lymphocyte-associated antigen) on cutaneous endothelial cells. TNF- α and IFN- γ are also strongly expressed by peripheral blood mononuclear cells early in the course of the reaction [84].

The overriding pathological finding in SJS and TEN is dermal cell apoptosis, which can be triggered by at least four pathways: Fas and FasL, TNF- α , TNF- α -related apoptosis-inducing ligand (TRAIL) and granzyme B. Levels of perforin, granzyme B and FasL have been shown to correspond to disease severity in delayed reactions to drugs [37]. Epidermal cells in the lesions of TEN overexpress FasL and are capable of killing Jurkat cells (cells from a lymphocyte line highly sensitive to FasL-mediated apoptosis). Elevated concentrations of FasL are present in the blood and blister fluid of patients with SJS or TEN. Since normal keratinocytes are known to express Fas, it was suggested that the coexpression of the death receptor and its ligand led to the 'collective suicide' of epidermal cells [85]. However, data on the expression of FasL on the surface of keratinocytes in SJS and TEN are somewhat controversial, with some authors unable to detect any expression in either SJS/TEN patient or control samples [86,87], whereas others report strong FasL expression on keratinocytes in biopsy samples of TEN patients, but not in those of healthy controls [85,88]. Increased serum soluble FasL concentration was demonstrated in patients with TEN and SJS compared with patients with drug-induced erythema multiforme and with healthy controls [87]. In a recent study, the soluble FasL from the sera of patients with SJS/TEN was significantly increased before the development of skin detachment, mucosal lesions or both. The authors suggest that the soluble FasL levels might be a useful marker to predict the onset of SJS/TEN [89].

Lesional skin from patients with SJS and TEN overexpresses TNF- α , IFN- γ , IL-2, IL-5, IL-13, CCR3, CXCR3 and CXCR4 [90].

Differential gene expression has been found in drug hypersensitivity reactions, depending on their severity. In the acute phase of SJS/TEN, overexpression of several alarmin genes has been found in peripheral blood mononuclear cells [91], therefore alarmins might play a role in the pathogenesis of these bullous diseases.

Recent studies have identified another cytotoxic molecule, granulysin, a cationic cytolytic protein primarily released by cytotoxic T cells and natural killer cells [92]. It has been suggested



Figure 2. Patient with a toxic epidermal necrolysis.

that granulysin can play an important role in inducing keratinocyte death in patients with SJS and TEN [92]. Granulysin in blister fluids has been found to correlate with the clinical severity of the disease [92]. In addition, serum granulysin levels are elevated during the early phases of SJS/TEN and it has been suggested that granulysin might enable early diagnosis of these entities [93].

There is a strong genetic association between HLA-B alleles and SJS/TEN to certain specific drugs and ethnicities. Studies in Han Chinese populations showed that the HLA-B*1502 allele is strongly associated with carbamazepine-induced SJS/TEN [94–95] whereas HLA-B*5801 is linked to allopurinol-induced reactions [96]. This strong association suggests a direct involvement of HLA molecules in the pathogenesis of the disease. This association between HLA-B*1502 and carbamazepine-induced SJS/TEN could not be demonstrated in Caucasians [97].

Fixed drug eruption

Fixed drug eruption usually appears as a solitary or a small number of pruritic, well-circumscribed, erythematous macules that evolve into edematous plaques; these lesions typically resolve after discontinuation of the offending drug, leaving hyperpigmentation at the site of lesions. The appearance of these lesions is often preceded and accompanied by a burning sensation. The lesions recur in exactly the same sites when rechallenged with each administration of offending drug. This entity can sometimes be greatly extended in the skin and become a serious disease [7]. Although β -lactam antibiotics may be causal agents, the most common drugs associated with FDE are sulfonamides, tetracyclines and NSAIDs [7].

Intraepidermal CD8⁺ T cells are likely to be involved in the pathogenesis of FDE; CD8⁺ T cells with cytotoxic potential can persist among basal keratinocytes in the skin lesion zone over prolonged periods of time after resolution. Even when resting, these cells express early activation markers (CD69) and a few hours after drug readministration they produce large amounts of IFN- γ , prior to the onset of apoptosis of epidermal cells [98]. In addition to producing IFN- γ , intradermal CD8⁺ T cells might also contribute to the development of FDE lesions by interacting directly with other inflammatory cells [99]. Thus, severe epidermal damage observed in the late stage of FDE lesion would be caused not only by the production of an early burst of IFN- γ from intraepidermal T cells but also by perforin, granzyme B and other cytokines produced secondarily by the rapid release of IFN- γ [7]. Fas–FasL interactions have also been shown to be

involved in the development of FDE lesions [100]. Recent studies have shown that CD8⁺ T cells identified in the blister fluid from patients with TEN share some phenotypic features with intraepidermal T cells residing in FDE lesions [83]. At present, the factors that determine whether patients have self-limited FDE or subsequently progress to TEN are unknown. In this regard, it should be kept in mind that the presence of intradermal CD8⁺ T cells was diluted by the prominent influx of CD4⁺ T cells (FoxP3⁺ and FoxP3⁻) into the epidermis in 1-day-old FDE lesions, whereas only low numbers of these cells were identified in the established lesions of TEN [83]. Although these studies were not performed with β -lactam antibiotics, a reasonable extrapolation can still be made.

Other nonimmediate reactions

β -lactam-induced contact dermatitis is another type of delayed reaction characterized by eczematous lesions caused by contact with the responsible drug, often in an occupational setting [8,101]. Unlike the reaction with aminopenicillins, which are the most common systemically administered drugs, in contact eczema, any β -lactam antibiotics responsible for occupational exposure can induce a reaction. Mechanisms proposed for contact dermatitis include a combination of IVa and IVc-type reactions [24].

Drug-induced hepatitis, nephritis and interstitial lung disease are other forms of immunologic nonimmediate reactions. Sometimes, eosinophilia helps to distinguish a drug reaction from other diseases and suggests a T-cell-mediated process, since these cells are the main source of the eosinophil-stimulating cytokine IL-5 [67].

Drug-induced cytopenias, including thrombocytopenia, neutropenia and anemia, are often associated with an immune-related increase in blood cell destruction. The mechanism for this varies and can involve direct antidrug antibodies, antibodies that are directed against the drug complexed with other proteins, or antibodies against a component of the blood cell independent of drug. The most common drugs associated with drug-induced hemolytic anemia in the 1970s were methyldopa and penicillin [102]; currently, cefotetan and ceftriaxone are the most frequently implicated drugs [103–107]. Antibodies react with red blood cells only in the presence of the drug and/or its metabolites, and predominantly cause complement-mediated intravascular hemolysis [108–109]. Usually, in penicillin-induced hemolytic anemia, the direct antiglobulin test is positive, whereas the indirect antiglobulin test is negative. Penicillin-induced thrombocytopenia may also occur [110] but immune neutropenia is rare.

The mechanism for serum sickness-like syndrome has been attributed to circulating immune complexes involving IgG or IgM antibodies with local deposition facilitated by the release of vasoactive amines, although this has yet to be demonstrated in humans [111].

In linear IgA dermatosis, autoantibodies against a 120-kDa soluble domain of bullous pemphigous antigen 180 (LAD-1) have been detected. The possible mechanism for this is that the drug may haptenize these proteins and produce neoantigens [112].

Evaluation of nonimmediate reactions to β -lactam antibiotics

In vivo diagnostic tests

Delayed intradermal tests and patch tests can be useful to diagnose nonimmediate reactions to β -lactam antibiotics, such as MPEs, TEN, DRESS syndrome, AGEP and generalized eczema [11,13,53,113–118]. The European Network for Drug Allergy has published recommendations to perform intradermal and patch testing in nonimmediate reactions to β -lactam antibiotics [5]. The penicilloyl-polylysine, minor determinant mixture (from Diater, Madrid, Spain, and only commercially available in Spain) and other soluble β -lactam antibiotics should be used. Readings in nonimmediate reactions should be performed after 48 and 96 h. In intradermal tests, any infiltrated erythema with a diameter above 5 mm is considered a positive reaction [5]. Patch-test reading should be made according to the European Environmental and Contact Dermatitis Research Group patch test classification (FIGURE 3) [119,120]. The evaluation of the sensitivity and specificity of such tests is limited because provocation tests in patients with positive skin tests or with negative tests but severe reactions are avoided owing to ethical reasons. Only a few studies have been carried out with series of subjects with nonimmediate reactions to β -lactam antibiotics, most of them with MPEs (TABLE 1) [11,13,22,115,116,121]. The sensitivity of skin testing in these reactions is low and drug provocation testing may be required to establish the diagnosis. The sensitivity of skin testing may be higher in more severe reactions, such as desquamative exanthemas [122].

In general, intradermal tests appear to be somewhat more sensitive than patch tests [116], which in turn are more specific than delayed reading intradermal tests [11]. False-negative tests could be due to several possible reasons: clinically compatible allergic reactions may, in fact, not be immunologically mediated; currently available tests may lack sensitivity; or the cofactors present at the time of the reaction may be absent when the patients are later evaluated (e.g., during drug provocation tests) in an appropriate clinical setting.

Concerning β -lactam antibiotic-induced contact eczema, reports indicate that patch testing is more sensitive than intradermal testing [113]. In a large series of patients with contact dermatitis due to different compounds, patch tests were positive to β -lactam antibiotics in more than 50% of all positive cases, the others involving a mixture of different compounds. The β -lactam antibiotics included a great diversity of compounds, which varied from aminopenicillins to fourth-generation cephalosporins [8].

In vitro diagnostic tests

The lymphocyte transformation test has been used for the study of β -lactam hypersensitivity with variable results and it has recently regained popularity as a method for not only understanding the mechanisms but also as a complementary diagnostic tool for skin testing [123,124]. As occurs with IgE-mediated reactions to β -lactam antibiotics, in nonimmediate reactions, a false-positive result may occur because lymphocytes recognize the drug and proliferate even though they belong to different subsets.

Lymphocyte toxicity studies have shown the capacity of drug-derived metabolites to kill lymphocytes in individuals with serum sickness-like reactions to cefaclor. These studies consist of an *in vitro* model using a murine microsomal system as a surrogate for hepatic drug biotransformation. Lymphocyte killing was very high compared with baseline values in these patients and to those with immediate hypersensitivity or exposed controls. No cross-reactivity with cephalexin occurred [9].

Drug provocation tests

In nonimmediate reactions to β -lactam antibiotics, intradermal or patch tests fail to have sufficient sensitivity and a large proportion of patients need to be given the drug to establish the diagnosis or, perhaps more often, confirm tolerance. In many cases, reported clinical histories are often vague or inconsistent [125], with many patients erroneously classified as allergic when in reality they are not. In other cases, particularly in MPE, patients often tolerate the drug after reaction. In some studies, most patients displaying negative results in skin tests tolerated the suspect antibiotic [11,116]. In the studies by Lammintausta *et al.* [126,127] and Waton *et al.* [128], the negative predictive values of drug skin tests in suspected cutaneous



Figure 3. Positive patch testing to aminopenicillins.

adverse drug reactions due to β -lactam antibiotics were 97.4 and 86.6%, respectively. The most frequent reactions in these studies were exanthemas. In a study carried out by our group that included 238 patients evaluated for a MPE, 19 (8%) showed a positive result in skin or challenge testing [116]. Intradermal tests were positive in ten patients (52.6%) and patch tests in five out of 12 cases (41.6%). However, eight subjects (45%)

Table 1. Summary of studies in patients with nonimmediate reactions to β -lactam antibiotics.

Study (year)	Patients	β -lactam implied	ST (%)	IDT	PT	DPT	Ref.
Romano <i>et al.</i> (2002)	101 allergic patients	AM: 95.9% PP: 4.1%	98 (97)	BP: 29.6% AM: 95.9% PP: 4.1%	BP: 18.3% AM: 91.8% PP: 4.1%	3 (3%)	[11]
Torres <i>et al.</i> (2004)	20 patients with positive skin tests	BP: 20% AM: 80%	20 (100)	AM: 20 (100%)	AM: 18 (90%)	ND	[115]
Padial <i>et al.</i> (2008)	22 allergic patients	NS	2 (9)	AM: 50% CLX: 50%	AM: 50% CLX: 50%	22	[22]
Moreno <i>et al.</i> (2008)	19 allergic patients	AM: 68.4% BP: 10.5% CEPH: 10.5% CLX: 5.2% UK: 5.3%	11 (55)	BP: 36.4% AM: 72.7%	BP: 9.1% AM: 45.4%	8 (45%)	[116]
Trcka <i>et al.</i> (2007)	71 allergic patients	AM: 75.2% UK: 24.8%	68 (95.8)	IDT or PT BP: 28.2% AM: 95.8%		3 (4.2%)	[23]
Lammintausta <i>et al.</i> (2005)	785 patients with exanthema by different drugs (not only β -lactam antibiotics)	NS	NS	0/31	BP: 6/152 (3.9%) PHP: 8/298 (2.6%) AM: 10/247 (4.0%) CEPH: 12/290 (4.1%) Others: 2/5 (40%)	BP: 8 (4.4%) AM: 11/53 (20.7%) AP: 1/9 (1.1%) CEPH: 2/45 (4.4%)	[126,127]
Blanca-López <i>et al.</i> (2009)	20 allergic patients	AM: 100%	1 (5)	NS	AM	19 (95%)	[121]

AM: Aminopenicillin; AP: Ampicillin; BP: Benzylpenicillin; CEPH: Cephalosporin; CLX: Cloxacillin; DPT: Drug provocation test; IDT: Intradermal tests; ND: Not done; NS: Not specified; PHP: Phenoximethylpenicillin; PP: Piperacillin; PT: Patch tests; ST: Skin tests (IDT or PT); UK: Unknown.

with negative skin tests showed an exanthematic reaction during challenge testing. All reactions were mild [116]. In other studies, a significant percentage of subjects with negative patch or intradermal tests reacted to drug provocation tests and diagnosis was established by this procedure [22,121]. In TABLE 1, the results of allergy tests in several studies are shown.

Drug provocation tests should never be performed in patients who have experienced severe nonimmediate reactions, such as SJS, TEN, DRESS or AGEP (FIGURE 1) [129]. Drug provocation testing is also contraindicated in patients who have experienced hematologic reactions and organ-specific reactions such as nephritis or hepatitis.

Expert commentary

Nonimmediate reactions to β -lactam antibiotics are more frequent than previously thought and some can be serious or even fatal. Prompt recognition and drug discontinuation can be of the utmost importance in the evolution of the reaction. The immunogenicity of β -lactam antibiotics in nonimmediate reactions and the underlying mechanisms are not well elucidated; however, MPEs are known to be the most frequent nonimmediate reactions caused by β -lactam antibiotics. A high percentage of patients with this type of reaction tolerate the antibiotic after reactions but a proportion of them will experience a reaction again. A combination of different type IV mechanisms (IVa–d) is probably responsible for most nonimmediate reactions to β -lactam antibiotics. Cytotoxic T cells play a fundamental role in most nonimmediate reactions, with a predominance of CD4⁺ T cells in MPEs and of CD8⁺ T cells in bullous reactions. Increased expression of of

Th1 cytokines, chemokines and chemokine receptors, and Th2 cytokines has been described. Other cells, like dendritic cells and eosinophils, have a role in nonimmediate reactions to β -lactam antibiotics and other drugs. Allergological evaluation is complicated and currently available tests show poor sensitivity and specificity. The development of more precise assays will enable us to study the mechanisms in depth and to consequently improve the diagnosis of nonimmediate reactions to β -lactam antibiotics.

Five-year view

Immunochemical studies of cells, cytokines and other mediators in nonimmediate reactions will enable us to increase our knowledge of the mechanisms of different nonimmediate reactions. This will permit the development of more sensitive assays in the diagnosis of nonimmediate reactions to β -lactam antibiotics and the development of more specific treatments. Studies concerning the influence of genetic variations between populations in the prevalence of reactions will enable us to prevent these reactions.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Key issues

- β -lactam antibiotics are the most widely prescribed antibiotics globally and are also the most frequently involved drug in immune adverse drug reactions. These reactions can be classified as immediate or nonimmediate according to the time interval between the last drug administration and their onset.
- Nonimmediate reactions to β -lactams include a broad number of clinical entities, with maculopapular exanthemas (MPEs) being the most common.
- Viral infections are well-recognized cofactors that participate in the elicitation of MPEs due to β -lactam antibiotics. Between 70 and 100% of patients receiving aminopenicillins during an Epstein–Barr virus infection develop a MPE. In many cases, Epstein–Barr virus-associated amoxicillin exanthema is reversible and patients tolerate the antibiotic after recovery from the reaction. However, in other patients, re-exposure can induce the reaction again.
- Nonimmediate reactions are predominantly T-cell-mediated, although the exact mechanisms are not well understood. A combination of different type IV mechanisms (IVa–d) is probably responsible for many nonimmediate reactions. Cytotoxic T cells play a principal role in most nonimmediate reactions, with a predominance of CD4⁺ T cells in MPEs and of CD8⁺ T cells in bullous reactions.
- In nonimmediate reactions, both the core structure of β -lactam antibiotics and the whole molecule are recognized by T cells; cross-reactivity between cephalosporins and penicillins seems to be rare.
- Delayed intradermal tests and patch tests can be useful to diagnose several nonimmediate reactions to β -lactam antibiotics, such as MPEs, toxic epidermal necrolysis, acute generalized exanthematous pustulosis and contact dermatitis. However, intradermal or patch testing fails to have sufficient sensitivity and a large proportion of patients need to be given the drug to establish the diagnosis or, perhaps more often, confirm tolerance.
- Drug provocation tests should never be performed on patients who have experienced severe nonimmediate reactions, such as Stevens–Johnson syndrome, toxic epidermal necrolysis, drug rash with eosinophilia and systemic symptoms, hematologic reactions and organ-specific reactions such as nephritis or hepatitis.

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