

Nonimmediate reactions to betalactams

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Purpose of review

Nonimmediate reactions to β -lactams include several clinical entities, from maculopapular rash to severe reactions such as Steven-Johnson syndrome. Toxic epidermal necrolysis and organ-specific reactions may also occur.

Recent findings

Progress has been made in understanding the role of the immunological system in nonimmediate reactions to β -lactams. Different T-cell subsets recognize β -lactams after haptenation of serum or cell proteins in the context of major histocompatibility complex. Studies using T-cell lines and clones have shown that a heterogeneous response is generated, with the expression of different cytokine profiles. Betalactams also act on dendritic cells, inducing changes that enable them to interact with naïve lymphocytes, becoming memory T cells. Tissue-activated CD4 and CD8 cells express perforin and other cytotoxic mediators that elicit the lesions. Studies on the clinical course of these entities indicate that cells migrate, establishing a recirculation with homing to the skin and back to the circulation. These cells thus participate not only in skin lesions but probably also in the repair process.

Summary

Understanding the immunological mechanisms involved in nonimmediate reactions to β -lactams has improved over the last few years, with better definition of the different T-cell subpopulations involved. Experimental studies and monitoring of the response support the implication of different cell subsets.

Keywords

β -lactams, immunological response, in-vitro test, in-vivo test, nonimmediate reaction

Abbreviations

CLA	cutaneous lymphocyte antigen
Ig	immunoglobulin
IL	interleukin
LTT	lymphocyte transformation test
NIRBL	nonimmediate reactions to β -lactams
SJS-TEN	Steven-Johnson syndrome/toxic epidermal necrolysis
CTAK	cutaneous T cell attracting chemokine
DR	class II-HLA antigen

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Introduction

Betalactams, the most widely prescribed antibiotics around the world, can conjugate spontaneously, *in vivo* and *in vitro*, to serum or cell membrane proteins that are recognized by the immune system [1] and induce either immunopathological responses or tolerance. Evidence which supports the immune-mediated nature of these reactions includes the following: occurrence following reexposure to the drug, positive intradermal and patch testing, and the ability of mononuclear cells from the peripheral blood and intralesional skin of affected individuals to be stimulated *in vitro* by the culprit penicillin [2,3].

Several groups have proposed classifying allergic reactions to β -lactams as immediate or nonimmediate, adapting the classification proposed by Levine many years ago who divided the reactions into immediate, accelerated or delayed [4]. Accelerated reactions include serum sickness-like syndrome, late onset urticaria and angioedema. In many instances, exanthematic or urticarial reactions appear within 24 h of drug intake, especially in cases in which the reaction occurs shortly after a previous reaction. Accordingly, both accelerated and delayed reactions can be categorized as nonimmediate reactions.

A correct classification of these reactions, therefore, requires knowledge of the time interval between drug intake and symptom onset, in addition to the type of clinical manifestations. These details are essential in establishing an initial diagnosis that tells us which allergological test to use in order to reach a definitive diagnosis.

Organ-specific reactions, excluding those that occur as part of organ involvement in systemic anaphylaxis, can also be considered as delayed reactions mediated by

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T-cell specific lymphocytes [5]. Amongst these, the best known is acute hepatitis to amoxicillin, others being acute pancreatitis or kidney involvement, though these are relatively less common. Instances also exist in which specific organ involvement accompanies skin manifestations, such as in maculopapular exanthema. This review deals with nonimmediate reactions to β -lactams (NIRBLs) with skin involvement, focusing mainly on mild NIRBLs, since an appropriate classification and identification of the relevant drug is necessary should antibiotic therapy be required in the future.

Classification

The group of NIRBLs includes a number of clinical entities, ranging from maculopapular rashes, the most frequent manifestation, to urticaria and to other less common but more severe entities such as drug rash with eosinophilia and systemic symptoms, acute exanthematic pustulosis, and Steven-Johnson syndrome/toxic epidermal necrolysis (SJS-TEN) [5]. Fixed drug eruption (FDE) is a unique clinical entity that can sometimes be very extended in the skin and become a serious disease [6]. Drug-induced contact dermatitis is almost an occupational disease that affects exposed persons working in the pharmaceutical industry, healthcare personnel, and veterinarians, amongst others [7–9]. Serum sickness-like reactions, mostly to cefaclor, have been reported [10], though similar reactions have also appeared after administration of amoxicillin or other β -lactams [4]. Cefaclor-induced serum sickness-like syndrome is very interesting because it does not necessarily require previous drug exposure [11]. Other clinical entities have also been described, for example the baboon syndrome, a clinical entity consisting of an erythematous reaction on the buttocks, inner thighs and axillae [12]. Cases of perianal intergluteal vesicular eruption after a course of ampicillin-sulbactam have been reported and immunopathological studies have classified it as a form of bullous pemphigoid, specifically linear immunoglobulin A (IgA) dermatosis [13]. The most common of all these entities is an exanthematic reaction, occurring in 25% of all cases with skin manifestations, followed to a lesser extent by urticaria. This contrasts with the much lower proportion of the other reactions, including immediate IgE-mediated reactions such as anaphylaxis [14].

Excluding reactions after occupational exposure to β -lactams, amoxicillin is undoubtedly the most frequent drug involved in NIRBLs [15,16], followed by cephalosporins, although NIRBLs have been reported for virtually all β -lactams, including clavulanic acid [17–26]. The true prevalence of these reactions is unknown. Several studies have reported the relevance of exanthematic reactions related with aminopenicillins [15,27]. Due to the great variation in antibiotic consumption between different countries, however, differences must certainly

exist in the prevalence and incidence of these reactions. In a study carried out in Europe, France had a consumption of β -lactams three to six times higher than the Netherlands. The consumption of amoxicillin was very high in France, Portugal, Spain and Belgium and cephalosporin in Greece [28]. Differences clearly exist for immediate reactions but for delayed reactions sufficient information is still unavailable.

Clinical considerations

The alarm signal for a reaction is the appearance of any skin manifestation in the course of drug treatment. In general, these manifestations occur from days to weeks after drug exposure, and in many cases the reaction occurs after a subsequent exposure, which often occurs a long time after the previous treatment. Cutaneous reactions can also appear during the course of different viral infections, especially with infectious mononucleosis [29,30]. After the infection resolves, the patient may again take the drug safely following negative provocation testing. Sometimes the maculopapular exanthema can be intense, with subcutaneous oedema, persisting for several weeks despite discontinuing the therapy [31]. Up to 20% of patients with maculopapular exanthema have hepatic involvement with elevation of enzyme levels [32].

When studies are based on retrospective information, it can sometimes be difficult to determine whether the reaction was exanthematic or urticarial, even if angioedema was present, because a certain oedematous component may exist in some severe exanthematic reactions.

Fixed drug eruption is another β -lactam-induced response, consisting of an erythematous lesion over a limited area of the skin that may become bullous and leave a pigmented residual lesion that persists for a long time [6,33].

Immunopathological mechanisms involved

Although the participation of specific immunological mechanisms seems clear, not all reactions are reproducible because other factors such as viruses or other aetiological agents may have contributed to exanthema or urticaria [32]. The same holds true for other clinical entities.

Results of immunological studies

T cells recognize a hapten peptide fragment that is generated by cellular uptake and processing of the hapten-carrier complexes and presentation to T cells in the presence of a major histocompatibility complex (MHC) [34]. 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 [34,35]. Peripheral blood lymphocytes from allergic patients proliferate *in vitro*

to free human serum albumin-conjugated β -lactams [34]. Functional analysis of T-cell clones from peripheral blood and from affected tissue shows different types of T-cell reactions that lead to different forms of drug-induced hypersensitivity [36].

Hertl *et al.* [35] found that T-cell clones derived from peripheral blood lymphocytes of allergic patients with maculopapular exanthemas were CD3+CD8+, producing the T helper type 1 (Th1) cytokines interleukin (IL)-2 and interferon (IFN)- γ [35]. Cutaneous T cells were identified as mainly CD8+ and when expanded *in vitro* expressed cytolytic activity against epidermal keratinocytes and autologous B cells, indicating that the skin was the target organ for CD8+ cells [36]. Brugnolo *et al.* [37], however, found high intracellular levels of the typical Th2 cytokines, IL-4, IL-5 and IL-13, in patients with NIRBLs. Yet others have found that the majority of specific T lymphocytes isolated from peripheral blood show a CD4+ phenotype. Antigen-specific T-cell clones isolated from blood were CD4+ or CD8+ and secreted a heterogeneous pattern of cytokines upon antigen stimulation [2,38]. Thus, these studies show that both CD4+ and CD8+ T cells can be activated with highly heterogeneous functions.

Recent studies have focused on the involvement of the innate immune response. Our group has shown that dendritic cells play a role in the induction of delayed T-cell responses. Dendritic cells from patients with NIRBLs to amoxicillin were able to present this drug to autologous T lymphocytes, inducing a specific response. Use of the lymphocyte transformation test (LTT) plus dendritic cells from the same patient showed the proliferative response to be higher than in the classical LTT in which B lymphocytes and monocytes were used as antigen-presenting cells. This enhanced response was only found in patients, not in healthy exposed controls in which lymphocytes did not proliferate in response to any of the β -lactams used [39]. Moreover, amoxicillin plays a fundamental role in triggering the maturational process in dendritic cells from allergic patients but not in controls. The reason why amoxicillin is able to induce this effect only in allergic individuals is still unknown. Whether it is due to an intrinsic property of dendritic cells or to factors present in the serum of patients is a matter of speculation [40]. Another possibility could be that amoxicillin generates danger signals in immature dendritic cells, which could induce the release of inflammatory mediators that activate dendritic cells [41]. As these possibilities need further examination we are considering refining this methodology for use with the *in-vitro* diagnosis of delayed drug reactions.

With regard to FDE, T cells are also the effector cells and CD8+ T cells with cytotoxic potential can persist among

basal keratinocytes in the skin lesion zone over prolonged periods of time after resolution. The characteristic T-cell phenotypes express $\alpha\beta$ receptor, CD3CD8, CD45RA and CD11b but not CD27 and CD56. These cells change from CD45RA+ to CD45RO+ upon activation [6]. Unlike the reaction of IgE responses to inhaled protein allergens, when there is often a combined delayed response with T-cell involvement, this has never been reported for hypersensitivity reactions to β -lactams, although strong immediate and delayed reactions to the same drug and in the same site have been seen with ceftriaxone in an occupational worker with a periorcular dermatitis due to ceftriaxone [42]. The possibility thus exists that when different haptens are applied to the skin, one can induce an immediate response and another a delayed response.

Only a few genetic studies on the susceptibility to β -lactam delayed hypersensitivity have been undertaken, one of which [43] showed a higher prevalence of HLA2 and DRw52 with a lower prevalence of DR4, specifically for delayed reactions to aminopenicillins.

Other immunological mechanisms

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, though this has yet to be shown in humans [44].

There are instances in which vesicular lesions may be produced by a linear IgA dermatosis. In these cases autoantibodies against a 120 kDa soluble domain of bullous pemphigous antigen 180 (LAD-1) have been detected. The possible mechanism is that the drug may haptenize these proteins and produce neoantigens [13].

Monitoring the immunological response

A logical approach to evaluating NIRBLs is to monitor the progression of reactions by obtaining peripheral blood and samples of affected tissue in order to observe the ongoing immunological mechanisms from the initiation of the reaction until its resolution. In cases in which bullae also appear, such as SJS-TEN, vesicle fluid can provide valuable additional information. For example, CD8 T lymphocytes are mainly detected in the vesicle fluid [45,46] whereas CD4 T lymphocytes are found in the skin and in peripheral blood [47]. Our group has performed several studies monitoring different entities of nonimmediate reactions. Although the studies were not always done with β -lactams, a reasonable extrapolation can still be made. In general, in patients with cutaneous reactions, the skin homing receptor, cutaneous lymphocyte antigen (CLA), is increased in both T-lymphocyte subsets, CD4 and CD8, in both the skin and the

peripheral blood [47]. Initially, it was thought that this molecule was expressed on the lymphocytes that were involved in the reactions. Careful evaluation of these cells from a patient with NIRBLs, however, enabled them to be classified into CLA+CD45RO+ and CLA-CD45RO+, with only the former subset showing the capacity to specifically proliferate in the presence of the β -lactam inducing the reaction [48]. Additional studies have shown that memory CLA+ cells can be CD4+ and CD8+ and in some instances persist for many days after the disappearance of the lesion. This indicates that other lymphocyte subpopulations, with activities different from the effector function, arrive at the skin. These cells can have a regulatory function and may contribute to repair the affected tissue. These circumstances can be detected by evaluating the cellular phenotype during different phases of the reaction.

In-vivo diagnostic tests

For immediate reactions evaluation with appropriate penicillin skin testing is accepted to be the standard approach [49] but in NIRBLs, although general recommendations have been provided [4], the results of studies are not the same. This may be because clinically compatible allergic reactions may, in fact, not be immunologically mediated, currently available tests lack sensitivity, or the cofactors present at the time of the reaction are absent when the patients are later evaluated (e.g. during drug provocation tests) in an appropriate clinical setting.

Intradermal and patch testing reactions have been used for the diagnosis of NIRBLs. The general procedure has been published by the European Network for Drug Allergy (ENDA) [14]. Series or individual cases involving intradermal or patch testing have been published, ranging from penicillins and cephalosporins to the uncommon drug clavulanic acid [22]. Although differences in sensitivity have been found with intradermal testing compared with patch testing [50], these are small and in those cases in which the reactions occurred after taking the drug by the systemic route, the sensitivity was never higher than 50–60%. As occurs with many other drugs, the determination of the threshold of irritancy is of great value for the performance of the test [50].

Although the specificity is generally very high with no false positive cases if the recommended concentrations for skin testing are used, the sensitivity of these tests is a matter of debate and most studies addressing this issue do not use well defined or sufficient numbers of positive controls to assess sensitivity. Many cases that were intradermal or patch test positive were not in fact challenged, whereas the remaining cases that were skin test negative and were challenged had good tolerance. In our experience, in studies using cases based on a positive

provocation test to different β -lactams, the sensitivity was lower than previously estimated [51]. Based on these data more studies are in progress in order to verify sensitivity values in patients with NIRBLs.

When β -lactams induce contact eczema, reports indicate that patch testing is more sensitive than intradermal testing [52]. Unlike the reaction with aminopenicillins, which are the most common systemically administered drugs, in contact eczema any β -lactam responsible for occupational exposure can induce the reaction. Out of a total of 7857 cases with contact dermatitis to different compounds, patch testing was positive to β -lactams in more than 50% of all positive cases, the others involving a mixture of different compounds. The β -lactams included a great diversity of compounds that varied from aminopenicillins to fourth generation cephalosporins [9].

Many studies have shown that positive intradermal testing to amoxicillin is often also positive to ampicillin, or in some instances to benzylpenicillin [53]. Cases also exist, however, of positivity just to benzyl penicillin. For cephalosporins, delayed hypersensitivity cross reactivity is very rare with close β -lactams, and in cases that were patch test positive to several cephalosporins, the side chain seems to contribute to the cross-reactivity; for example, individuals tested were positive to cefuroxime, cefotaxime, ceftazidime, ceftriaxone and cefuroxime [8]. All these studies also support the role of a side chain-specific contribution to the complex structure recognized by the T-cell receptor.

The concentration of penicillins used for intradermal or patch testing varies in different studies, although specific recommendations have been published [4,54,55]. Concentrations ranging from 375 to 1 mg for intradermal testing and from 20 to 1% in sodium petrolatum for prick testing have been used [53,56]. We performed a study with 20, 2 and 0.2 mg in saline for intradermal testing and 50, 25 and 5% in patch testing, showing that for intradermal testing the concentration of 20 mg was optimal, although most cases also responded with the other concentrations. For patch testing, the concentration of 5% w/w in sodium petrolatum was optimal and preferable to the application of solution diluted in saline [56].

In-vitro diagnostic tests

For many years, the LTT was used for the study of β -lactam hypersensitivity with variable results, and it has recently gained popularity again as a method not only for understanding the mechanisms but also as a complementary diagnostic tool for skin testing [7,57]. In general, lymphocytes proliferate to the β -lactam haptenated proteins, though further information is required for a more detailed determination of the proliferative subpopulations. Thus, LTT was positive in 87% of cases

with immediate reactions and in 84% of those with nonimmediate reactions [7].

In our experience, peripheral blood mononuclear cells from 64% of patients proliferated *in vitro* to at least one of the penicillins tested. The sensitivity in individuals with NIRBLs was 57%, which was higher compared with the sensitivity of the in-vivo test (37%) [58]. Heterogeneity in the response was observed because some cases with selective in-vivo responses to amoxicillin responded *in vitro* to both amoxicillin and benzylpenicillin or only to benzylpenicillin, even though the drug that induced the reaction was amoxicillin. As occurs with IgE-mediated reactions to β -lactams, in NIRBLs the possibility exists of a person with a positive clinical history having a negative skin test but positive LTT. This may be because lymphocytes recognize the drug and proliferate even though they belong to different subsets, such as regulatory cells.

Lymphocyte toxicity studies have shown the capacity of drug-derived metabolites to kill the 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 [11].

As stated above, exposed individuals to β -lactams may develop immunological responses [59]. The presence of IgG or antibodies of other isotypes, or lymphocytes that proliferate and respond to free or conjugated β -lactams has been reported [58–61], explaining why quantitation of IgG antibodies has no value in these cases. This limitation must therefore be taken into account when T-lymphocyte-based tests are used.

Histopathology

T cells are implicated in all cutaneous reactions, but because they differ in their manifestation, severity, progression and response to treatment, distinct histopathological features can be inferred. These features include the way dendritic cells interact with the drug, the signal that they give in the lymph node to naive lymphocytes to become mature cells [39], the mechanism by which these cells migrate to the skin by orchestrated homing at which point adhesion molecules, integrins and chemokine receptors, after interaction with their corresponding ligands, enable the cells to reach the tissue [45,46]. Yet further measurable interactions occur after arrival at the tissue. More detailed immunochemical and molecular biology studies will probably elucidate the differences between these processes, leading to greater precision in the identification of the different entities.

A hallmark of histopathology studies is a mononuclear cell infiltrate formed predominantly by T cells, which are activated expressing DR antigens, CD69 activation markers and the skin homing receptor CLA in both CD4 and CD8 subsets, with CD4 generally predominating over CD8 cells. With respect to their skin localization, mostly CD4 are found in the perivascular dermis whereas both CD4 and CD8 are found in the dermoepidermal junction [32]. In exanthematic reactions, a predominant perivascular mononuclear cell infiltrate with increased numbers of eosinophils in the papillary dermis has been found. Up to 20% of the cells express perforin and granzyme B with enhanced expression of IL-12 [62]. Similar patterns occur in bullous exanthematic reactions with a high percentage of perforin-positive CD8 T cells, particularly in the epidermis. These CD8 T cells kill keratinocytes by both MHC-I and MHC-II class-restricted mechanisms [32].

The histological characteristics of SJS-TEN present many dead keratinocytes with scarce T-cell infiltration, probably because of loss of superficial layers of skin [63]. The expression of CTAK in keratinocytes from patients with bullous drug reactions such as SJS-TEN has been found with the concomitant expression of CCR10 in peripheral lymphocytes that returned to baseline values after disease resolution [64]. In acute generalized exanthematic pustulosis, keratinocytes express high levels of IL-8, a chemokine that recruits neutrophils to the epidermis.

In-situ hybridization has shown Epstein–Barr virus gene products in B and T lymphocytes in the skin [65]. This reaction has also been shown for cytomegalovirus and human herpes virus 6, among others [66].

Drug provocation tests

Causality criteria to identify NIRBLs are often based on general rules from which a likelihood can be generated. These are biological plausibility, temporal association and previously reported effects. If algorithms are developed based on these conditions, however, false positives are often diagnosed [4]. In our experience, and particularly in the case of exanthematic reactions, patients often tolerate the drug. In many other cases, clinical histories reported are often vague or not particularly consistent [67], with many patients erroneously classified as allergic when in reality they are not. These patients are then given drugs that are more toxic and are placed at risk of infections or colonization by resistant organisms; for instance, the use of vancomycin in patients mislabelled as β -lactam allergic. In daily allergological practice, therefore, many patients are challenged with β -lactams [68,69] often following negative in-vivo and in-vitro tests.

As occurs with nonimmediate reactions to other drugs, in patients with NIRBLs, intradermal or patch testing fails to have sufficient sensitivity and an important proportion of patients need to be given the drug to establish the diagnosis or, perhaps more often, confirm tolerance. In a study carried out by our group in 1995 [53], 65% of the cases were skin test positive, but the remaining cases required controlled administration to confirm cross reactivity. The definition of a positive group in this study was based on previously known positive cases. To avoid this selection bias we performed a study in 2004 [51] evaluating 146 patients who developed a reaction compatible with a nonimmediate reaction. In only 22 cases (15%), were we able to induce symptoms compatible with a delayed reaction, either exanthematic or urticarial. Surprisingly, the skin tests were positive in only 9% of this well characterized group. These data suggest that we need to reevaluate the role of intradermal skin testing in nonimmediate reactions to β -lactams.

Controlled administration is achieved by performing intradermal testing as recommended [4]. If this elicits a positive response we stop but if it is negative we proceed to the oral administration of the drug by giving the patient incremental doses of 5–50 and 100 mg during one morning at a minimum of 1 h intervals. After an accumulated dose of 155 mg, the patient remains under observation for 8 h. If no symptoms appear in a resting period of 2 days, a dose of 300 mg every 12 h is given and after an additional period of observation of 48 h, we proceed to administer 500 mg in the same manner.

Conclusion

Increasing evidence supports the role of T cells in inducing the different immunological entities described under the group of NIRBLs. In addition to T cells, dendritic cells also participate and they orchestrate a complex system that requires adhesion molecules, including integrins, chemokine receptors and other molecules that interact with their corresponding ligands, enabling them to reach the affected tissue. Following further interactions, different immunopathological lesions develop which are subsequently reflected into different clinical entities. In the future, more details of the trafficking mechanism in parallel with the tissue response will permit a better definition and classification of these entities. Progress in molecular biology and genetics, as well as the development of more sophisticated assays, will enable us not only to study the mechanisms better but also to develop more precise assays to diagnose NIRBLs.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 361).

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