

Immunoglobulin E binding determinants on β -lactam drugs

Brian A. Baldo and Nghia H. Pham

Purpose of review

Allergies to penicillins and cephalosporins remain an important clinical problem, but structural and immunochemical knowledge of the allergenic structures involved has tended to lag behind the heavy usage, consequent adverse reactions and introduction of new therapeutic members of these two families of antibiotics. Evidence of the increasing incidence of reactions to cephalosporins and to 'minor' determinants of the β -lactams is accumulating. Also, although numerous reports detail unique allergic recognitions of individual members of the two families, particularly the cephalosporins, information remains predominantly clinical. The present review summarizes the most recent advances in studies of structural aspects of β -lactams as allergens.

Recent findings

For the cephalosporins, a pyrazinone allergenic degradation product of cefaclor and cephalexin has been identified and characterized. The widely used cephalosporin cephalothin was shown to cross-react allergenically with benzylpenicillin and the common cross-reacting structure was identified. The fine structural features on the amoxicillin molecule recognized by antibodies that distinguish 'major' and 'minor' determinants were identified, and steric factors were used to explain antibody recognition of the amoxicillin determinants. A recent study elucidated the molecular basis of some cases of the multiple drug allergy syndrome and its relationship to β -lactam allergy.

Summary

Findings of the type described in the present review provide fundamental insights into the nature and size of antigenic determinants on 'small' molecules such as drugs and other chemicals. At the clinical level, such structure/activity findings have implications for our understanding of drug allergenic cross-reactions, for selection for therapy of an appropriate member from a family of structurally related drugs and, ultimately, for desensitization of drug-allergic patients.

Keywords

allergen structure, beta-lactam allergens, cephalosporins, drug allergens, penicillins

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Research Laboratory, NSL Health Limited, Melbourne, Victoria, Australia

Correspondence to Brian A. Baldo, PhD, 11 Bent Street, Lindfield, NSW 2070, Australia
Tel: +612 9880 9757; e-mail: babaldo@optusnet.com.au

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Abbreviation

IgE immunoglobulin E

Introduction

Allergic reactions to antibiotics, particularly of the β -lactam group, which includes the penicillin and cephalosporin families, are still under-evaluated [1] and present daily problems to general practitioners and allergists. Although the value of employing skin testing for clinical diagnosis of allergies to β -lactams, particularly the penicillins, is widely acknowledged, the lack of comprehensive panels of testing agents that cover the numerous different penicillins and cephalosporins in both 'major' and 'minor' antigenic forms is a serious deficiency for successful diagnosis [2]. The value of skin testing, even with the available penicillin testing agents, has been further emphasized by Solensky *et al.* [3]. Those investigators concluded that a large proportion of patients who have penicillin-specific immunoglobulin E (IgE) antibodies have only vague allergy histories, and that such patients should therefore undergo penicillin skin testing before they are administered that agent. In considering the risks associated with skin testing, a timely reminder about testing without minor determinants has recently been given by Macy and coworkers [4,5], who pointed out that testing without 'minor determinant mix' may miss some allergic individuals and that this incomplete testing drives much of the use of antibiotics such as vancomycin and may contribute to antibiotic resistance.

The importance of taking into account structural and immunochemical considerations in the in-vivo and in-vitro diagnosis of allergy to penicillins and cephalosporins has been emphasized [2,6–9]. However, the immunochemistry and serology of 'minor' determinants of penicillins and of cephalosporin antigens and allergens remain sorely under-researched.

Different or unique allergenic determinants on β -lactam drugs

In what is probably a reflection of current usage, allergic reactions to amoxicillin, particularly minor determinants, are now more common than reactions to benzylpenicillin [2,8,10,11]. Recent serological studies employing tests for serum IgE antibodies to major and minor determinants of benzylpenicillin and amoxicillin [2,8] indicated that cross-reactions, particular between the minor determinants, often occur between these two penicillins [2,8]. Routine tests conducted in our laboratory for β -lactam reactive IgE antibodies in the sera of patients with suspected allergy to a β -lactam drug(s) [12•] have also revealed a higher incidence of recognition of cephalosporins than of penicillins.

Although often lacking precise immunochemical findings, reports of allergic reactions to individual β -lactams continue to appear frequently, indicating specific recognition of all or parts of individual penicillins or cephalosporins. For example, Romano *et al.* [13] outlined a case of anaphylactic shock and skin test positivity to ceftazidime in which skin tests, radioallergosorbent tests and challenge tests to some other β -lactams [including benzylpenicillin (major determinant and minor determinant mix), ampicillin, amoxicillin, cephalothin, cephalixin, cefaclor and cefixime] were negative. Even in the absence of quantitative structure/activity investigative findings, this led those authors to conclude that the person's sensitization appeared to be due to allergic recognition of the entire ceftazidime molecule; this conclusion was also reached for some patients who were allergic to cefaclor in an earlier immunochemical quantitative hapten inhibition study [14]. A similar case report of an anaphylactic reaction to ceftriaxone [15] emphasizes yet again the possible selective nature of reactions to individual β -lactams.

Increased recognition in recent years of the importance of side chain determinants in IgE-mediated reactions to penicillin is leading to greater awareness of possible structural similarities between β -lactams, including cephalosporins, and consequent possibilities of allergic cross-reactions [6–9]. With this in mind, Novalbos *et al.* [16] studied 41 well characterized penicillin-allergic patients by skin and provocation testing. Those investigators concluded that risk for an allergic reaction to a cephalosporin was low if the cephalosporins used contained different side chains to the penicillin responsible for the allergic reactions. In an assessment of IgE antibody responses in persons with immediate hypersensitivity reactions to injected cephalosporins, Romano *et al.* [17] found that most patients with a history of reactions to cephalosporins are sensitized to a cephalosporin(s). Some of the patients reacted only to the administered cephalosporin but others reacted to different cephalosporins. Those authors concluded that these findings can be explained in terms of responses to unique determinants or to cross-reactions but, again, no experimental immunochemical data, qualitative or quantitative, were advanced to support the conclusion.

The essentially clinical studies of the type outlined above do little to improve our understanding of the allergenic determinants and structures that are responsible for cross-reactions. Such an improvement will only be achieved by undertaking detailed and systematic quantitative immunochemical fine structure/activity studies [7,11,18,19••].

Some recent structure/activity insights

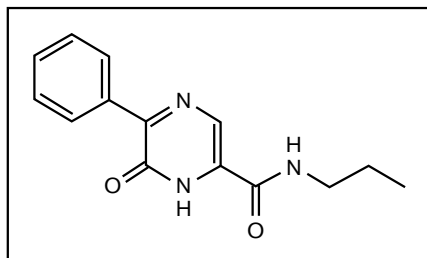
Much remains to be done if we are to identify precisely, at the fine structure level, the IgE antibody binding

determinants on β -lactam drugs, particularly the cephalosporins. However, some recent studies have revealed some unexpected and fascinating insights into β -lactam allergenic structures.

Selective and unexpected reactions to amoxicillin were exhibited by sera from allergic persons that distinguished between the amoxicilloyl (major) and the amoxicillanyl (minor) determinants [19••]. Results of direct antibody binding and detailed quantitative hapten inhibition studies in serum that recognized the amoxicillanyl ('-anyl') but not the amoxicilloyl ('-oyl') determinant led to the conclusion that the open β -lactam ring of the '-oyl' determinant allows increased flexibility and rotation around C-6 and C-7, permitting close steric association between the hydroxyaminobenzyl side chain and the peptide carrier. H-bonding resulting from the close association between the -OH on the side chain and the lysine carrier subsequently interferes with the binding of antibodies to the side chain. By contrast, rigidity conferred on the amoxicillin molecule by the intact β -lactam ring in the '-anyl' form (i.e. when it is linked to the carrier via the C-2 carboxyl group at the other end of the molecule) permits free and uninhibited access of antibodies to the side chain. Quantitative hapten inhibition experiments revealed that antibodies reacting only with the '-oyl' determinant recognized at least part of the hydroxyaminobenzyl side chain distinct from the hydroxy group and the thiazolidine ring carboxyl, and that such recognition is possible when amoxicillin is in the '-oyl' but not in the '-anyl' antigenic form. These results are an example that again demonstrates the importance of diagnostic testing with both major and minor β -lactam antigens. In these patients, employment of only one form would have missed clear-cut and potentially dangerous sensitivities [2,19••].

In a preliminary study of potential cephalosporin allergens, Venemalm [20••] found that cephalosporins such as cefaclor and cephalixin with an α -amino group on the 7- β -acyl substituent of the cephalosporin molecule undergo aminolysis with formation of a pyrazinone degradation product (Fig. 1). This was shown by allowing cefaclor or cephalixin to react with propylamine in alkaline aqueous solution and the resultant solid left to stand in phosphate buffer at 37°C for 2 weeks. When cefaclor was conjugated to human serum albumin or poly-L-lysine under mild alkaline conditions and stored, an analogous degradation occurred, leading to the formation of a conjugated pyrazinone derivative. A corresponding pyrazinone-polylysine conjugate was prepared from the pyrazinone acid and tested for IgE antibody binding with sera from 31 persons with suspected β -lactam allergies. Fifteen of the samples were positive to cefaclor in the cefaclor Immuno CAP test (Pharmacia, Uppsala, Sweden) and

Figure 1. Pyrazinone product formed from the degradation of the cephalosporins cefaclor and cephalexin



nine proved positive to the pyrazinone linked conjugate, showing that this hapten is recognized by antibodies in the serum of some cefaclor-allergic persons.

An earlier study showed allergenic cross-reactions between penicillins and cephalosporins, in particular cephalothin [21]. In an extension to that study, Zhao *et al.* (Zhao Z, Baldo BA, Rimmer J, unpublished data) noticed that a high proportion of routine tests for β -lactam-reactive IgE antibodies in the sera of 1797 persons with suspected β -lactam drug allergy suggested the possibility of allergenic cross-reaction between bezympenicillin and cephalothin. Immunochemical investigations revealed a single population of cross-reacting IgE antibodies that recognized both drugs. Those investigations also indicated that the common complementary allergenic structure was primarily the side chain methylene group with some additional, and weaker, recognition of neighbouring structures, probably the attached amide group and perhaps even all, or part of, the β -lactam ring. Such subtle and small common structural determinants are probably involved in allergic reactions to other β -lactams and indeed to other nonrelated drug allergies [22,23**].

Multiple drug allergy syndrome and β -lactam antibiotics

Multiple drug allergy syndrome is a clinical condition in which patients display allergic sensitizations to a variety of drugs that may be both pharmacologically and structurally unrelated [24]. Although antibiotics, including the β -lactams, have been implicated in this syndrome [24,25], the mechanism that underlies the recognition of diverse structures had not been investigated until recently, in a study reported by Pham *et al.* [23**]. Drug recognition by IgE antibodies in a wide variety of drugs was shown to be related to the presence of alkyl amino groups but only if the alkyl groups were 'small' (i.e. methyl, or perhaps ethyl). Those groups are present in many different pharmacological agents. Separate populations of antibodies to drugs that contain alkyl amino groups and to β -lactam antibiotics were

demonstrated in the sera of patients, and this finding is in accord with findings reported by Khoury and Warrington [26], who concluded that the development of penicillin and of non- β -lactam allergies in multiple drug allergy syndrome are not associated and occur independently.

Conclusion

Precise identification of allergenic structures on penicillins and cephalosporins has important implications for both therapy and desensitization (immunotherapy) in β -lactam allergic patients. However, immunochemical and structural allergenic definitions of these two related families of drugs has not kept pace with the relatively high incidence of allergic reactions arising from the frequent and widespread use of these drugs. This is particularly true for the cephalosporins.

Identification of 'small' parts of drug molecules as allergenic determinants, and demonstration of the importance of steric factors in some cases of allergic recognition, has provided insight into the nature of allergic cross-reactions and the basis of patient allergic responses to structurally related, and sometimes apparently structurally unrelated, drugs.

Chemical definitions of cephalosporins as allergens are still lacking, and the recent demonstration of an allergenic degradation product reinforces the belief that the cephalosporins as a group will not necessarily parallel the so-called 'major' and 'minor' allergenic determinant structures seen with the penicillins. As with the penicillins, however, reaction conditions and storage will no doubt influence the nature of the resultant cephalosporin allergenic structures.

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

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