

Immediate allergic reactions to betalactams: facts and controversies

Miguel Blanca Gomez^a, Maria J. Torres^a, Critobalina Mayorga^a, Ezequiel Perez-Inestrosa^b, Rafael Suau^b, Maria I. Montañez^b and Carlos Juarez^c

Purpose of review

To analyse the available data in the field of immediate allergic reactions to β -lactams, with particular emphasis on more recent studies, and to comment on the future role of this group of antibiotics.

Recent findings

The world of β -lactams has become more complex than initially thought, due to the increased number of chemical structures available, the wide variety of indications for their use in treating different infectious diseases, and possibly also due to the interaction of other as yet undetermined factors. Benzyl penicillin, the original inducer of allergic reactions, has now largely been replaced by amoxicillin and, to a lesser extent, by cephalosporins in inducing IgE-mediated allergic reactions. These structures often share extensive cross-reactivity, eliciting clinical reactions to many compounds, especially amongst penicillins. In other circumstances selective responses are observed which are restricted to one group or one single compound, as occurs in the group of cephalosporins. The application of new determinants for skin testing and the use of adapted in-vitro studies have enabled these findings to be confirmed in detail.

Summary

Results indicate that evaluation of immediate reactions to β -lactams requires the use of several determinants for both in-vitro and in-vivo testing, and which must reflect the relevant drug involved in eliciting the response. This tendency will be strengthened in the future if use of benzyl penicillin continues to decrease as a drug to which populations are exposed.

Keywords

β -lactams, drug allergy, major determinant, minor determinant, cephalosporin

Curr Opin Allergy Clin Immunol 4:261–266. © 2004 Lippincott Williams & Wilkins.

^aAllergy Service, Carlos Haya Hospital, Malaga, Spain; ^bDepartment of Organic Chemistry, Faculty of Science, University of Malaga, Malaga, Spain; and ^cInternal Medicine Department, Carlos Haya Hospital, Malaga, Spain

Correspondence to Dr Miguel Blanca Gomez, Laboratorio de Investigacion, Pabellon 5, Planta sótano, Hospital Civil, Malaga ES-29008, Spain
E-mail: miguel.blanca.sspa@juntadeandalucia.es

Current Opinion in Allergy and Clinical Immunology 2004, 4:261–266

Abbreviations

BPO benzyl penicilloyl
HSA human serum albumin
RAST radioallergosorbent test

© 2004 Lippincott Williams & Wilkins
1528-4050

Introduction

Allergic reactions to β -lactam represent the most frequent cause of immunological drug reactions mediated by specific immunological mechanisms [1–3]. They constitute a model for understanding drug allergy and immunology [3] and have provided the largest number of manuscripts, resulting in many questions which have opened up new fronts [2,4]. Improvements in the production process of β -lactams aimed at reducing allergenicity and immunogenicity have been accompanied by a variation in the chemical structure, required to increase the antibiotic spectrum or bypass the action of β -lactams [2,5]. This has resulted in a number of reactions remaining important and new determinants being recognized by the immunological system, with the existence of cross-reactors and selective responders within both immediate IgE- and T-cell mediated responses. Although the genetic basis for this is unknown, the highly variable patterns of consumption from one country to another, with the common denominator of a high consumption of amoxicillin and the progressive disappearance of benzyl penicillin, influence the response [5].

An increasing number of manuscripts, many differing from the classical viewpoint, have contributed over the last two decades to changes in the views held on allergic reactions to β -lactam [6–17]. Although these include both clinical and basic research with IgE- and T-cell mediated responses [18], this review will focus exclusively on IgE-mediated responses.

Three periods can be identified from examination of the voluminous literature available. The first dates back to the 1940s, when a huge number of immunochemical studies identified the chemical structures which were candidates for recognition [2,19–21]. Very soon after, several reports of reactions appeared after their clinical use [22–25]. These led to a consistent understanding, which in turn has provided the background necessary to evaluate allergic reactions over several decades [4,24].

An initially slow, progressive change in the patterns of consumption, together with the introduction of new structures, produced a limited number of observations suggesting that a broader pattern of recognition was taking place [26–28]. The long-held classical dogma was that benzyl penicillin conjugates to a carrier, inducing

the benzyl penicilloyl (BPO) determinant. cross-reactivities between different penicillins were quite high, side-chain specific responses were negligible, in-vivo tests with major and minor determinants would provide the diagnosis in more than 90% of cases, and the oral route was not relevant in inducing an allergic reaction [4]. By the 1980s, however, a number of clinical observations, together with some immunochemical work, were questioning these views [6–17].

Because this classical view was held for so long, other possibilities were not initially considered seriously [2,26–28]. The generalized use of penicillin V in Scandinavian countries motivated the inclusion of penicillin V for in-vitro testing [29], but unfortunately no clinical studies were carried out to determine whether selective reactions occurred. The similarities between penicillin V and penicillin G, however, are not that significant so we use both drugs in our protocols for evaluating IgE responses to penicillin G [7]. The last period is characterized by variations in patterns of response from country to country, but as the same protocols were not used by all groups, comparison of results was hampered [15,18,30].

Up to now two main blocks of opinion have existed: the initial position still supported by American investigators who maintain the classical view [4] and the European experience, mostly provided by our group, whose opinions have been reflected in a recently published position paper [31,32••]. We will now look at some of these controversies and disagreements [33–35] and suggest ideas for using similar methodologies to evaluate a problem in which, despite differences in observations, a number of similarities do exist that are in agreement with the patterns of consumption of β -lactams [32••].

The clinical problem

Initial observations indicated that there were patients who developed severe anaphylactic reactions to amoxicillin but who had good tolerance to benzyl penicillin [6,7]. In-vitro studies indicated that these patients developed IgE antibodies which recognized preferentially the side chain structure [17,36,37]. Further observations indicated that skin testing with major and minor determinants was not sufficient to establish the diagnosis, thus invalidating the idea that if a patient had a positive history but a negative test, the likelihood of good tolerance was greater than 90% [4]. Another observation was that amoxicillin taken orally was sometimes a cause of anaphylaxis, contrary to published views [4,38].

Analysis of the consumption of antibiotics during this period showed that in 1995, amoxicillin was prescribed twice as much as erythromycin and tenfold more than ampicillin. Since 1984 the trends for antimicrobial drug

prescribing and penicillin G have decreased and the role of cephalosporins has increased [39]. In a recent study carried out in patients allergic to penicillins, amoxicillin was the drug most frequently reported as inducing allergic reactions [34]. This raises questions as to whether amoxicillin, a very widely used drug, induces different rates of reaction in allergic patients. Differences in prescription habits may account for a low number of cases, but these do not explain the overall pattern of response. It has therefore become necessary to include amoxicillin in routine skin testing [4,32••]. Because skin testing has no optimal sensitivity, in-vitro testing might be required as a complementary test [7,32••]. Even so, nearly 30% of patients will require controlled administration of the antibiotic [40,41], though we do not recommend controlled administration in patients who are highly susceptible [31,32••].

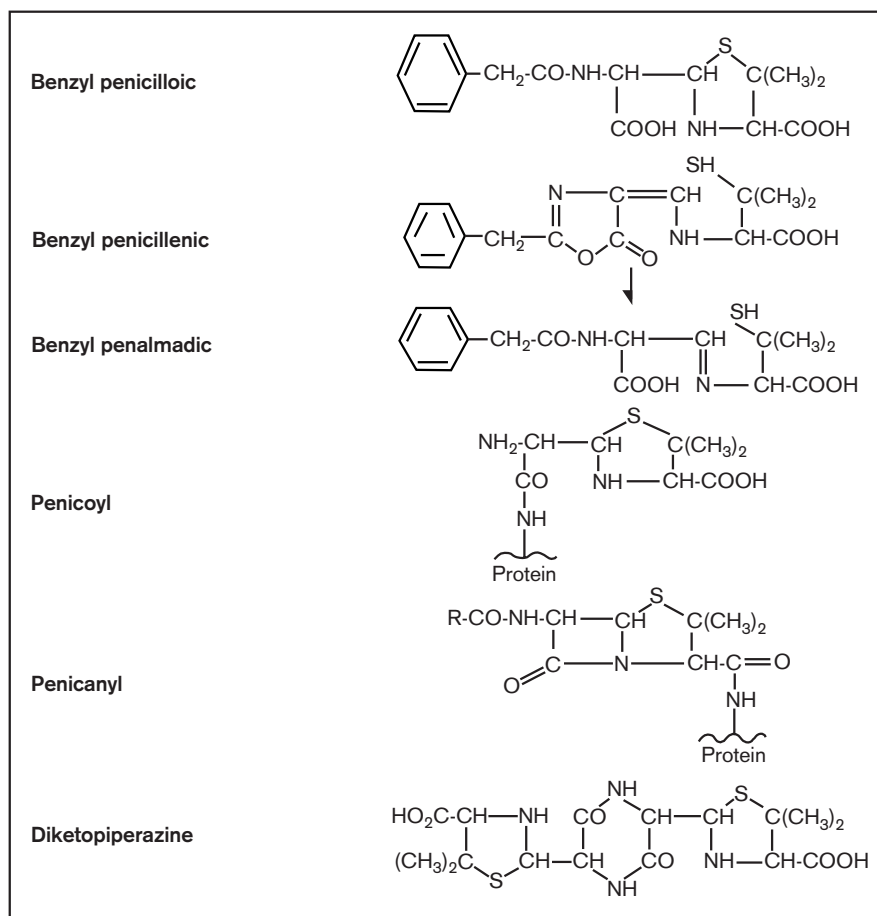
What are the penicillin determinants?

A critical issue in understanding immediate hypersensitivity reactions to β -lactams has been the definition of the epitope or the antigenic determinant [2,22,23,42]. From the outset, the best identified structure was the BPO determinant that was recognised by IgE and IgG antibodies (Abs) [22]. However, the generation by penicillin of different metabolites with different possibilities for conjugation led researchers to seek other determinants. The structures considered were benzyl penicilloic, benzyl penicillenilic, benzyl penamaldate, benzyl penaldate, benzyl penicoil and benzyl penicilanyl [2]. A detailed description is shown in Fig. 1. Advanced immunochemical analyses concerning IgE specificities are lacking. A recent study has shown that the equivalent structure to penicilloyl and penicanyl in penicillin V and amoxicillin did not contribute significantly to the recognition. When several combinations were used for studying in-vitro IgE antibodies, however, an improvement in the diagnostic sensitivity of the test was apparent [43].

In-vitro testing showed from the very beginning that a number of patients were skin test negative to BPO but positive to minor determinant mixture (MDM). The definition of minor determinants was made without an in-vitro immunochemical validation. Consequently, much confusion exists in the literature concerning comparison between the original minor determinants described and the in-vivo determinants used.

The concept of major and minor determinants has been associated with the appearance of urticaria and anaphylaxis, respectively [2]. It has been proposed that anaphylactic patients have IgE antibodies that mainly recognize the minor determinants whereas patients with urticaria have, in addition to IgE antibodies, IgG antibodies to BPO. We have been unable to corroborate

Figure 1. Proposed minor penicillin determinants



this, and the presence of IgG antibodies does not appear to distinguish between patients with anaphylaxis or urticaria and exposed controls [44].

What are the haptenic determinants in cephalosporins?

Because cephalosporins are subjected to nucleophilic attack at the same position as benzyl penicillin, the cephalosporoyl determinant was compared with the corresponding BPO [45]. Most of the information available comes from in-vitro studies [46]. Chemical differences in cephalosporins are recognized by IgE antibodies with different patterns [47]. However, large systematic studies including clinical data are unavailable. Experimental prototypes have recently been produced, but with no information about the specific technical details and with only single cases having been tested [48,49]. Only one series has been validated in which sera from different individuals included those with a response to cephalosporins [50]. This lack of knowledge hinders our understanding of cross-reactivity between penicillins and cephalosporins [51].

Despite structural similarities between penicillins and cephalosporins, both groups of drugs differ in the product obtained from the nucleophilic opening of the β -lactam ring. In penicillins the nucleophilic opening takes place very quickly and effectively, making a stable conjugate, but cephalosporins suffer an extensive fragmentation process in the dihydrothiazine portion, leading to a large number of degradation products [52]. The C7 substitution plays a dominant role in the formation of the epitope. A number of studies consider that R1 and R2 contribute to determinants and that these must be taken into account when assessing cross-reactivity. The fact that the R2 determinant is lost during conjugation, however, means it is impossible for this structure to contribute to the constitution of the antigenic determinant [47].

Lack of understanding of the exact chemical structure of cephalosporin antigenic determinants has hindered clinical interpretation of adverse reactions to these drugs and delayed our understanding of the mechanisms involved in the specific recognition and binding of IgE

molecules to these antigenic determinants. A series of chemically well defined structures comprising the entire acyl side chain R1 and the aminoacidic residue included in the β -lactam moiety of the cephalosporins have been synthesized and linked as amide functions to an aliphatic (*n*-butyric) chain. They were used to study the molecular basis of the recognition of different cephalosporins by IgE antibodies from patients allergic to cephalosporins. Results obtained in radioallergosorbent test (RAST) inhibition studies correlated well with skin tests. Fine structural recognition was detected between IgE antibodies in different sera. Thus, these synthesized compounds incorporate the appropriate epitope recognizable by IgE antibodies and provide the basis for understanding the pathway of cephalosporin conjugation to carrier proteins, to determine the structural requirements needed to be recognized by IgE antibodies, and, finally, to obtain an efficient in-vitro test to detect these antibodies. Modifications in the chemical structure are now underway in our laboratories to enhance the specificity and sensitivity of the recognition process.

The problem of cross-reactivity between β -lactams

Whether a patient with an allergic reaction to penicillins can safely take β -lactams is often questioned. Of these, cephalosporins are the most important, although other β -lactams may often be involved [5,31]. Most studies addressing the question of cephalosporins have been carried out by selecting more or less well defined groups who were originally allergic to penicillin. Thus, the question to be solved is what probability has a patient of tolerating a penicillin if he or she is allergic to cephalosporin. A major limitation is that these studies use a low number of cases with a rather heterogeneous selection of patients [53,54]. This problem is not negligible, because many patients who develop an IgE response to cephalosporin prove to be allergic to penicillin after completing the study. Furthermore, we have been unable to show the co-existence of sensitivity, and neither are we able to identify *a priori* which factors help in establishing cross-reactivity. It has been suggested that immunochemical studies may predict cross-reactivity [12,47], but this has not been fully corroborated [11,55]. IgE antibodies to BPO recognize other β -lactam structures, including monobactams, but in spite of this immunochemical recognition, good tolerance has been observed in patients who are highly sensitized to benzyl penicillin [53,56].

What do we expect from in-vitro studies?

One of the main problems we are now facing is that sensitivity of in-vivo and in-vitro tests is decreasing, and this tendency is expected to continue because of the reduction in the use of penicillin G. Another problem is that to achieve optimal sensitivity with amoxicillin

determinants, concentrations as high as 20 mg/ml must be used, with the consequent risk of systemic reactions [40,41]. New or improved in-vitro tests are therefore necessary. For many years, human serum albumin (HAS) has been used as an adequate carrier for in-vitro testing [29], but in order to find solid phase assays with a higher capacity for hapten fixation, poly-L-lysine or amino spacers have been successfully applied [36,37]. These in-vitro studies have limitations, however, because not all cases are diagnosed and because a very high number of cases still remain which are skin test positive but RAST negative [7,40,41]. Furthermore, some cases can be RAST positive but skin test negative, so that both approaches are often necessary for testing [57].

For interested clinicians and for many researchers, in-vitro studies have been used as a model for defining in detail many of the specificities described and also for understanding cross-reactivities. Our experience with large groups of sera indicates that three main groups of patients who are allergic to penicillins can be identified: those who recognize the common structure, those who recognize the side chain of penicillin with a very high degree of cross-reactivity with other penicillins, and those who mainly recognize the side chain of amoxicillin [58].

Limitations with both in-vivo and in-vitro studies have led investigators to propose alternative tests. The cellular allergosorbent test assay is based on the property of basophils releasing histamine and sulfidopeptide leukotrienes after in-vitro stimulation with allergens or drugs [59]. Although a number of studies have been carried out with allergens, sufficient experience is not yet available for IgE responses to β -lactams. Further evidence is now available, however, concerning the monitoring by flow cytometry of basophil activation. The number of cells can be quantitated by measuring IgE or its receptors and CD63 on the basophil surface and making a comparison with a control to establish whether the response is positive or negative [60]. This technique has been used in individual cases and limited series of drug allergy patients [61–63]. When used to study allergy to β -lactams, a number of studies indicate that sensitivity is similar or better than that for specific IgE determination [60]. Both Basotest and cellular allergosorbent test, however, are not as reproducible as immunoassays, making their routine use difficult. The second unresolved question concerns whether these tests are able to define selective or cross-reactive responders, as has been shown with in-vitro methods.

Is it possible to generate new in-vitro or in-vivo conjugates to improve understanding and diagnosis?

Efforts to characterize hapten carrier conjugates have been unable to provide information concerning the true

situation. Despite the initial use of HSA [29], it was soon ascertained that polylysine was very useful for in-vivo diagnosis [36,37], and therefore HSA has been replaced by other spacers [64]. This in-vitro conjugate, however, does not represent the original conjugate generated *in vivo*.

The approach developed by our group has been to create a structure with a high hapten density in the carrier [36,37]. The technology consists of the use of polyamidoamino dendrimers (G_nP , $n=0-6$), with a globular structure similar to HSA, which can be densely penicilloylated with precisely defined chemical structures. This represents significant progress compared with conventional benzyl penicilloyl conjugates (BPO-HSA and BPO-polylysine) [65]. Preliminary RAST inhibition studies have confirmed the usefulness of penicilloylated polyamidoamino dendrimers as conjugates for the recognition of IgE antibodies from patients allergic to penicillin. We are now ready to evaluate the relevance of the size of the hapten-carrier conjugate and hapten density profile in recognition, simply by changing the generation of the starting dendrimer. Thus, presenting many penicillin determinants to specific IgE antibodies using structures able to carry many hapten molecules would increase in-vitro sensitivity. Consequently, these compounds can be considered promising candidates for use in the development of a new in-vitro test, with increased sensitivity for the diagnosis of allergy to β -lactam antibiotics compared with the tests used until now. Three-dimensional architecture is a factor of remarkable importance due to the strict requirement for optimal recognition processes. To exploit more widely the biological advantages of large molecule drug-polymer conjugates and to examine more thoroughly how structure, molecular weight, and spatial architecture correlate with biological profile it is essential to develop definite conjugates that have a restrained molecular weight composition, the same molecular distribution, and uniform pendent structure [66]. Currently, we have been successful in developing an in-vitro assay with this structure and the possibility of conjugating different penicillin determinants simultaneously, which will permit improvement of the in-vitro assay. To create an in-vitro conjugate that mimics the original, however, is a difficult task and at present we are far from finding a solution.

Conclusion

Allergy to β -lactams, the most important cause of allergic drug reactions, has become a more complicated problem due to the contribution of different chemical structures in inducing clinically relevant specificities. This has been extensively demonstrated for IgE-mediated reactions, leading to revision of the role of current in-vitro and in-vivo tests for the diagnosis of immediate reactions.

Nevertheless, further re-evaluation and readaptation analyses need to be carried out. Variations in the patterns of recognition between different countries mean this research must be undertaken by different groups.

Because β -lactams are used to treat different processes and denial of these drugs may have a detrimental effect, falsely labelling patients as allergic or nonallergic should be avoided wherever possible by application of the knowledge acquired. Advances made over recent years enable a more rational approach to this problem, although further clinical and laboratory research, together with a broader view of the situation, is still required in order to solve daily diagnostic problems. Bearing in mind the progress made over the last decade, the future looks promising, provided several groups work on the problem and researchers are able to generalize the practice of evaluating allergic reactions to β -lactams in accordance with these recommendations.

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
- 1 Amos HE. The problem of adverse drug reactions. In: Amos HE, editor. Allergic drug reactions. London: Arnold; 1976. pp. 1-5.
 - 2 Dewdney JM. Immunology of the antibiotics. In: Sela M, editor. The antigens, vol 5. New York: Academic Press; 1977. pp. 173-245.
 - 3 Ahlstedt S, Kristofferson A. Immune mechanism for induction of penicillin allergy. *Prog Allergy* 1982; 30:67-134.
 - 4 Weis ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 1988; 18:515-540.
 - 5 Blanca M, Vega JM, García J, et al. New aspects of allergic reactions to betalactams: cross-reactions and unique specificities. *Clin Exp Allergy* 1994; 24:407-415.
 - 6 Blanca M, Perez E, Garcia J, et al. Anaphylaxis to amoxicillin but good tolerance for benzylpenicillin. *Allergy* 1988; 43:508-510.
 - 7 Blanca M, Vega JM, García J, et al. Allergy to amoxicillin with good tolerance to other penicillins: study of the incidence in patients allergic to betalactams. *Clin Exp Allergy* 1990; 20:475-481.
 - 8 Fernandez-Rivas M, Perez Carral C, Cuevas M, et al. Selective allergic reactions to clavulanic acid. *J Allergy Clin Immunol* 1995; 95:748-750.
 - 9 Candarso AI, Saez Jiménez SA, Vidal C, Rodríguez M. Aztreonam induced anaphylaxis. *Lancet* 1990; ii:746-747.
 - 10 Marcos Bravo C, Luna Ortiz I, Gonzalez Vazquez R. Hypersensitivity to cefuroxime with good tolerance to other betalactams. *Allergy* 1995; 50:359-361.
 - 11 Baldo BA, Harle DG. Identification of penicillin allergenic determinants that bind IgE in the sera of patients with penicillin allergy. *Mol Immunol* 1990; 27:1063-1071.
 - 12 Baldo BA. Penicillins and cephalosporins as allergens: structural aspects of recognition and cross-reactions. *Clin Exp Allergy* 1999; 29:744-749.
 - 13 Romano A, Blanca M, Mayorga C, et al. Immediate hypersensitivity to penicillins: studies on Italian subjects. *Allergy* 1997; 52:89-93.
 - 14 Demoly P, Messaad D, Sahla H, et al. Immediate hypersensitivity to ceftriaxone. *Allergy* 2000; 55:418-419.
 - 15 Audicana M, Bernal G, Urrutia I, et al. Allergic reactions to betalactams: studies in a group of patients allergic to penicillin and evaluation of cross-reactivity with cephalosporin. *Allergy* 1994; 49:108-113.

- 16 Sastre J, Quijano LD, Novalbos A, *et al.* Clinical cross-reactivity between amoxicillin and cephadroxil in patients allergic to amoxicillin and cephadroxil in patients allergic to amoxicillin and with good tolerance of penicillin. *Allergy* 1996; 51:383–386.
- 17 Blanca M, Mayorga C, Sanchez F, *et al.* Differences in serum IgE antibody activity to benzylpenicillin and amoxicillin measured by RAST in a group of penicillin allergic subjects. *Allergy* 1991; 46:632–638.
- 18 Blanca M. Allergic reactions to penicillins: a changing world? *Allergy* 1995; 50:777–782.
- 19 Torres MJ, Blanca M. Letter to the editor. *Allergy* 2003; 58:452.
- 20 Batchelor FR, Dewdney JM, Gazzard D. Penicillin allergy: the formation of the penicilloyl determinant. *Nature* 1965; 206:362–364.
- 21 Parker CW, de Weck AL, Shapiro J, *et al.* The preparation and some properties of penicillenic acid derivatives relevant to penicillin hypersensitivity. *J Exp Med* 1962; 115:803–819.
- 22 Levine BB, Price VH. Studies on the immunological mechanisms of penicillin allergy: II. Antigenic specificities of allergic wheal-and-flare skin responses in patients with histories of penicillin allergy. *Immunology* 1964; 36:542–56.
- 23 de Weck AL. Immunopathological mechanisms and clinical aspects of allergic reactions to drugs. In: de Weck AL, Bundgaard H, editors. *Allergic reactions to drugs*. Berlin: Springer; 1983. 63:75–133.
- 24 Parker CW. Drug allergy. *N Engl J Med* 1975; 292:732–736.
- 25 Adkinson NF Jr, Thompson WL, Maddrey WC, Lichtenstein L. Routine use of penicillin skin testing on an inpatient service. *N Engl J Med* 1971; 285:22–24.
- 26 Green GR, Roseblum AH, Sweet LC. Evaluation of penicillin hypersensitivity: value of clinical history and skin testing with penicilloyl-polylysine and penicillin G. A cooperative prospective study of the Penicillin Study Group of the American Academy of Allergy. *J Allergy Clin Immunol* 1977; 60:339–345.
- 27 Van Dellen RG, Gleich GJ. Penicillin skin tests as predictive and diagnostic aids in penicillin allergy. *Med Clin North Am* 1970; 54:997–1007.
- 28 Solley GO, Gleich GJ, Van Dellen RG. Penicillin allergy: clinical experience with a battery of skin test reagents. *J Allergy Clin Immunol* 1982; 69:238–244.
- 29 Juhlin L, Wide L. Detection of penicillin allergy by radioallergosorbent test. *Lancet* 1979; 2:261.
- 30 Ahlstedt S, Kristofersson A. Experimental evidence of decreased incidence of penicillin allergy through the use of pure penicillins. *Infection* 1979; 7:499–502.
- 31 Blanca M, Mayorga C, Torres MJ, *et al.* Side chain specific reactions to betalactams: 14 years later. *Clin Exp Allergy* 2002; 32:192–197.
- 32 Torres MJ, Blanca M, de Weck A, *et al.*, ENDA and the EAACI Interest Group on Drug Hypersensitivity. Diagnosis of immediate allergic reactions to betalactam antibiotics. *Allergy* 2003; 58:961–972.
- The position statement of the ENDA Committee of the European Academy of Allergy is provided in this manuscript, including recommendations and algorithms for evaluating patients with immediate allergic reactions to β -lactams.
- 33 Adkinson NF. Side-chain specific beta-lactam allergy. *Clin Exp Allergy* 1990; 20:445–447.
- 34 Macy E, Burchette RJ. Oral antibiotic adverse reactions after penicillin skin testing: multi-year follow-up. *Allergy* 2002; 57:1151–1158.
- 35 Blanca M, Torres MJ, Mayorga C, Radial A. Penicillin allergy is a global problem: the European experience. *J Allergy Clin Immunol* 2003; 112:1014–1015.
- 36 Blanca M, Mayorga C, Perez E, *et al.* Determination of IgE antibodies to the benzylpenicilloyl determinant: a comparison between poly-L-lysine and human serum albumin as carriers. *J Immunol Methods* 1992; 153:99–105.
- 37 Blanca M, Moreno F, Mayorga C, *et al.* The nature of the carrier in the RAST assay influences the capacity for detecting IgE antibodies to penicillins. *J Clin Immunoassay* 1994; 17:166–170.
- 38 Macy E, Ritcher PK, Falkkoff R, Zeiger R. Skin testing with penicilloate and peniloate prepared by an improved method: amoxicillin oral challenge in patients with negative skin tests responses to penicillin reagents. *J Allergy Clin Immunol* 1997; 100:586–591.
- 39 McCaig L, Hughes JM. Trends in antimicrobial drug prescribing among office-based physicians in the United States. *JAMA* 1995; 273:214–219.
- 40 Torres MJ, Romano A, Mayorga C, *et al.* Diagnostic evaluation of a large group of patients with immediate allergy to penicillins: the role of skin testing. *Allergy* 2001; 56:850–856.
- 41 Torres MJ, Mayorga C, Leyva L, *et al.* Controlled administration of penicillin to patients with a positive history but negative skin and specific IgE antibodies. *Clin Exp Allergy* 2002; 32:270–276.
- 42 Batchelor FR, Dewdney JM, Feinberg JG, Weston RD. A Penicilloylated protein impurity as a source of allergy to benzylpenicillin and 6 aminopenicillanic acid. *Lancet* 1967; 1:1175–1177.
- 43 Daxon Z, Adriansson J, Martin A. Diagnostic capabilities of penicillin-based structures as tools in the in vitro diagnosis of penicillin allergy. *J Invest Clin Immunol* 1992; 2:240–246.
- 44 Torres MJ, Mayorga C, Pamies R, *et al.* Immunologic response to different determinants of benzylpenicillin, amoxicillin and ampicillin: comparison between urticaria and anaphylactic shock. *Allergy* 1999; 54:936–943.
- 45 Hamilton-Miller JMT, Newton GGF, Abraham EP. Products of aminolysis and enzymic hydrolysis of the cephalosporins. *Biochem J* 1970; 116:371–384.
- 46 Hamilton-Miller JMT. Changes in proton magnetic-resonance spectra during aminolysis and enzymic hydrolysis of cephalosporins. *Biochem J* 1970; 116:385–395.
- 47 Harle DG, Baldo BA. Drugs as allergens: an immunoassay for detecting IgE antibodies to cephalosporins. *Int Arch Allergy Appl Immunol* 1990; 92:439–444.
- 48 Romano A, Quarantino D, Venuti A. Selective immediate allergic response to cefuroxime. *J Allergy Clin Immunol* 1998; 101:564–565.
- 49 Romano A, Quarantino D, Venemalm L, *et al.* A case of immediate IgE hypersensitivity to ceftriaxone. *J Allergy Clin Immunol* 1999; 104:1113–1114.
- 50 Romano A, Mayorga C, Torres MJ, *et al.* Immediate allergic reactions to cephalosporins: cross-reactivity and selective responses. *J Allergy Clin Immunol* 2000; 106:1177–1183.
- 51 Romano A, Quarantino D, Aimone-Gastin I, *et al.* Cephalosporin allergy: characterization of unique and cross-reacting cephalosporin antigens. *Int J Immunopathol Pharmacol* 1997; 10:187–191.
- 52 Sánchez-Sancho F, Perez-Inestrosa E, Suau R, *et al.* Synthesis, characterization and immunochemical evaluation of cephalosporin antigenic determinants. *J Mol Recognit* 2003; 16:148–156.
- 53 Blanca M, Fernández J, Miranda A, *et al.* Cross-reactivities between penicillins and cephalosporins: clinical and immunological studies. *J Allergy Clin Immunol* 1989; 83:381–385.
- 54 Anne S, Reisman RE. Risk of administering cephalosporin antibiotics to patients with histories of penicillin allergy. *Ann Allergy* 1995; 74:167–170.
- 55 Baldo B, Pham NH. Detection and side-chain specificity of IgE antibodies to flucloxacillin in allergic subjects. *J Mol Recognit* 1995; 8:1–7.
- 56 Miranda A, Blanca M, Vega JM, *et al.* Cross-reactivity between a penicillin and a cephalosporin with the same side chain. *J Allergy Clin Immunol* 1996; 98:671–677.
- 57 Torres MJ, Mayorga C, Cornejo-Garcia JA, *et al.* IgE antibodies to penicillin in skin test negative patients. *Allergy* 2002; 57:963.
- 58 Moreno F, Blanca M, Mayorga C, *et al.* Studies of the specificities of IgE antibodies found in sera from subjects with allergic reactions to penicillins. *Int Arch Allergy Immunol* 1995; 108:74–81.
- 59 Bircher AJ, Haldiman D, Weber J. CAST-ELISA testing for the diagnosis of immediate-type allergic reactions to betalactam antibiotics. *ACI Int* 1996; 8:109–110.
- 60 Sanz ML, Sánchez G, Vila L, *et al.* Ag-induced basophil activation: CD63 cell expression detected by flow cytometry in patients allergic to Dermatophagoides pteronyssinus and Lolium perenne. *Clin Exp Allergy* 2001; 31:1007–1013.
- 61 Monneret G, Gutowski MC, Bienvenu J. Detection of allergen-induced basophil activation by expression of CD63 antigen using a tricolour flow cytometric method. *Clin Exp Immunol* 1999; 115:393–396.
- 62 Abuaf N, Rajoely B, Ghazouani E, *et al.* Validation of flow cytometry assay detecting in vitro basophil activation for the diagnosis of muscle relaxant allergy. *J Allergy Clin Immunol* 1999; 104:411–418.
- 63 Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. *Clin Exp Allergy* 1997; 27:175–181.
- 64 Parker CW, de Weck AL, Shapiro J, *et al.* The preparation and some properties of penicillenic acid derivatives relevant to penicillin hypersensitivity. *J Exp Med* 1962; 115:803–819.
- 65 Sanchez-Sancho E, Perez-Inestrosa E, Suau R, *et al.* Dendrimers as carrier protein mimetics for IgE antibody recognition: synthesis and characterisation of densely penicilloylated dendrimers. *Bioconjugate Chem* 2002; 13:647–653.
- 66 Perez-Inestrosa E, Suau R, Blanca M, *et al.* Multivalent hapten-carrier complexes with dendrimers as mimetic carrier protein. Patent No. P200302737; 2003.