

REVIEW

Penicillins and cephalosporins as allergens — structural aspects of recognition and cross-reactions

B. A. BALDO

Molecular Immunology Unit, Kolling Institute of Medical Research, Royal North Shore Hospital of Sydney and Department of Medicine, University of Sydney, Sydney, New South Wales, Australia

Although a wide range of chemically different antibacterials are used clinically, the β -lactam drugs, principally the penicillins and cephalosporins, are the most commonly prescribed antibiotics and the most common drug allergies seen clinically.

Penicillins

'Major' and 'minor' determinants

From the perspective of drug allergy, penicillins are the best known and most intensively studied β -lactam antibiotics. All penicillins contain both a β -lactam and a thiazolidine ring and individual penicillins are distinguished by the nature of the side-chain group R (Fig. 1). Extensive investigations over a period of more than 30 years [1–4] identified a number of ultimately proven, and some putative, antigenic determinants and, on the basis of ring modifications and the point of attachment of protein carrier, determinants have been designated 'major' or 'minor' [5] (Fig. 1). These are quantitative terms referring to the amounts of drug conjugates formed. Approximately 95% of penicillin molecules that become covalently bound to protein under physiological conditions form penicilloyl groups and it is this quantitative predominance rather than clinical or immunological importance that the term 'major' refers to. A variety of other conjugates including penicillenate, penicilloic acid, penicillanyl, penamaldate, penaldate, D-penicillamine and penicoyl are classified as 'minor' determinants and although the direct involvement of some of these determinants in provoking immediate hypersensitivity reactions and IgE responses in man has not been demonstrated [4,6], it has been claimed that immediate reactions to penicillins are more often a response to 'minor' than to 'major' determinants [5,7].

Spectrum of penicillin determinants

In recent years, a view of penicillin allergens based on fine

structural detail of the IgE antibody-binding determinants has been advanced [6,8–10]. This approach, which is not necessarily incompatible with the view of penicillin antigens as differently linked protein-bound conjugates, has built up a picture of penicillin allergenic determinants as a spectrum of specificities covering the entire molecule and varying from discrete side chain [8–11] and thiazolidine ring determinants to compound antigens embracing either, or both, of these groupings with the β -lactam ring [8,9].

Specific detection of side-chain determinants

Since it is the side-chain group that distinguishes the many different penicillins, and since the side-chain groups are recognized by IgE antibodies in many allergic reactions, it is clear that specific tests for individual penicillins are often required rather than simply employing 'major' and 'minor' determinant preparations made from benzylpenicillin. This was demonstrated in a recent study where prick test and immunoassays established that skin and serum reactivities were directed toward the side-chain determinant of a family of structurally closely related compounds including flucloxacillin, the drug responsible for provoking the clinical reactions. Penicillins with structurally unrelated side-chains such as benzylpenicillin, phenoxymethylpenicillin, ampicillin, ticarcillin, etc. did not react [10] (Fig. 2). These results accord with the findings of Solley *et al.* [12] who showed that a battery of different penicillins detected an increased number of skin test-positive subjects and Moss *et al.* [13] who found that some patients with cystic fibrosis had positive skin tests to ticarcillin and piperacillin even when skin tests to benzylpenicillin and penicilloyl-polylysine were negative. Moss *et al.* [13] concluded that these patients appeared to be distinguished by development of side-chain-specific IgE antibodies rather than antibodies to metabolites of benzylpenicillin. More recently, IgE antibody reactivity, as measured by RAST and skin tests, was shown to be directed to the acyl side-chain of amoxicillin and the allergenic importance of side-chain determinants on penicillins has been emphasized by Blanca *et al.* [11,14].

Correspondence: B. A. Baldo, Molecular Immunology Unit, Kolling Institute of Medical Research, Royal North Shore Hospital of Sydney, St Leonards NSW, 2065, Australia.

Clinical consequences of the heterogeneity of penicillin allergenic determinants

Since all penicillins share the β -lactam and thiazolidine rings, allergenic recognition of these structures means that all penicillins have the potential to cause an allergic reaction in sensitive subjects. A further requirement for reaction may be determined by the point of linkage of the drug to the carrier, for example attachment to protein via opening of the β -lactam ring in the penicilloyl determinant or via coupling through the thiazolidine ring carboxyl in the penicillanyl determinant (Fig. 1).

Some reactions, however, are a consequence of side-chain group recognition with or without the participation of all or part of the adjacent β -lactam ring. Since side-chain structures, unlike the ring structures, distinguish the many different penicillins, such reactions therefore usually show restricted cross-reactivity or absence of cross-reactivity with other penicillins. A consequence of these findings and conclusions is that individual skin prick tests and RIAs will be needed for the direct detection of IgE antibodies to the many different penicillins, that is, employment of skin tests and solid phases for only penicillins G and V, for example, will miss many penicillin-reactive IgE antibodies and clinical sensitivities.

With the above conclusions in mind, it is clear that with a knowledge of the structures of the different penicillins, in particular the side-chain structures, it may be possible to avoid likely cross-reactions in some penicillin-allergic subjects. For

example, dicloxacillin, flucloxacillin and oxacillin would be prescribed with caution in a subject known to react to cloxacillin. Likewise, cross-sensitivity may exist between ampicillin and amoxicillin and, in the absence of skin test or RIA findings, a penicillin with a structurally different side chain may be selected. Of course, if the latter reaction was due to recognition of regions other than the side-chain, all penicillins, not only ampicillin and amoxicillin, may have to be avoided. Figure 3 sets out structural similarities and differences of side-chains found on a range of penicillins.

Cephalosporins

Studies on the cephalosporins as allergens have been nowhere near as numerous and intense [4,15,16] and the determinants on cephalosporins causing allergic reactions in man are in the early stages of study [17]. Consequently, questions on allergenic cross-reactivities between cephalosporins, and between cephalosporins and penicillins, which often arise cannot currently be answered with a great deal of confidence and this raises difficulties in the selection of antibiotics for some penicillin- and/or cephalosporin-allergic subjects. It has been said that antibodies to side chain structures on cephalosporins appear to be immunologically important and, compared with penicillins, there appears to be less cross-reactivity among cephalosporins and even less cross-reactivity between cephalosporins and penicillins [15]. It should be noted that cross-reactions between cephalosporins and penicillins have been

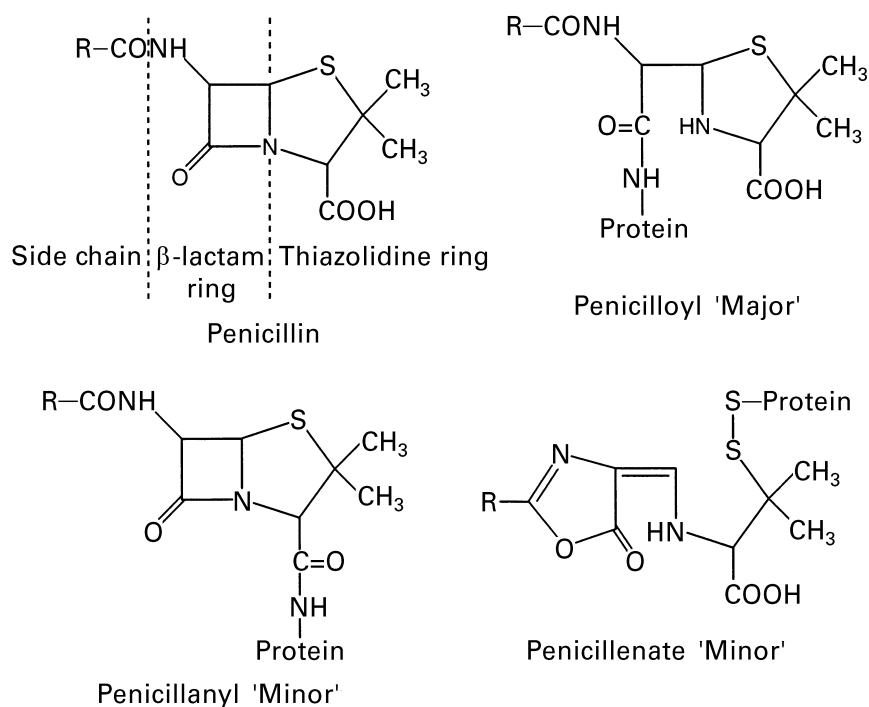


Fig. 1. General structure of penicillins (top, left) and structures of the so-called 'major' and two of the 'minor' penicillin antigenic-allergenic determinants showing points of attachment of the drug to the carrier.

R group recognized R group not recognized

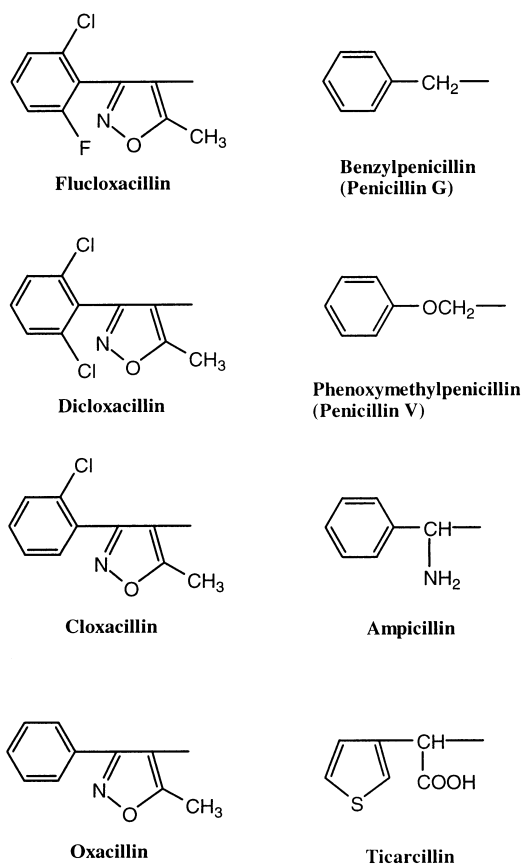


Fig. 2. Example of specific allergic recognition of methyl-phenyl-isoxazolyl side-chain determinants on penicillin by IgE antibodies in the sera of subjects who reacted to flucloxacillin [10].

observed in both laboratory animals [18–20] and in man [21–24]. In support of side chain recognition, the 2-thiophene group of cephalothin with particularly strong recognition of the methylene substituent was implicated in binding to IgE antibodies from allergic subjects [25].

Recognition patterns of cephalosporin-reactive IgE antibodies

A recent study [17] has shown that subjects allergic to the widely used cephalosporin antibiotic cefaclor have serum IgE antibodies that react with the drug. Quantitative hapten inhibition studies employing sera from subjects allergic to cefaclor revealed fine structural recognition differences between the combining site specificities of cefaclor-reactive IgE antibodies in the sera of different subjects. Unlike penicillins, where discrete side chain or thiazolidine ring determinants alone may be recognized,

IgE-binding determinants on cefaclor encompassed the entire molecule. Fine structural recognition specificity differences at positions R₁ (side chain) and R₂ (substituent attached to dihydrothiazine ring) (Fig. 4) were detected between IgE antibodies in different sera. Some antibodies showed clear preferential recognition of the aminobenzyl group at position R₁ and Cl at R₂ while with others, a greater degree of recognition tolerance was seen at R₁ where, for example, the aminohydroxybenzyl or aminodihydrobenzyl groups were recognized, and at R₂ where a methyl or even an ester group was tolerated. Three main IgE antibody recognition patterns were distinguished reflecting the fine structural recognition differences found in the population of cephalosporin-reactive antibodies studied. The three main groups were: group 1 — clear preferential recognition of cefaclor alone, group 2 — cefaclor and cephaloglycin preferentially recognized and group 3 — equal recognition of cefaclor, cephadrine and cefadroxil. For structures of side-chains on different cephalosporins see Fig. 4.

It already seems clear then that, as with the penicillins, cephalosporins as allergens cannot simply be considered as a group of compounds with a common allergenic determinant structure. IgE antibodies that bind to cefaclor show great heterogeneity indicated by clear, fine structural differences in recognition of the R₁ and R₂ side-chain groups on the drug (Fig. 4).

Summary and conclusions

Both *in vivo* (skin tests) and *in vitro* (immunoassays) tests may be employed together with a carefully gathered case history to aid the diagnosis of suspected immediate hypersensitive (IgE-mediated) reactions to a wide variety of penicillins and cephalosporins.

Skin prick testing is considered the safest and most specific, but not necessarily the most sensitive procedure (for example, in comparison with intradermal injection), but there is no general and widespread agreement yet on the selection of drug antigens and haptens that should be employed. The 'major' determinant (penicilloyl) and 'minor' determinant mix (generally prepared from benzylpenicillin [penicillin G]), may be employed but such a selection frequently takes no account of reactions specifically due to the side chain determinants on individual penicillins and cephalosporins. In some cases, the free individual drug may be employed as a skin test agent to detect IgE antibodies reactive with the side chain group (see for example [10]) but a positive reaction to the free drug may not always occur in a sensitive subject and the mechanism for positive skin reactions to the unconjugated drug remains obscure. A better selection for a panel of skin tests for subjects suspected of being allergic to a penicillin might be the 'major' determinant, and a 'minor'

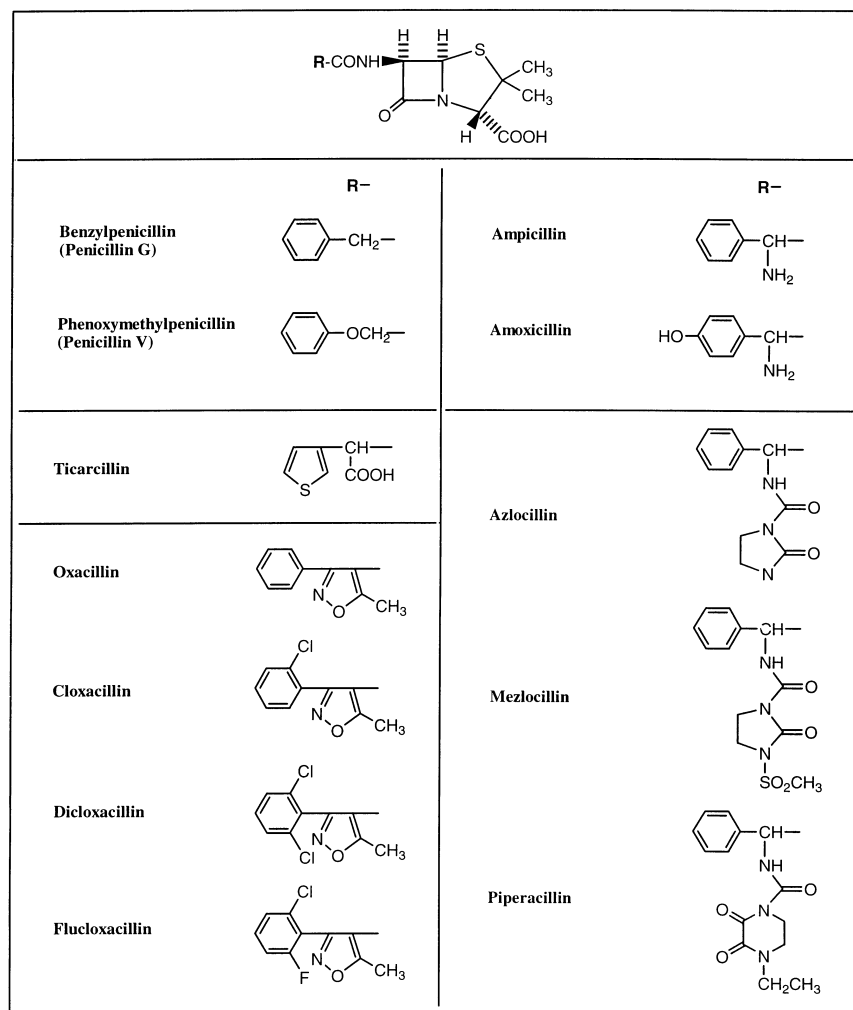


Fig. 3. Structural similarities and differences of penicillin.

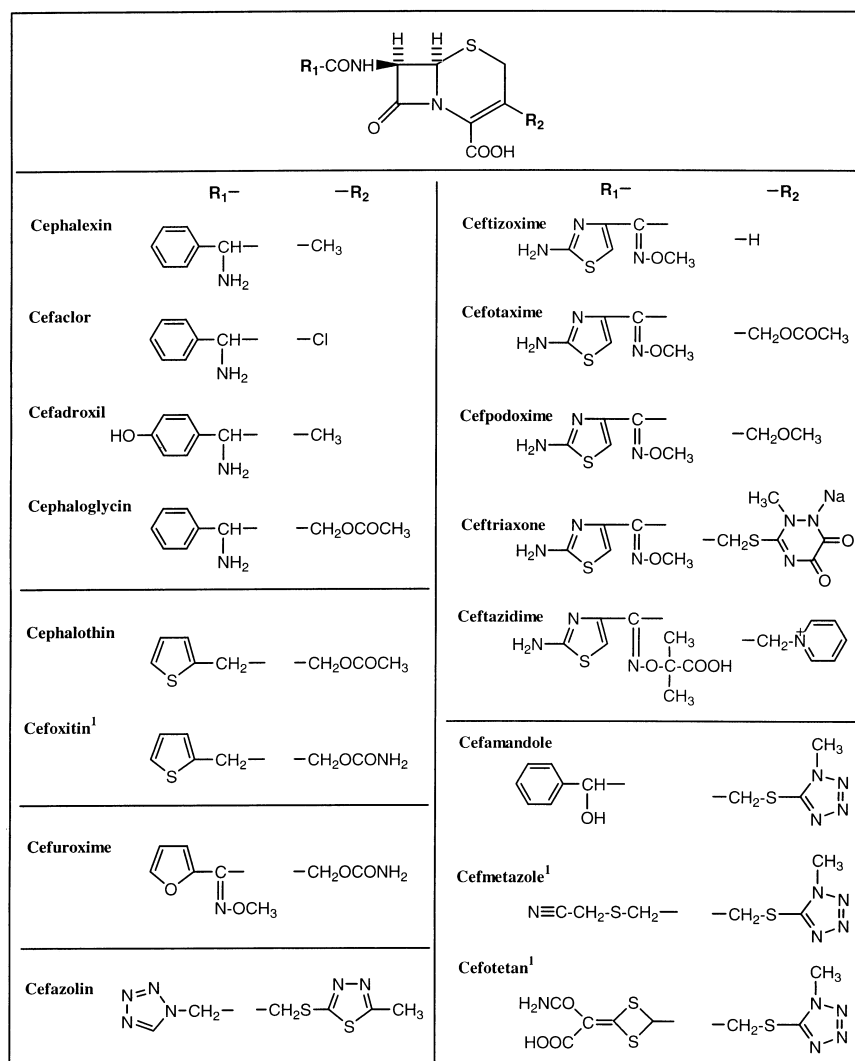
determinant, for example, the penicillanyl specificity, prepared from benzylopenicillin and/or the particular penicillin suggested by the history. Analogous preparations may be suitable for the cephalosporins but little information is available at present.

β -lactam conjugates may be prepared from poly-L-lysine [3] or human serum albumin and although penicilloyl-polylysine has been employed for skin testing for many years, other penicillin antigens, for example, the penicillanyl specificity, have not been widely used *in vivo* or *in vitro*. Routine and widespread use of conjugated β -lactam antigens will require standardized methods of preparation and adequate characterization, including determination of the number of haptenic groups per mole of carrier. Procedures currently employed in our laboratory for characterization of penicilloyl and penicillanyl antigenic conjugates with polylysine include penamaldite assay [26], determination of free amino groups in the conjugate [27,28], the employment of radio-labelled drug as tracer and nuclear

magnetic resonance spectroscopy [29, Batley, Zhao, Baldo, unpublished]. Interestingly, IgE antibodies that react with the penicillanyl, but not the penicilloyl, determinant are found in the sera of some subjects with symptoms and/or a history of penicillin allergy [Zhao, D'Ambrosio, Baldo, unpublished]. This finding highlights the need for a systematic clinical and laboratory study of the relevance and relative importance of the penicillanyl, and a range of 'minor' determinants.

With respect to allergenic cross-reactivity of penicillins, all penicillins share the β -lactam and thiazolidine rings and antibodies to these structures may react with all penicillins. Cross-reactions will generally not occur when antibodies recognize side chain groups since the side chains distinguish the different penicillins. Cross-reactions via side chain recognition, however, may occur where there are structural similarities, for example, ampicillin and amoxicillin or flucloxacillin and cloxacillin.

Immediate allergic reactions to cephalosporins and the



¹ Cephamycin with an α -methoxy group ($-\text{OCH}_3$) at the 7-position

Fig. 4. Structural similarities and differences of cephalosporins.

nature of cephalosporin allergens are far less studied and understood but the incidence of reactions is unlikely to be less than the penicillins and our *in vitro* tests shows the incidence to be significantly higher [Zhao, Pham, Baldo, unpublished]. IgE antibody reactivity has been demonstrated with both ends of the cephalosporins molecule, that is, the side chain group (R_1 substituent) and the ring structures with the attached R_2 group [17]. As with penicillins, antigens for testing may be prepared by coupling via an opened β -lactam ring or the dihydrothiazine ring carboxyl, conjugation through the ring S or, when a suitable functional group is present, linkage of carrier to the side chain group.

The principles of allergenic cross-reactions between cephalosporins are similar to the penicillins, viz. recognition of the core ring structures may result in cross-reactivity with

all cephalosporins regardless of side chain structure but because the R_2 , as well as the R_1 , side chain group may differ between different cephalosporins, the situation is more complicated. Cross-reactions may occur via R_1 recognition where side chain groups are the same (for example cefaclor, cephalexin and cephaloglycin) or similar (cefaclor and cefadroxil) or they may be contributed to by R_2 recognition (cephalexin and cephradine, cephalothin and cefotaxime).

The β -lactam ring, and sometimes the side chain group, are structural features common to penicillins and cephalosporins (for example ampicillin and cefaclor) so, theoretically at least, allergenic cross-reactions between some penicillins and cephalosporins seem likely. However, studies so far have been too few to provide reliable and detailed data for this question. Even so, where an allergic sensitivity

to a particular penicillin or cephalosporin is known or suspected, substitution of an alternative β -lactam should only be done after a careful consideration of structural similarities and differences.

The selection of penicillin and cephalosporin antigens currently available for allergic diagnostic use is too narrow and does not always permit the detection of specific sensitivities to individual β -lactams. More conjugates for skin prick testing in particular should be trialed and made available. The relevance and efficacies of skin prick tests and *in vitro* immunoassays as diagnostic tests with the expanded range of test reagents should be compared.

References

- Levine BB. Immunochemical mechanisms of drug allergy. In: Miescher PA, Muller-Eberhard HJ, eds. Textbook of Immunopathology, Vol. 1, 2nd edn. New York: Grune and Stratton, 1976:403–19.
- Dewdney JM. Immunology of the antibiotics. In: Sela M, ed. The Antigens, Vol. IV. New York: Academic Press, 1977:73–228.
- Parker CW. Drug allergy. In: Parker CW, ed. Clinical Immunology, Vol. II. Philadelphia: Saunders, 1980:1219–60.
- de Weck AL. Penicillins and cephalosporins. In: de Weck AL, Bundgaard H, eds. Allergic Reactions to Drugs Berlin: Springer, 1983:423–82.
- Levine BB. Immunologic mechanisms of penicillin allergy. A haptenic model system for the study of allergic diseases in man. *N Engl J Med* 1966; 275:1115–25.
- Baldo BA, Harle DG. Drug allergenic determinants. In: Baldo BA, ed. Molecular Approaches to the Study of Allergens Monographs in Allergy, Vol. 28, Basel: Karger, 1990:11–51.
- Levine BB, Redmond AP. Minor haptenic determinant-specific reagents of penicillin hypersensitivity in man. *Int Arch Allergy Appl Immunol* 1969; 35:445–55.
- Harle DG, Baldo BA. Identification of penicillin allergenic determinants that bind IgE antibodies in the sera of subjects with penicillin allergy. *Molec Immunol* 1990; 27:1063–71.
- Baldo BA, Pham NH. Structure-activity studies on drug-induced anaphylactic reactions. *Chem Res Toxicol* 1994; 7:703–21.
- Baldo BA, Pham NH, Weiner J. Detection and side-chain specificity of IgE antibodies to flucloxacillin in allergic subjects. *J Molec Recognition* 1995; 8:171–7.
- Blanca M, Vega JM, Garcia J et al. Allergy to penicillin with good tolerance to other penicillins; study of the incidence in subjects allergic to beta-lactams. *Clin Exp Allergy* 1990; 20:475–81.
- Solley GO, Gleich GJ, Van Dellen RG. Penicillin allergy: Clinical experience with a battery of skin-test reagents. *J Allergy Clin Immunol* 1982; 69:238–44.
- Moss RB, Rabin S, Hsu Y-P, Blessing-Moore J, Lewiston NJ. Allergy to semisynthetic penicillins in cystic fibrosis. *J Pediatr* 1984; 104:460–6.
- Blanca M, Vega JM, Garcia J, Miranda A, Carmona MJ, Juarez C. New aspects of allergic reactions to betalactams: cross-reactions and unique specificities. *Clin Exp Allergy* 1994; 24:407–15.
- Kishiyama JL, Adelman DC. The cross-reactivity and immunology of β -lactam antibiotics. *Drug Safety* 1994; 10:318–27.
- Weiss ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 1988; 18:515–40.
- Pham NH, Baldo BA. β -Lactam drug allergens: Fine structural recognition patterns of cephalosporin-reactive IgE antibodies. *J Molec Recognition* 1996; 9:287–96.
- Brandriss MW, Smith JW, Steinmann HG. Common antigenic determinants of penicillin G, cephalothin and 6-aminopenicillanic acid in rabbits. *J Immunol* 1965; 94: 696–704.
- Batchelor FR, Dewdney JM, Weston RD, Wheeler AW. The immunogenicity of cephalosporin derivatives and their cross-reaction with penicillin. *Immunol* 1966; 10:21–33.
- Gralnick HR, McGinnis MH. Immune cross-reactivity of penicillin and cephalothin. *Nature* 1967; 216:1026–7.
- Thoburn R, Johnson JE, Cluff LE. Studies on the epidemiology of adverse drug reactions. IV. The relationship of cephalothin and penicillin allergy. *J Am Med Assoc* 1966; 198:345–8.
- Grieco MH. Cross-allergenicity of the penicillins and the cephalosporins. *Archs Intern Med* 1967; 119:141–6.
- Abraham GN, Petz LD, Fudenberg HH. Immunohaematological cross-allergenicity between penicillin and cephalothin in humans. *Clin Exp Immunol* 1968; 3:343–57.
- Delafuente JC, Panush RS, Caldwell JR. Penicillin and cephalosporin immunogenicity in man. *Ann Allergy* 1979; 43:337–40.
- Harle DG, Baldo BA. Drugs as allergens: an immunoassay for detecting IgE antibodies to cephalosporins. *Int Arch Allergy Appl Immunol* 1990; 92:439–44.
- Rolli H, Schneider CH. Penamaldite assay: Improved methodology for the quantitation of penicilloic acids, penicilloyl amides and penicilloyl esters. *Arzneim-Forsch/Drug Res* 1984; 34:1247–9.
- Edwards RG, Spackman DA, Dewdney JM. Development and use of three new radioallergosorbent tests in the diagnosis of penicillin allergy. *Int Archs Allergy Appl Immunol* 1982; 68:352–7.
- Snyder SL, Sobocinski PZ. An improved 2, 4, 6-trinitrobenzenesulfonic acid method for the determination of amines. *Anal Biochem* 1975; 64:284–8.
- Caneva E, Di Gennaro P, Farina F, Orlandi M, Rindone B, Falagiani P. Synthesis and characterization of a penicillin-poly(L-lysine) which recognizes human IgE anti-penicillin antibodies. *Bioconjugate Chem* 1993; 4:309–13.