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IDENTIFICATION OF PENICILLIN ALLERGENIC DETERMINANTS THAT BIND IgE ANTIBODIES IN THE SERA OF SUBJECTS WITH PENICILLIN ALLERGY

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Abstract—Much of the literature on penicillin hypersensitivity is devoted to the identification of penicillin antigens rather than allergens. Human IgE-binding determinants on different penicillins have rarely been closely investigated with the view of defining fine structural allergenic features and differences. We have developed radioimmunoassays employing ampicillin, amoxicillin and ticarcillin solid phases for the detection of penicillin-reactive IgE antibodies. Quantitative hapten inhibition studies employed to identify IgE-binding regions on the penicillin molecules revealed a heterogeneous group of allergenic determinants consisting exclusively, or in part, of the α -aminobenzyl and benzyl side chain groups and the β -lactam and thiazolidine rings of the penicillin nucleus.

INTRODUCTION

Life-threatening anaphylactic (type I) reactions to penicillin are well recognised and reported to be the most frequent cause of anaphylaxis in man (Idsoe *et al.*, 1968). A number of *in vivo* and *in vitro* techniques are available for the diagnosis of immediate allergic reactions to penicillin including skin tests and detection of circulating IgE antibodies in patients' sera. For the detection of penicillin-reactive IgE antibodies, both the radioallergosorbent test (RAST) (Adkinson, 1975; Kraft and Wide, 1976; Kraft *et al.*, 1977; Spath *et al.*, 1979; Juhlin and Wide, 1979; Jarish *et al.*, 1981) and ELISA (de Haan *et al.*, 1979) have been used with the RAST and reported to produce excellent correlations with skin test results using penicilloyl-polylysine (Ahlstedt, 1984).

The penicilloyl determinant is said to be the major antigenic determinant in penicillin allergy (Levine and Ovary, 1961; Parker *et al.*, 1962; de Weck, 1962; Dewdney, 1977; Ahlstedt *et al.*, 1980). Many so-called "minor" determinants have also been identified including the penicillenate determinant, penicilloic acid, the penicillanyl determinant, penamaldate and penaldate determinants, D-penicillamine, penicoyl derivatives and penicillin polymers, although their involvement in human allergic reactions is unclear (Dewdney, 1977; Parker, 1980; Ahlstedt and Kristofferson, 1982; de Weck, 1983; Weiss and Adkinson, 1988). Limited information is available concerning the allergenicity of penicillins in man, especially with regard to fine structural allergenic differences between the penicillins. Indeed, with the exception of the RAST studies of Juhlin and Wide (1972), and preliminary reports by de Haan *et al.*,

(1985) and de Haan and van Ketal (1987) employing both human sera and mouse monoclonal antibodies, few investigations have made any attempt to identify which regions of the penicillin molecule are complementary to the combining sites of penicillin-reactive IgE antibodies in sera from allergic patients.

Bearing in mind our current inadequate knowledge of penicillin allergenic determinants, we attempted to produce penicillin complexes suitable for use in solid phase assays to detect penicillin-reactive IgE antibodies and to employ the assays to identify IgE-binding determinants on different penicillins.

MATERIALS AND METHODS

Subjects and sera

On the basis of case histories and skin tests to the penicillin in question and/or penicilloyl-polylysine, patients Mo, El, Fo, Di, Ca, Ba, Wa, Cr, Sm and McC were diagnosed as clinically allergic to penicillins. Patient Go reacted clinically to D-penicillamine.

Sera from adults allergic to house dust mites and/or grass pollens were obtained after clinical diagnosis, made on the basis of case history and skin prick tests, had been confirmed by the detection of allergen-specific IgE antibodies in the RAST. These sera showed radioactive uptakes of 10–45% with the corresponding allergen discs. Sera from non-allergic, healthy adults were collected from hospital staff members. Cord sera were obtained from the Department of Obstetrics, Royal North Shore Hospital.

Drugs and chemicals

D-Penicillamine, phenethicillin potassium, ticarcillin disodium, 6-aminopenicillanic acid, cephalothin

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sodium and ethanolamine were obtained from Sigma Chemical Co. (St. Louis, MO). The following drugs were donated: ampicillin sodium (Beecham Research Laboratories, N.S.W.); amoxicillin sodium (CSL, Vic.); benzylpenicillin sodium (Glaxo Australia, Vic.); phenoxymethylpenicillin potassium (Abbott Australia, N.S.W.); epicillin (E. R. Squibb and Sons, Vic.); mezlocillin sodium (Bayer Australia, N.S.W.); piperacillin sodium (Cyanamid Australia, N.S.W.); cloxacillin sodium and flucloxacillin sodium (Alpha-pharm Pty. Ltd, N.S.W.).

Preparation of drug-solid phase covalent complexes

For the preparation of each drug-solid phase support, conditions for optimising the coupling of the drug to the activated solid phase were determined by investigating the pH, temp and time of incubation. Optimum conditions are given for the preparation of the most reactive supports.

(1) Ampicillin

(a) *Ampicillin-epoxy-activated-Sepharose 6B (support 1)*. Ampicillin sodium (60 mg) was dissolved in water (4 ml) and mixed with epoxy-activated-Sepharose 6B (500 mg). The pH was adjusted to 10.8 with 1 M NaOH and the mixture gently shaken at 25°C for 20 hr. After washing with water, 0.1 M borate buffer pH 8 and 0.1 M acetate buffer pH 4, the remaining free activated groups on the gel were blocked by incubation with 1 M ethanolamine pH 9 at room temp for 4 hr. The gel was then washed as described above and washed finally with water.

(b) *Ampicillin-divinylsulphone-activated-Sepharose 6B (support 2)*. Divinylsulphone-activated Sepharose 6B was prepared as previously described (Baldo and Fisher, 1983a). Ampicillin sodium (60 mg) was dissolved in water (4 ml) and mixed with divinylsulphone-activated Sepharose 6B (1.5 ml sedimented gel). The pH was adjusted to 10.0 with 1 M NaOH and the mixture gently shaken at 25°C for 16 hr. The gel was then washed and blocked as in (a) above.

(2) Amoxicillin

(a) *Amoxicillin-epoxy-activated-Sepharose 6B (support 1)*. Amoxicillin sodium (60 mg) was coupled to epoxy-activated-Sepharose 6B (500 mg) as in 1(a) above except the pH of coupling was 11.6.

(b) *Amoxicillin-divinylsulphone-Sepharose 6B (support 2)*. Amoxicillin sodium (60 mg) was coupled to divinylsulphone-activated Sepharose 6B (1.5 ml sedimented gel) as in 1(b) above except the pH of coupling was 9.0.

(3) Ticarcillin

Ticarcillin disodium (50 mg) was coupled to epoxy-activated-Sepharose 6B (500 mg) as in 1(a) above except the pH of coupling was 11.8.

(4) D-Penicillamine

D-Penicillamine (60 mg) was coupled to epoxy-activated-Sepharose 6B (500 mg) as in 1(a) above except the pH of coupling was 12.2.

(5) Ethanolamine-Sepharose complex

This was prepared by using 300 mg of ethanolamine and 500 mg of epoxy-activated-Sepharose 6B (Baldo and Fisher, 1983b).

Detection of drug-reactive IgE antibodies by radioimmunoassay

Antibodies were detected as previously described (Harle *et al.*, 1987). Briefly, serum (50 μ l) was incubated with 6 mg of the solid-phase complex for 3 hr. Tubes were then washed and centrifuged three times with phosphate-buffered saline containing 0.05% Tween-20 before adding 30,000 cpm of ^{125} I-anti-human IgE. After 18 hr, tubes were again washed and counted in a Packard Auto-Gamma Spectrometer. The presence of specific IgE antibodies was determined by the per cent radioactive uptake of ^{125} I-anti-human IgE, i.e. per cent of counts added.

Inhibition assay

Serum (50 μ l), appropriately diluted was incubated for 1 hr with 50 μ l of a solution being tested before addition of the solid phase (6 mg in 100 μ l). The assay was then performed as above (Harle *et al.*, 1987).

RESULTS

Detection of IgE antibodies that react with penicillins

Table 1 summarises results obtained from direct binding radioimmunoassay studies in which ampicillin, amoxicillin ticarcillin and D-penicillamine solid phase complexes, control supports and Pharmacia penicillins V and G and HSA control discs were used with sera from control subjects and subjects clinically allergic to penicillins. With the ampicillin-, amoxicillin-, ticarcillin-, D-penicillamine- and control-Sepharose supports, a serum was regarded as positive in the radioimmunoassay when the radioactive uptake of labelled second antibody was greater than three times the cord/normal value (Wide *et al.*, 1967). An uptake of 2-3 times the cord/normal figure was considered to be a borderline positive.

With ampicillin-epoxy-activated-Sepharose 6B (support 1), sera from 9 patients Ba, Ca, Cr, El, Fo, McC, Mo, Sm and Wa produced significant uptakes of ^{125} I-anti-human IgE which were between five and 25 times the uptakes obtained with cord serum, sera from "normal" non-allergic subjects and from subjects allergic to house dust mites and/or grass pollens. The uptakes with sera Mo (14.9%) and Ca (10.9%) can be regarded as strongly positive. Serum from subject Go, who reacted clinically to D-penicillamine, gave an uptake of 1.6% which was regarded as a

Table 1. Results of tests for the presence of penicillin-reactive IgE antibodies in the sera of control subjects and subjects clinically allergic to penicillin(s)

Sera	Radioactive uptake (%) of ¹²⁵ I-anti-human IgE ^a with Sepharose linked to						D-Penicillamine	Ethanolamine ^d	Sepharose alone	Commercially-available supports		
	Ampicillin		Amoxicillin		Ticarcillin	Penicillin G				Penicillin V	HSA	
	Support 1 ^b	Support 2 ^c	Support 1	Support 2								
Controls												
Cord	0.6	0.4	0.6	0.4	0.7	0.2	0.5	0.4	0.5	0.5	0.4	0.4
Normals ^e	0.7 ± 0.1 ^f	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	0.5 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Allergics ^g	0.7 ± 0.1	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Patients												
Ba	4.2	4.0	3.6	2.4	— ^h	1.0	0.7	0.3	1.7	1.7	1.5	1.5
Ca	10.9	12.9	8.9	7.4	10.8	0.6	0.7	0.5	1.3	3.4	0.6	0.6
Cr	8.6	12.0	5.9	11.2	9.2	1.0	0.5	0.3	19.5	6.9	1.7	1.7
Di	1.2	0.7	1.4	1.2	1.9	0.8	0.6	0.5	2.2	3.0	2.9	2.9
El	3.6	2.6	1.0	3.0	6.7	0.2	0.6	0.4	11.2	11.6	0.6	0.6
Fo	2.4	3.9	2.5	3.0	8.9	1.3	0.5	0.4	1.9	3.0	0.9	0.9
Go ⁱ	1.6	0.4	0.5	0.9	1.6	0.8	0.6	0.4	1.1	1.8	1.5	1.5
McC	3.7	2.7	8.6	5.7	19.9	1.0	1.2	1.0	1.0	1.1	1.0	1.0
Mo	14.9	2.3	5.9	1.9	—	1.0	0.5	0.6	0.8	1.0	0.6	0.6
Sm	6.0	6.0	4.9	5.0	10.1	1.6	0.9	0.8	15.6	7.8	1.0	1.0
Wa	7.3	6.9	6.2	4.9	—	0.5	0.7	0.4	1.5	2.6	0.6	0.6

^aDirectly proportional to the amount of IgE antibodies bound to the solid phase.^bSupport 1: drug coupled to epoxy-activated Sepharose 6B.^cSupport 2: drug coupled to divinylsulfone-activated Sepharose 6B.^dEthanolamine-epoxy-activated Sepharose 6B.^e10 adult subjects (eight female/two male).^fMean ± SD.^g10 adult subjects allergic to house dust mites and/or pollens but not drugs.^hNot done.ⁱPatient Go reacted clinically to D-penicillamine.

borderline positive. Serum from subject Di, with an uptake of 1.2% proved negative with the ampicillin support. Background binding levels of 0.6–0.7% were obtained with control sera. With control supports of ethanolamine-Sepharose and Sepharose alone, both the penicillin positive sera and control sera gave low uptakes in the range 0.3–0.7%.

Uptakes of ^{125}I -anti-human IgE with ampicillin-divinylsulphone-Sepharose 6B (support 2, Table 1) for 9 sera Ba, Ca, Cr, El, Fo, McC, Mo, Sm and Wa were considered positive with uptakes between 5 and 32 times the values obtained with control sera. In particular, sera Ca and Cr were strongly positive with uptakes of 12.9 and 12.0%, respectively. In contrast, the control sera produced low uptakes (0.4–0.5%). Sera Di and Go were regarded as negative with uptakes of 0.7% and 0.4%, respectively.

Sera from eight patients Ba, C, Cr, Fo, McC, Mo, Sm and Wa were positive with amoxicillin-Sepharose (support 1). Serum Ca showed the highest uptake (8.9%) of ^{125}I -anti-human IgE while serum Di was borderline and sera El and Go negative with uptakes of 1.0% and 0.5%, respectively. Control sera again gave low uptakes of 0.6%.

When amoxicillin was coupled to divinylsulphone-activated-Sepharose 6B, 9 sera Ba, Ca, Cr, El, Fo, McC, Mo, Sm and Wa proved positive with serum Cr (11.2%) having the highest uptake of ^{125}I -anti-human IgE. Serum Di again proved to be a borderline positive while results with control sera were in the range 0.4–0.5%.

With the ticarcillin-Sepharose support, serum McC from a subject who reacted clinically to ticarcillin, gave an uptake of 19.9%. Of the seven other sera tested, five were positive while sera Di and Go proved to be borderline positives. In contrast, uptakes obtained with control sera were again low (0.7–0.9%).

With D-penicillamine-epoxy-activated-Sepharose 6B, control sera gave low uptakes of 0.2–0.3% while sera from six penicillin reactors Ba, Cr, Fo, McC, Mo and Sm and serum from Go who reacted clinically to D-penicillamine, were all positive. Sera Ca, Di and Wa were borderline and serum El proved negative.

Table 1 also summarises results obtained with the Pharmacia Phadebas RAST penicillin solid supports. In the "Directions for use" supplied with the Pharmacia penicillin discs, it is suggested that a ratio of the uptake of penicillin G or V discs relative to the HSA disc of 2 or higher can be considered a positive result whereas a ratio lower than 2 is considered negative. Based on this criterion, sera from five penicillin-allergic patients Ca, Cr, El, Sm and Wa would be considered positive with the commercial penicillin G discs whilst sera from six patients Ca, Cr, El, Fo, Sm and Wa would be regarded as positive with the penicillin V discs. Based on our more stringent protocol for positivity, namely, a ratio of three times background, only three sera, Cr, El and Sm would be

clear positives with the Pharmacia penicillin G assay, whilst six sera, Ca, Cr, El, Fo, Sm and Wa would be regarded as positives with the penicillin V assay.

Hapten inhibition studies with penicillins and cephalothin

Results of inhibition studies where sera Mo, Cr, Ca, and El were preincubated with a number of penicillins and cephalothin before the addition of ampicillin-epoxy-activated-Sepharose 6B (support 1) are summarised in Table 2. The quantities (nmol) required to produce 50% inhibition of IgE antibody binding are compared side-by-side for each of the compounds tested. Detailed inhibitory dose-response profiles are given in Figs 1–4 for sera Mo, Cr, Ca and McC respectively. With serum Mo, three penicillins, ampicillin, benzylpenicillin and amoxicillin were clearly the best inhibitors producing 50% inhibition with 75, 100 and 125 nmol, respectively, of each compound. The next best inhibitors were phenoxymethylpenicillin, phenethicillin, cloxacillin, mezlocillin and epicillin with activities ranging from 230 to 360 nmol, respectively, for 50% inhibition, while piperacillin and flucloxacillin showed the weakest inhibitory activity of the penicillins tested (Fig. 1). Cephalothin proved to be a weak inhibitor of IgE antibody binding with 50% inhibition not being achieved with 1500 nmol of drug whilst 6-aminopenicillanic acid, the weakest inhibitor tested, produced only 16% inhibition with 2000 nmol of compound.

With serum Cr (Fig. 2), benzylpenicillin was the most potent inhibitor of IgE binding (150 nmol for 50% inhibition) while phenoxymethylpenicillin, piperacillin and ampicillin proved to be the next best inhibitors and these compounds were better than amoxicillin, phenethicillin, epicillin and mezlocillin, respectively. Both cloxacillin and flucloxacillin proved to be relatively poor inhibitors whilst 6-aminopenicillanic acid produced 20% inhibition with 2000 nmol of compound. Cephalothin was the weakest inhibitor of all compounds tested with less than 10% inhibition being achieved with 1500 nmol of drug.

Although benzylpenicillin (280 nmol for 50% inhibition) proved to be the most potent inhibitor of the IgE antibodies in serum Ca, a different profile of inhibitory potencies to that of sera Mo and Cr was obtained. Piperacillin was the second best inhibitor (350 nmol for 50% inhibition) with mezlocillin, ampicillin, phenoxymethylpenicillin, phenethicillin, amoxicillin, cloxacillin, epicillin and flucloxacillin ranging from 380 nmol to 720 nmol respectively for 50% inhibition. As shown in Fig. 3, all the penicillins tested were similar in inhibitory potency and the dose-response profile was markedly different to that obtained with serum Mo (Fig. 1). Unlike the findings with sera Mo and Cr, 6-aminopenicillanic acid proved to be a moderately good inhibitor producing 49% inhibition with 2000 nmol of compound. As with sera Mo and Cr, cephalothin again proved to be

Table 2. Inhibition of IgE antibody binding to ampicillin-Sepharose by penicillins and cephalothin in penicillin-allergic patients

Compound	Structure	Amount (nmol) of compound needed for 50% inhibition of the uptake of ¹²⁵ I-anti-human IgE with serum ^a			
		Mo	Cr	Ca	El
Benzylpenicillin		100	150	280	200
Phenoxymethylpenicillin		230	200	540	
Ampicillin		75	260	500	360
Amoxicillin		125	320	560	520
Phenethicillin		270	320	550	
Epicillin		360	380	610	
Mezlocillin		300	490	380	
Piperacillin		580	230	350	
Cloxacillin		290	680	610	
Flucloxacillin		580	940	720	
6-Aminopenicillanic acid		> 1500 ^b	> 1500 ^b	> 1500 ^b	
Cephalothin		> 1500 ^b	> 1500 ^b	> 1500 ^b	

^aSera Mo, Cr, and Ca were used at a dilution of 1:2. Serum El was used undiluted.^b50% inhibition of IgE binding was not achieved (see Figs 1, 2 and 3).

a very weak inhibitor of IgE antibody binding (20% inhibition with 1500 nmol of drug).

With serum El, benzylpenicillin (200 nmol for 50% inhibition) was considerably more potent an inhibitor of IgE antibody binding than ampicillin and amoxicillin (360 and 520 nmol for 50% inhibition, respectively). Unfortunately, lack of sera prevented further investigations with other penicillins and cephalothin.

Using ticarcillin-Sepharose, IgE binding was inhibited best when serum McC was preincubated with ticarcillin (470 nmol for 50% inhibition) (Table 3,

Fig. 4). The next best inhibitors ampicillin, phenethicillin, amoxicillin and benzylpenicillin ranged from 600–1050 nmol for 50% inhibition, respectively. Cephalothin although the weakest inhibitory compound tested, proved to be weakly active with 39% inhibition being achieved with 2000 nmol of drug.

DISCUSSION

The exact chemical nature of each of the drug-solid phases prepared and used in this study is not known.

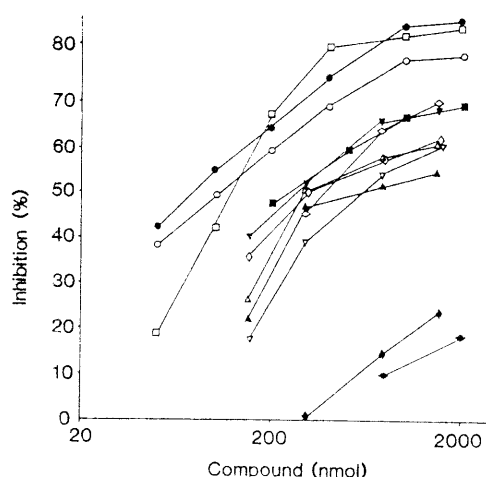


Fig. 1. Inhibition by penicillins and cephalothin of IgE binding in serum Mo to ampicillin-Sepharose complex. Serum Mo was used at a dilution of 1:2. Key to symbols: (○) benzylpenicillin; (●) ampicillin; (□) amoxicillin; (■) phenoxymethylpenicillin; (△) cloxacillin; (▲) flucloxacillin; (▽) piperacillin; (▼) phenethicillin; (◇) mezlocillin; (◆) cephalothin; (◆) 6-aminopenicillanic acid; (◇) epicillin.

In particular, the nature, number and ratio of the linkages involved in the attachment of the ligands to Sepharose has yet to be defined. However, with the bis-oxirane- and divinylsulfone-activated supports, nucleophilic addition is likely to occur via the *R* (side chain) primary amino groups on ampicillin and amoxicillin and the thiophene ring of ticarcillin. Addition via the phenolic —OH of amoxicillin seems less likely. The great difficulties involved in precisely defining the complexes chemically, and in quantitative terms, leaves an element of doubt in trying to precisely identify the different populations of penicillin-reactive IgE antibodies present in the sera. Nevertheless, the findings reported here clearly

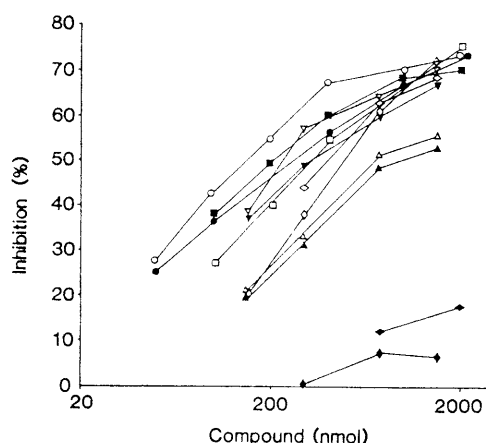


Fig. 2. Inhibition by penicillins and cephalothin of IgE binding in serum Cr to ampicillin-Sepharose complex. Serum Cr was used at a dilution of 1:2. Key to symbols: (○) benzylpenicillin; (●) ampicillin; (□) amoxicillin; (■) phenoxymethylpenicillin; (△) cloxacillin; (▲) flucloxacillin; (▽) piperacillin; (▼) phenethicillin; (◇) mezlocillin; (◆) cephalothin; (◆) 6-aminopenicillanic acid; (◇) epicillin.

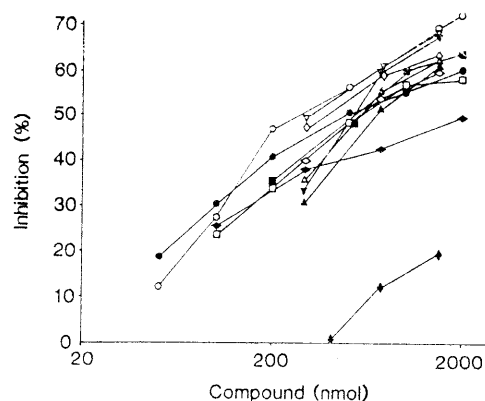


Fig. 3. Inhibition by penicillins and cephalothin of IgE binding in serum Ca to ampicillin-Sepharose complex. Serum Ca was used at a dilution of 1:2. Key to symbols: (○) benzylpenicillin; (●) ampicillin; (□) amoxicillin; (■) phenoxymethylpenicillin; (△) cloxacillin; (▲) flucloxacillin; (▽) piperacillin; (▼) phenethicillin; (◇) mezlocillin; (◆) cephalothin; (◆) 6-aminopenicillanic acid; (◇) epicillin.

demonstrate that penicillin-reactive IgE antibodies can be detected with the drug-solid phases employed and the specificities of at least these populations of antibodies can be defined with confidence. This is because conclusions on antigen-binding specificity of the IgE antibodies are obtained from experiments involving pre-incubation of serum antibodies with *free*, and not chemically coupled, drug.

The penicillin-solid phases we have developed and employed for the detection of penicillin-reactive IgE antibodies, proved to be at least as useful as, if not more valuable than, the commercially available penicillin G and V solid supports. This was illustrated by results obtained with sera from 11 different subjects diagnosed as clinically allergic to penicillin. With the latter drug-solid phases, six of the 11 sera were found to be positive whereas 9 positive sera were identified

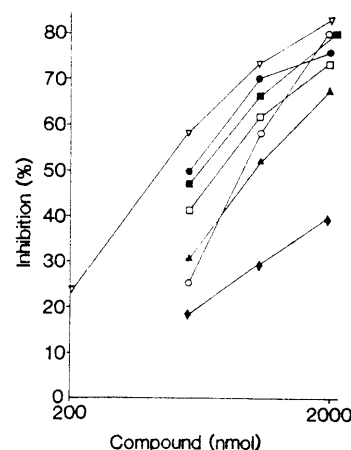


Fig. 4. Inhibition by penicillins and cephalothin of IgE binding in serum McC to ticarcillin-Sepharose complex. Serum McC was used at a dilution of 1:2. Key to symbols: (○) benzylpenicillin; (●) ampicillin; (□) amoxicillin; (■) phenethicillin; (◆) cephalothin; (▲) piperacillin; (▽) ticarcillin.

Table 3. Inhibition of IgE antibody binding in serum MoC^a to ticarcillin-Sepharose by penicillins and cephalothin

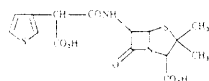
Compound ^b	Amount (nmol) for 50% inhibition of the uptake of ¹²⁵ I-anti-IgE ^c
Ticarcillin ^d	470
Benzylpenicillin	1050
Ampicillin	600
Amoxicillin	830
Phenethicillin	660
Piperacillin	1150
Cephalothin	>2000

^aSerum used at a dilution of 1:2.

^bFor structures other than ticarcillin see Table 2.

^cDirectly proportional to the amount of IgE antibodies bound to the solid phase.

^dTicarcillin:



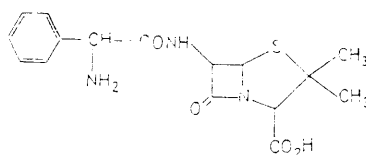
with the ampicillin and amoxicillin supports. Six of the eight sera tested with the ticarcillin support were positive with the highest uptake (19.9%) being recorded with sera from the subject who was clinically allergic to the drug. The remaining two sera examined were borderline positives. Results therefore reinforced earlier conclusions that penicillin RAST assays can be a valuable adjunct to the case history and skin tests in the diagnosis of an immediate allergic reaction to penicillin(s) (de Weck, 1983).

A number of the sera tested showed positive reactions with more than one of the different penicillin-solid phases regardless of the chemical nature of the side chain of the penicillin molecule attached to the solid support. On the other hand, the vastly different uptakes of ¹²⁵I-anti-IgE recorded by the same sera (for example sera McC and Mo) when used with different penicillin-solid phases, suggested the presence of other IgE antibodies that preferentially recognize determinants present, or available for reaction, on only one of the supports. In an attempt to obtain information on the fine-structural specificities of some of the penicillin-reactive IgE antibodies, detailed hapten inhibition studies were performed with 5 sera. With serum Mo, it was clear that the IgE antibodies were primarily directed to the alkyl side chain (*R* group) of the penicillin molecule. Thus, ampicillin, benzylpenicillin and amoxicillin were considerably more potent inhibitors of IgE binding than other penicillins which, although they contained an aromatic benzene group, had substitutions at the primary amino group (mezlocillin, piperacillin) or incorporated five membered oxazole rings (cloxacillin, flucloxacillin) adjacent to the benzene ring or contained ethers in the side chain (phenoxymethylpenicillin, phenethicillin). Since all the penicillins tested in the inhibition experiments varied in the *R* group only, it was clear that the differences in inhibitory potencies were due entirely to the composition of this group. The greater inhibitory potency of ampicillin compared to amoxicillin suggested that the

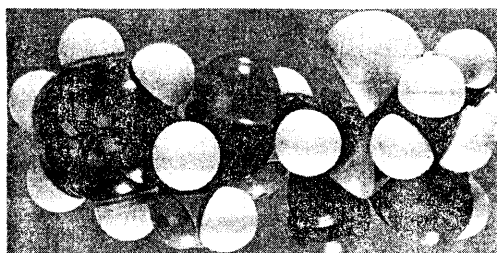
IgE antibodies in the serum of patient Mo were directed towards an unsubstituted benzene ring. The far weaker inhibition of epicillin, which contains a cyclohexadiene ring, relative to ampicillin confirmed that a benzene ring is an important feature of the complementary allergenic determinant. In addition, the much better inhibition exhibited by benzylpenicillin compared to phenoxymethylpenicillin suggested that the O atom in the latter compound was not recognised by the complementary IgE antibodies. The fact that mezlocillin and piperacillin were considerably weaker inhibitors than ampicillin indicated that the amino group of ampicillin rather than a substituted amino group was also important for complementary IgE antibody recognition. Further confirmation that the specificity of the subject's IgE antibodies was directed towards the *R* group was the extremely poor inhibitory activity of 6-aminopenicillanic acid which contains the β -lactam and thiazolidine rings of the penicillin nucleus. Thus, for serum Mo, the allergenic determinant recognised by the complementary IgE antibodies appears to be almost exclusively the α -aminobenzyl group of the ampicillin molecule (Fig. 5). With serum Cr, a slightly different specificity pattern was observed. In this case, the IgE antibodies appeared to be primarily complementary to the benzyl group of benzylpenicillin with a small degree of recognition of the β -lactam ring. This conclusion was reached after comparing the results obtained with benzylpenicillin with the inhibitory activities of, on the one hand, ampicillin and piperacillin, compounds containing α -substituted benzyl groups and, on the other, phenoxymethylpenicillin and phenethicillin both of which contain a phenoxyalkyl group (Table 2). Addition of a substituent to the aromatic ring as in amoxicillin led to a decrease in antigen-antibody complementarity as shown by comparing the inhibition potencies of ampicillin and amoxicillin. The very weak inhibition exhibited by 6-aminopenicillanic acid again confirmed that the *R* group was particularly important for IgE antibody binding.

With serum from subject Ca, a third pattern of IgE antibody specificity was observed. Although benzylpenicillin was marginally the best inhibitor, all of the other penicillins tested proved to have similar inhibitory potencies. These results seem to indicate a more important role for the "backbone" β -lactam and thiazolidine rings (Fig. 5) than was the case with the allergenic determinants complementary to the IgE antibodies in sera Mo and Cr. This conclusion was supported by the clear recognition of 6-aminopenicillanic acid by the subject's IgE antibodies. The relatively weak inhibitory activity of cephalothin on the other hand suggested that the thiazolidine ring is the region of the molecule that is most complementary to the IgE antibodies in the sera of subject Ca.

The finding that ticarcillin was the best of the inhibitors tested with serum McC was consistent with the clinical diagnosis that the patient was allergic to



Ampicillin



Space-filling model of ampicillin

Outline of model with allergenic

Sera	Allergenic determinant identified	determinant shaded
Mo		
Cr		
Ca		

Fig. 5. Allergenic determinants recognised by IgE antibodies in the sera of three penicillin-allergic subjects. The determinants were identified in hapten inhibition studies using an ampicillin solid phase. The hatched and dotted shadings refer to regions of the molecule showing high and low degrees of complementarity respectively to the patients' IgE antibodies.

this drug. Although cephalothin proved to be the weakest inhibitor, significant inhibition was obtained over the concentration range 600–2000 nmol per tube (Fig. 4). Ticarcillin and cephalothin have a 3-thiophene and 2-thiophene moiety respectively as the *R* side chain with the former compound having a carboxyl group on the α -C of the side chain. The weaker inhibitory activity of cephalothin is probably best explained by the presence of the dihydrothiazine ring instead of the thiazolidine ring that occurs in the penicillins (Table 2, Fig. 5).

Although lack of sera precluded detailed inhibition studies on serum E1, benzylpenicillin was the most potent inhibitor of the three penicillins examined and,

as with sera Mo and Cr, it seems likely that the *R* group is an important feature of the allergenic determinants recognized by IgE antibodies in these three subjects. Overall, findings with the sera of all five subjects indicate that, as with a number of other drug studies so far (Harle *et al.*, 1987; Smal *et al.*, 1988), the specificity of the penicillin reactive IgE antibody response is heterogeneous.

In a recent study, two patients who reacted clinically to amoxicillin were found to be skin test and RAST positive to the drug, RAST negative to benzylpenicilloyl and skin test negative to benzylpenicilloyl and a minor determinant mixture. RAST inhibition showed that with both sera the IgE responses were

directed to the acyl side chain of amoxicillin (Blanca *et al.*, 1988).

The presence of a β -lactam ring and an amide linkage to the R group side chain in cephalosporins suggests the possibility of cross-reactivity with the penicillins and this was, in fact, observed in the present investigation. A study of cephalosporin-reactive IgE antibodies in the sera of subjects allergic to cephalosporins and the cross-reactivities of these antibodies with penicillins is currently underway (Harle and Baldo, unpublished).

It is clear that the immunoassays described here for the detection of IgE antibodies that react with a number of different penicillins, are valuable additions both to the penicillin RASTs currently available and as supplementary diagnostic aids to case histories and skin tests. Further application of the immunoassays combined with hapten inhibition studies of the type detailed here will help to identify and define more precisely the number and nature of allergenic, as distinct from antigenic, determinants on the many different penicillins available for use.

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