

M.J. Torres
C. Mayorga
R. Pamies
J.L. Rodriquez
C. Juarez
A. Romano
M. Blanca

Immunologic response to different determinants of benzylpenicillin, amoxicillin, and ampicillin. Comparison between urticaria and anaphylactic shock

Authors' affiliations:

M.J. Torres, C. Mayorga, R. Pamies,
J.L. Rodriquez, C. Juarez, M. Blanca, Research
Unit for Allergic Diseases, Internal Medicine
Department, Carlos-Haya Hospital, Málaga,
Spain
A. Romano, Department of Internal Medicine and
Geriatrics, UCSC, Allergy Unit, C.I. Columbus,
Rome and IRCS Oasi Maria S.S., Troina, Italy

Correspondence to:

Dr Miguel Blanca
Allergy Laboratory
Carlos Haya Hospital
Avd Carlos Haya s/n
29010-Málaga
Spain

Key words: anaphylactic shock; IgE antibodies; IgG antibodies; penicillins; skin tests; urticaria.

Background: Subjects with IgE-mediated allergic reactions to penicillins can develop urticaria or anaphylactic shock. Urticaria is mainly associated with positivity to the major determinant of benzylpenicillin (BPO), and anaphylactic shock with minor determinants (MDM). The presence of IgG antibodies to BPO is thought to be mainly associated with urticaria, possibly protecting from anaphylactic shock. We aimed to study the skin test response to BPO and MDM, amoxicillin (AX), and ampicillin (AMP) in a group of subjects allergic to penicillins, and to evaluate the role of specific IgG.

Methods: We studied a group of patients with immediate allergic reactions to penicillins, comparing urticaria and anaphylactic shock. Skin tests were done with BPO, MDM, AX, and AMP. Specific IgE and IgG antibodies to benzylpenicilloyl-poly-L-lysine (BPO-PLL) and amoxicilloyl-poly-L-lysine (AXO-PLL) were determined by RAST and ELISA, respectively.

Results: Fifty-nine patients were studied (30 with anaphylactic shock and 29 with urticaria). Skin test positivity to BPO was associated with urticaria ($P<0.001$), and positivity to MDM, AX, and AMP with anaphylactic shock ($P=0.006$, $P<0.001$, and $P=0.002$, respectively). Specific anti-BPO-PLL and AXO-PLL IgG values were higher in patients than controls ($P<0.001$), but no differences were observed between urticaria and anaphylactic shock.

Conclusions: Positivity to minor determinants of penicillins is associated more with anaphylactic shock than urticaria, but the role of IgG antibodies in helping to prevent the development of anaphylactic shock could not be confirmed.

Abbreviations: BPO: benzylpenicilloyl; MDM: minor determinant mixture; AX: amoxicillin; AMP: ampicillin; AXO-PLL: amoxicilloyl-poly-L-lysine; BPO-PLL: benzylpenicilloyl-poly-L-lysine.

Date:

Accepted for publication 6 April 1999

To cite this article:

Torres M.J., Mayorga C., Pamies R., Rodriquez J.L.,
Juarez C., Romano A. & Blanca M. Immunologic response
to different determinants of benzylpenicillin, amoxicillin,
and ampicillin. Comparison between urticaria and
anaphylactic shock.
Allergy 1999; 54, 936–943.

Copyright © Munksgaard 1999

ISSN 0105-4538

The mechanisms involved in allergic reactions to penicillins can be antibody dependent or T-cell mediated (1), although antibody production is a phenomenon controlled by T cells. Classically, antibody-mediated reactions are induced by IgE antibodies and are classified as immediate reactions. Other antibodies have been detected in sera from patients with allergic reactions to β -lactams, and while some studies have claimed that these antibodies can be involved in the reaction (2), others suggest that the presence or absence of this antibody may influence the type of reaction (3). However, the role of IgG antibodies in inducing or modifying allergic reactions has not been sufficiently clarified (4).

Two main clinical manifestations exist in immediate allergic reactions to penicillins: anaphylactic shock and urticaria (3). In anaphylactic shock, several of the following symptoms may appear: generalized erythema, systemic pruritus, dyspnea, gastrointestinal pain, nausea, or vomiting, as well as hypotension. In other instances, symptoms are mainly limited to the skin and consist of urticaria and/or angioedema. Although overlapping may exist and urticaria can be present in anaphylactic shock, this differentiation is usually accepted for evaluating immediate allergic reactions to β -lactams (5).

Skin tests are the most used and validated method for evaluating IgE-mediated reactions to penicillins, and allergic subjects are usually positive to major and/or minor determinants of benzylpenicillin (6). The RAST is not generally part of the initial diagnostic procedure of IgE-mediated penicillin reactions because of both its lower sensitivity and lower specificity than skin tests (7). A number of studies have shown that the use of determinants of other penicillins, such as amoxicillin (AX) and ampicillin (AMP), can be of value for evaluating immediate reactions to β -lactams (8–11). Subjects can respond to just one or a combination of any of these determinants (6, 10).

The concept of minor determinant response is mainly based on skin test studies (12–14) and when these results have been correlated with *in vitro* tests, no definite conclusions have been obtained (4). However, it is clear that allergic patients can be classified into several categories according to their skin test response (5, 14). Subjects with skin test positivity to minor determinants are reported to be more prone to developing anaphylactic shock after administration of penicillin, but subjects with positivity to the major determinant of benzylpenicillin (BPO) mainly develop urticaria (5, 13). The reason for this has been related, as stated above, to the ability to produce IgG antibodies to BPO, which can act as blocking antibodies and therefore prevent the development of systemic reactions (12).

In this paper, we report the skin test response to BPO and minor determinants of benzylpenicillin (MDM), AX, and AMP in patients with urticaria and anaphylactic shock, and establish the relationship between the presence of IgG antibodies to benzylpenicillin and AX and the clinical manifestations.

Material and methods

Subjects

We evaluated a group of patients who had had an IgE-mediated reaction to a penicillin derivative and were skin test positive to at least one of the determinants mentioned above. Subjects were divided into two groups according to their history; group A comprised patients with symptoms limited mostly to the skin, consisting of urticaria and/or angioedema, and group B comprised patients having several of the following symptoms: generalized erythema, systemic pruritus, dyspnea, tachycardia, abdominal cramps, nausea, vomiting, and hypotension. This second group was considered to have had anaphylactic shock. This evaluation was made by patient interviews or consulting clinical records when subjects had been treated in the emergency department of our hospital.

Skin test

The following determinants were used: BPO 5×10^{-5} mM/l and MDM 2×10^{-2} mM/l, both provided by Allergopharma, Merck (Darmstadt, Germany); AX 20 mg/ml from Beecham (Toledo, Spain); and AMP 20 mg/ml from Antibiotic SA (León, Spain). Skin tests were done by prick, and if they were negative, intradermal tests were carried out. Subjects were classified as positive or negative as previously described (15).

Specific IgG antibodies

These were measured by ELISA as previously described (16, 17). Microtiter plates (Dynatech Laboratory Ltd, Sussex, UK) were coated with conjugates of amoxicilloyl-poly-L-lysine (AXO-PLL) or benzylpenicilloyl-poly-L-lysine (BPO-PLL) at 100 μ g/ml. Alkaline phosphate goat anti-human conjugated IgG (Sigma) was used as antibody. The optical density was read at 405–650 nm.

As a negative control group, we used serum from 100 subjects aged between 6 months and 2 years who had not been exposed to any penicillin derivative. Those IgG values

having an absorbance greater than 0.400 nm were considered positive, this value itself being greater than the mean plus 2SD of the negative control group.

Specific IgE antibodies

These were made by RAST to BPO-PLL and AXO-PLL as previously described (18). Blood samples were obtained at the moment that patients were evaluated, and sera was kept at -20°C until assayed. All samples were made in parallel, and values were considered positive if they were higher than

Table 1. Age, sex, drug involved in reaction, and time elapsed since development of reaction and evaluation of patient in all subjects in group A

Patient no.	Age (years)	Sex	Drug involved	Time elapsed (days)
1	57	F	AMP	UK
2	30	M	AMP	UK
3	52	F	AMP	1250
4	61	F	AMP	780
5	49	M	AX	UK
6	42	F	AX	224
7	63	M	AX	120
8	36	M	AX	UK
9	36	M	AX	UK
10	53	F	AX	54
11	38	F	AX	365
12	18	F	AX	2160
13	37	F	AX	270
14	29	M	AX	UK
15	30	F	AX	UK
16	56	F	BP	4173
17	74	M	CEPH	8462
18	60	M	CEPH	UK
19	68	F	PENI	1080
20	56	M	PENI	5400
21	54	F	PENI	UK
22	32	F	PENI	3295
23	62	M	PENI	3000
24	53	F	PENI	285
25	68	M	PENI	2880
26	35	M	PENI	5000
27	67	F	PENI	UK
28	52	F	PENI	UK
29	52	M	PENI	915
30	54	F	PENI	5405

AMP: ampicillin, AX: amoxicillin, BP: benzylpenicillin, CEPH: cephalosporin, PENI: unspecified penicillin, UK: unknown.

2.5 % of label uptake, which was the mean plus 2SD (19) of the negative control group with a total IgE of 8–1300 kU/l.

Statistical analysis

The percentage of penicillin derivatives involved in both groups and the proportion of skin tests positive to all the determinants tested were compared by chi-square analysis. The time elapsed between the occurrence of the reaction and the patient evaluation when the sera were obtained was compared by the *t*-test for independent groups. IgG values in groups A and B and the control group were compared by ANOVA and, in the case of an association, the Bonferroni test. The IgE responses to AX and benzylpenicillin were divided into two categories: presence (positive) or absence (negative) of specific antibodies, according to a cutoff point of 2.5, which was over the mean plus 2SD of a negative control group. The IgG response was also divided into the same two categories, according to a cutoff point of 0.400 nm, which was over the mean plus 2SD of a negative control group.

Results

Subjects

We selected a group of 59 subjects who developed an immediate reaction to a penicillin derivative and were skin test positive to at least one of the penicillin determinants. According to the criteria established above, 30 subjects belonged to group A and 29 to group B. Results for group A are presented in Table 1. There were 13 men and 17 women, with an age range of 18–74 years (49.13 ± 14.13). Results for group B are presented in Table 2. There were 17 men and 12 women, with an age range of 23–82 years (46.45 ± 14.01). Comparison of the age between both groups showed no significant differences.

In 19 subjects of group A, the time interval between the occurrence of the allergic episode and the evaluation of the patient and taking of sera was known, and the mean value was 2374 ± 2387 days. In 15 subjects of group B, the time interval was also known, and the mean value was 1049 ± 1195 days. Although these values showed differences, they were not quite significant ($P=0.06$).

Table 2. Age, sex, drug involved in reaction, and time elapsed since development of reaction and evaluation of patient in all subjects in group B

Patient no.	Age (years)	Sex	Drug involved	Time elapsed (days)
1	60	M	AMP	UK
2	56	M	AMP	120
3	38	M	AX	75
4	32	M	AX	60
5	47	M	AX	2400
6	52	M	AX	52
7	53	M	AX	UK
8	35	F	AX	UK
9	37	M	AX	UK
10	59	F	AX	1900
11	23	M	AX	UK
12	38	M	AX	UK
13	52	F	AX	3500
14	33	F	AX	365
15	36	F	AX	720
16	23	F	AX	UK
17	33	F	AX	183
18	69	M	AX	3300
19	63	F	AX	UK
20	39	M	BP	94
21	54	F	CEPH	930
22	55	M	PENI	UK
23	49	M	PENI	UK
24	36	M	PENI	UK
25	82	F	PENI	UK
26	62	M	PENI	600
27	40	F	PENI	UK
28	36	M	PENI	1440
29	55	F	PENI	UK

AMP: ampicillin, AX: amoxicillin, BP: benzylpenicillin, CEPH: cephalosporin, PENI: unspecified penicillin, UK: unknown.

Drugs involved in the reaction

In group A, the drug most frequently involved was an unspecified penicillin in 12 subjects, followed by AX in 11, AMP in four, cephalosporins in two, and benzylpenicillin in one (Table 1). The cephalosporins involved were cephadroxil in one patient and cefuroxime in another. The term “unspecified penicillin” is used for those patients who, although sure it was a penicillin, were unable to specify the derivatives involved in the reaction. In group B, the drug most frequently involved was AX in 17 subjects, followed by unspecified penicillin in 12, AMP in two, benzylpenicillin

in one, and cephalosporins in one (Table 2). The cephalosporin involved was cefuroxime. There were no statistical differences in the β -lactam involved in the reaction between the groups.

Skin test

The skin test results from group A (Table 3) showed that the determinant most frequently inducing positivity was BPO in 17 subjects, followed by the combination of BPO and MDM in five patients. In the eight remaining subjects, there was positivity to one or more penicillin determinants. In group B (Table 4), the combination MDM plus AX plus AMP was positive in 10 patients, followed by MDM alone in six subjects and AX plus AMP in five. Positivity to BPO appeared in only three subjects.

Evaluation of the percentage of positivity to each determinant showed that, in group A, 76.7% of the patients were skin test positive to BPO, 33.3% to MDM, 20% to AX, and 20% to AMP. In group B, 13.8% of the patients were positive to MDM, 69% to AX, 65.5% to AMP, and 58.6% to BPO. These differences were statistically significant for all haptens (BPO<0.001, MDM=0.006, AX<0.001, and AMP=0.002).

Specific IgE antibodies

When we evaluated the specific IgE antibodies, we found that in group A (Table 3), 12 (40%) patients were positive to BPO-PLL and four (13.3%) to AXO-PLL, and in group B (Table 4) seven (24.1%) were positive to BPO-PLL and eight (27.6%) to AXO-PLL. The mean IgE value $\pm$ SD to BPO-PLL was 2.85 ± 3.0 in group A and 3.7 ± 7.1 in group B, the *t*-test analysis showing no significant differences. The mean IgE value $\pm$ SD to AXO-PLL was 1.67 ± 3.12 for group A and 6.37 ± 12.7 for group B. Comparison, although not significant, was very close ($P=0.055$). No correlation was found between total IgE levels and RAST values in either group.

Specific IgG antibodies

A graphical representation of IgG values to both haptens with the median and the interquartile range of the three groups is presented in Fig. 1. The mean IgG value $\pm$ SD to BPO-PLL in group A was 0.516 ± 0.355 ; for group B, it was 0.413 ± 0.281 ; and for the negative control, it was 0.166 ± 0.098 . The ANOVA showed statistical differences between the groups ($P<0.001$). However, when groups A and B were compared by the Bonferroni test, there were no differences ($P=0.22$). The

Table 3. Skin test results to BPO, MDM, AX, and AMP, and specific IgE and IgG levels to BPO-PLL and AXO-PLL in all patients in group A

Patient no.	Skin test				IgE		IgG	
	BPO	MDM	AX	AMP	BPO-PLL	AXO-PLL	BPO-PLL	AXO-PLL
1	+	–	–	–	1.77	0.41	0.208	0.950
2	–	–	+	+	0.01	0.11	0.270	0.236
3	+	–	–	–	0.01	0.01	0.305	0.502
4	+	–	–	–	4.00	0.10	0.379	0.998
5	–	–	+	+	0.7	0.2	0.570	0.620
6	–	+	–	–	0.27	0.10	0.403	0.649
7	+	–	–	–	3.5	1.3	0.890	0.970
8	+	–	+	+	6.60	7.30	1.183	1.310
9	–	+	+	+	3.6	1.2	0.730	0.620
10	+	+	–	–	1.1	0.7	0.412	0.215
11	+	–	–	–	4.37	1.70	0.550	1.415
12	+	–	–	–	6.9	3.0	0.825	0.630
13	–	+	+	+	0.7	4.5	0.201	0.400
14	–	+	+	+	0.20	0.10	0.769	0.708
15	+	–	–	–	7.2	0.3	0.360	0.470
16	+	–	–	–	0.95	0.48	0.121	0.141
17	+	+	–	–	0.01	16.20	0.290	0.483
18	+	–	–	–	6.3	0.7	0.520	0.312
19	+	–	–	–	0.2	0.3	0.430	0.390
20	–	+	–	–	4.63	0.83	1.406	1.220
21	+	–	–	–	0.89	1.21	1.457	1.709
22	+	+	–	–	0.3	0.1	0.208	0.197
23	+	–	–	–	10.82	1.26	0.139	0.160
24	+	–	–	–	8.3	1.3	0.390	0.415
25	+	–	–	–	1.39	0.25	0.771	0.725
26	+	–	–	–	0.71	0.67	0.225	0.364
27	+	–	–	–	0.71	0.57	0.414	0.539
28	+	+	–	–	6.4	1.3	0.655	0.620
29	+	–	–	–	1.7	1.9	0.215	0.218
30	+	+	–	–	1.3	1.6	0.212	0.185

BPO: benzylpenicilloyl, MDM: minor determinant mixture, AX: amoxicillin, AMP: ampicillin, AXO-PLL: amoxicilloyl-poly-L-lysine, BPO-PLL: benzylpenicilloyl-poly-L-lysine.

mean IgG value $\pm$ SD to AXO-PLL was 0.612 ± 0.402 for group A, 0.593 ± 0.391 for group B, and 0.193 ± 0.101 for the negative control. The ANOVA also showed significant differences ($P < 0.001$). However, when groups A and B were compared, there were no differences ($P = 0.846$).

When we established two categories of IgG, positive or negative according to the previously established cutoff point, we found that 53% of the patients were positive to BPO and 70.6% to AXO in group A, and that 34% were positive to BPO and 65.5% to AXO in group B. Although there were more

positive subjects in the urticaria group than in the anaphylactic shock group, these differences were not significant ($P = 1.45$ for BPO and $P = 0.723$ for AXO).

Discussion

The purpose of our study was to evaluate the skin test positivity to major and minor determinants of benzylpenicillin and minor determinants of AX and AMP in subjects

Table 4. Skin test results to BPO, MDM, AX, and AMP, and specific IgE and IgG levels to BPO-PLL and AXO-PLL in all patients in group B

Patient no.	Skin test				IgE		IgG	
	BPO	MDM	AX	AMP	BPO-PLL	AXO-PLL	BPO-PLL	AXO-PLL
1	+	–	–	–	4.50	1.82	0.621	1.087
2	–	–	–	+	0.70	1.70	0.332	0.270
3	–	+	+	+	0.10	0.10	0.336	0.486
4	–	+	+	+	1.19	1.39	0.177	1.011
5	–	–	+	+	0.22	0.40	0.231	0.478
6	+	–	–	–	18.05	13.59	0.155	0.385
7	–	+	+	+	0.50	49.70	0.378	0.623
8	–	–	+	+	26.11	1.00	0.871	0.863
9	–	+	+	+	1.67	1.14	0.215	0.331
10	–	–	+	+	0.4	0.2	0.905	1.348
11	–	+	+	–	0.20	2.0	0.531	0.615
12	–	–	+	–	0.15	0.94	0.648	0.529
13	–	+	–	–	0.20	0.30	1.233	1.404
14	–	+	+	+	0.06	0.02	0.409	1.010
15	+	+	–	–	0.81	0.02	0.208	0.404
16	–	+	–	–	0.6	0.02	0.479	0.634
17	–	–	+	+	1.49	1.01	0.185	0.226
18	–	+	+	+	1.30	5.70	0.348	0.406
19	–	+	–	–	5.35	3.22	0.364	0.502
20	–	–	+	+	7.12	33.40	0.712	0.784
21	–	+	+	+	0.08	0.04	0.180	0.417
22	–	+	–	–	0.09	0.07	0.910	0.771
23	–	+	–	–	8.26	5.90	0.154	0.321
24	–	+	+	+	25.5	29.0	0.205	0.291
25	–	+	+	+	0.40	0.50	0.210	0.377
26	+	+	+	+	2.00	0.01	0.165	0.379
27	–	+	–	–	0.16	0.21	0.260	0.284
28	–	+	+	–	0.80	0.10	0.361	0.682
29	–	+	+	+	0.01	28.40	0.206	0.305

BPO: benzylpenicilloyl, MDM: minor determinant mixture, AX: amoxicillin, AMP: ampicillin, AXO-PLL: amoxicilloyl-poly-L-lysine, BPO-PLL: benzylpenicilloyl-poly-L-lysine.

with urticaria and anaphylactic shock to β -lactams. Since a number of studies have indicated that semisynthetic penicillins can also induce skin test positivity (11, 20), it seems reasonable to include these two new determinants in both skin testing and laboratory studies. We divided the clinical response into two categories: urticaria and anaphylactic shock, although we agree with others that some overlapping exists (1, 5, 14) and that it is sometimes difficult to classify subjects because their symptoms are mostly described retrospectively.

Skin test reactivity to MDM was observed soon after the first use of penicillins together with their ability to improve the diagnostic capacity of these tests (12, 13). The importance of semisynthetic penicillins in inducing allergic reactions has also been documented (11, 20), and recent studies indicate that they contribute significantly to the number of positive skin test responses (8–11, 20). MDM not only improves the capacity of diagnostic tests, but also is usually associated with a greater risk of developing anaphylactic shock (5, 13, 21).

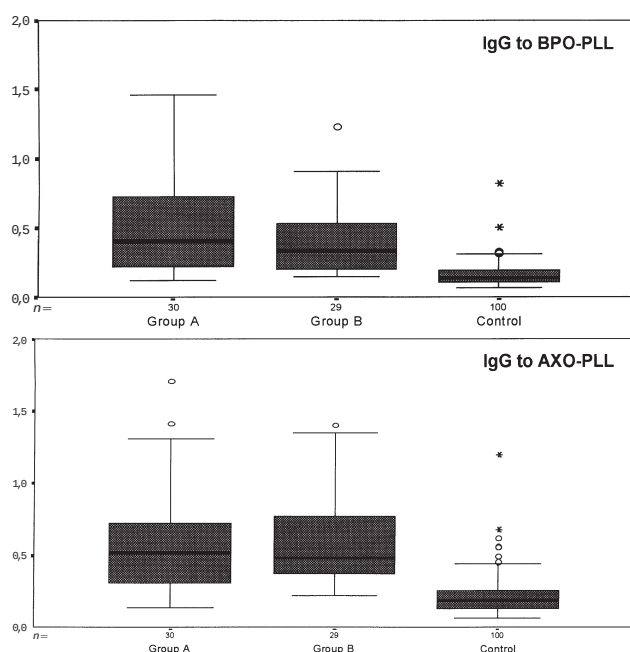


Figure 1. Box-plot analysis and off-layers of IgG levels to BPO (top) and AXO (bottom) in three groups: urticaria, anaphylactic shock, and controls.

The results of our study show that patients who developed an anaphylactic reaction were more frequently skin test positive to MDM, AX, and AMP than those with urticaria, and the latter were more frequently skin test positive to BPO. Nevertheless, these responses were not exclusive, since some patients with urticaria had skin positivity to minor determinants and others with anaphylactic shock were also positive to BPO (Tables 3 and 4). The positivity to MDM alone in our group was important and similar to another study (22). In our study, 33.3% of the patients from the urticaria group were positive to MDM in comparison with 69% of the anaphylactic shock group.

When we compared the RAST values to BPO-PLL and AXO-PLL in both groups, there was a tendency to detect more positive responses to AX in group B than in group A (24% vs 13%). On the other hand, positivity to BPO was 27% in group B vs 40% in group A. These data approached statistical significance, supporting the data found with skin test results.

The role of IgG antibodies was also evaluated. The presence of specific IgG antibodies to penicillins in the sera of subjects, whether or not allergic to penicillin, indicates immunity to penicillins (2, 23). It has also been suggested that they may be able to prevent anaphylactic shock in penicillin-allergic subjects by their blocking ability (3). IgG antibodies to penicillin have been studied by different authors using different methods and with variable results (24–26). Levine et al. observed that 100% of subjects receiving penicillins developed IgG antibodies (14). Adkinson found that 60% of subjects taking a short course of penicillin developed IgG antibodies (23). Other authors have found a lower percentage (27). Differences in the results may be related to the method used, the patient characteristics, and the clinical manifestations. In our study, in order to avoid or minimize background noise or nonspecific binding, we selected a control group of children aged between 1 month and 2 years who had not yet been exposed to penicillins, establishing a cutoff point of 0.400 nm for the presence or absence of IgG antibodies. With this level, important differences were observed in the mean values between groups A and B and the controls, indicating that in both urticaria and anaphylactic shock IgG antibodies were present, but no differences were found. Therefore, we see that the presence or absence of IgG antibodies does not favor the appearance of urticaria or anaphylactic shock, respectively, in subjects with immediate response to β -lactams.

In summary, we conclude that skin test positivity to minor determinants of penicillin, including AX and AMP, seems to be more frequent in cases of anaphylactic shock than urticaria and that the presence of IgG antibodies does not seem to prevent the appearance of anaphylactic shock. Other mechanisms should therefore be investigated in order to explain these differences.

Acknowledgments This work was supported in part by CICYT Grant SAF-240/96, BIOTECH ERB BIO4-CT96-0246 EEC Grant, Hospital Carlos Haya Foundation, and OASI María Santísima Foundation, Troina, Italy. We also thank Ian Johnstone for help with the English language version of the text.

References

1. Amos HE. Antigenic determinants of the penicillin molecule. In: Amos HE, editor. *Allergic drug reactions*. Oxford: Edward Arnold, 1976:64–74.
2. de Haan P, Kalsbeek GL. Induction of benzylpenicilloyl-specific antibodies, including IgE, by long-term administration of benzylpenicillin. *Clin Allergy* 1983;13:563–569.
3. Levine BB. Immunologic mechanisms of penicillin allergy. A haptenic model system for the study of allergic diseases of man. *N Engl J Med* 1966;275:1115–1125.

4. Saxon A, Beall GN, Rohr AS, Adelman DC. Immediate hypersensitivity reactions to betalactam antibiotics. *Ann Int Med* 1987;**107**:204–215.
5. Weiss ME, Adkinson NF. Immediate hypersensitivity reactions to penicillin and related antibiotics. *Clin Allergy* 1988;**18**:515–540.
6. Levine BB, Zolov DM. Prediction of penicillin allergy by immunological tests. *J Allergy Clin Immunol* 1969;**43**:231–244.
7. Gruchalla RS. Antibiotic drug allergy: challenges of diagnosis and treatment. 8th International Paul Ehrlich Seminar. 1997;**91**:237–249.
8. Blanca M, Perez E, García J, et al. Anaphylaxis to amoxicillin with good tolerance to benzylpenicillin. *In vivo* and *in vitro* studies of specific IgE antibodies. *Allergy* 1988;**43**:508–510.
9. Silviu Dan F, McPhillips S, Warrington R. The frequency of skin test reactions to side chain penicillin determinants. *J Allergy Clin Immunol* 1993;**91**:694–701.
10. Moss RB, Babin S, Hsu YP, Blessing-Moore J, Lewiston NJ. Allergy to semisynthetic penicillins in cystic fibrosis. *J Pediatr* 1984;**104**:460–466.
11. 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.
12. 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;**7**:542–548.
13. Levine BB, Redmond AP. Minor haptenic determinant specific reagins of penicillin hypersensitivity in man. *Int Arch Allergy Appl Immunol* 1969;**35**:445–455.
14. Levine BB, Redmond AP, Fellner MJ, Voss HE, Levytska V. Penicillin allergy and the heterogeneous immune response of man to benzylpenicillin. *J Clin Invest* 1966;**45**:1895–1906.
15. Blanca M, Vega JM, García JJ, et al. Allergy to penicillin with good tolerance to other penicillins. Study of the incidence in patients allergic to betalactams. *Clin Exp Allergy* 1990;**20**:475–481.
16. Mayorga C, Obispo T, Jimeno L, et al. Epitope mapping of betalactam antibiotics with the use of monoclonal antibodies. *Toxicology* 1995;**97**:225–234.
17. Torres MJ, González FJ, Mayorga C, et al. IgG and IgE antibodies in subjects allergic to penicillins recognize different parts of the penicillin molecule. *Int Arch Allergy Appl Immunol* 1997;**113**:342–344.
18. 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 carrier. *J Immunol Methods* 1992;**153**:99–105.
19. 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.
20. van Dellen RG, Walsh WE, Peters GA, Gleich GJ. Differing patterns of wheal and flare skin reactivity in patients allergic to the penicillins. *J Allergy* 1971;**47**:230.
21. Blanca M. New aspects of allergic reactions to betalactams. Cross-reactions and unique specificities. *Clin Exp Allergy* 1994;**24**:407–415.
22. Gadde J, Spence M, Wheeler B, Adkinson FN. Clinical experience with penicillin skin testing in a large inner-city STD clinic. *JAMA* 1993;**270**:2456–2463.
23. Adkinson NF. Quantitative studies of the IgE and IgG immune response to penicillin administration in man. *Ann Allergy* 1977;**39**:73–76.
24. Assem ESK, Vickers MR. Investigation of the response to some haptenic determinants in penicillin allergy by skin and *in vitro* allergy tests. *Clin Allergy* 1975;**1**:43–57.
25. Strannegård I-L, Majeed HA, Ahlstedt S. Antibodies to penicillin in children receiving long-term secondary prophylaxis for rheumatic fever. *Allergy* 1987;**42**:502–506.
26. de Haan P, Bruynzeel DP, van Ketel WG. Onset of penicillin rashes: relation between type of penicillin administered and type of immune reactivity. *Allergy* 1986;**41**:75–78.
27. Christie G, Coleman JW, Newby S, et al. A survey of the prevalence of penicillin-specific IgG, IgM and IgE antibodies detected by ELISA and defined by hapten inhibition, in patients with suspected penicillin allergy and in healthy volunteers. *Br J Clin Pharmacol* 1988;**25**:381–386.