

Decrease of selective immunoglobulin E response to amoxicillin despite repeated administration of benzylpenicillin and penicillin V

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Summary

Background Subjects with IgE responses to betalactams can develop selective or cross-reactive responses after the administration of penicillin derivatives. After the reaction, however, the hapten induces a boosting phenomenon, which may increase the titre and the affinity of the antibody, with the resulting risk of developing allergic reactions to other penicillins.

Objective To determine in subjects with selective responses to amoxicillin (AX) and good tolerance to benzylpenicillin (BP) and penicillin V (PV) whether the administration of these compounds induced any change in specificity, measured by either skin or *in vitro* testing, which could predict the appearance of cross-reactivity.

Methods Ten subjects with a selective response to AX were followed-up for 2 years with the periodic administration of penicillin G and V (Group A) and compared with another group composed of 10 persons with identical clinical characteristics but without repeated penicillin administration (Group B). Periodic *in vitro* and *in vivo* measurements of specific IgE antibodies were performed at 6-month intervals. Patients were randomized to Group A or B according to their order of inclusion.

Results In both groups, skin test reactivity tended to decrease, and although greater in Group A, the difference was not significant compared with Group B. Median RAST values also decreased over time and showed no differences in the exposed group compared with the controls. One patient in Group A became positive to benzylpenicilloyl (BPO), despite becoming negative to AX.

Conclusion Subjects with selective IgE responses to side-chain-specific determinants seem to become negative, with no influence from subsequent administration of a closely related penicillin.

Keywords allergic reactions, betalactams, boosting, IgE, immediate reactions, *in vitro* tests, penicillin, skin test

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Introduction

Subjects with immediate reactions to penicillins develop IgE antibodies to the benzylpenicilloyl (BPO) structure [1]. This structure results from the spontaneous conjugation of benzylpenicillin (BP) to the amino group of a protein carrier [2] and represents 95% of the antibody recognition. The remaining percentage is formed by a number of different structures produced after metabolism. These include benzylpenicillenate, benzylpenicilloic acid, benzylpenicillanyl, benzylpenamaldate, benzylpenaldate, D-benzylpenicillamine and benzylpenicilloyl [3]. Although some of these structures have been shown to induce a skin test-positive response, these determinants have not been fully defined immunochemically [4].

In a subgroup of allergic patients, the formation of amoxicillin (AX) conjugates with the appropriate carrier induces IgE antibodies that preferably recognize the side chain and have good tolerance to other penicillins [5,6].

Information from both clinical and laboratory studies has been reported concerning the IgE response to amoxicilloyl (AXO) and BPO determinants [7].

The IgE antibody response is not permanent over time, and increased or decreased antibody levels may occur months to years after the occurrence of the reaction [8]. Questions therefore remain regarding whether subjects with a selective response will react to BP or to other closely related betalactams in subsequent therapeutic courses. It is therefore relevant to determine whether subjects with a selective response to betalactams would tolerate closely related penicillin determinants.

We followed-up a group of patients with a selective response to AX who were given a full therapeutic dose of BP plus a course of penicillin V (PV) for 3 days at regular intervals. The results indicated that the patients did not usually respond with *in vitro* and/or *in vivo* tests to the subsequent penicillin administered, nor did they show any difference in the rate of negativization compared with the control group. The selective response thus seems to be restricted to the side chain and no further conversion to BPO seems to occur.

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Material and methods

Patient selection

A group of subjects with a selective response to AX were studied. The evaluation was carried out as published [9]. In order to consider the cases as selective, the skin test had to be negative to benzylpenicilloyl-poly-L-lysine (BPO-PLL) and minor determinant mixture (MDM), and no *in vitro* IgE antibodies of the BPO group had to be present. Furthermore, good tolerance to one million units of BP by the parenteral route (i.m.) and to one capsule of 500 mg of PV every 8 h for 3 days by the oral route was confirmed.

Follow-up studies

A group of 20 cases were randomly assigned to two groups: A and B. These subjects were selected based on the diagnosis of a selective response to AX with a good tolerance to BP. Group A was composed of 10 subjects to whom BP was given at one single dose of 10^6 U followed by 500 mg of PV every 8 h for 3 days 12, 18 and 24 months after the initial evaluation. Each patient received a total of three therapeutic courses after the initial evaluation. The mean time interval between the occurrence of the reaction and the first drug provocation was 4.9 months (ranging from 1 to 18 months). Group B was composed of another 10 patients with equivalent clinical characteristics but without repeated penicillin administration. In both groups, the periodic measurement of *in vitro* and *in vivo* specific IgE antibodies was performed at the time-points mentioned above. The whole follow-up period lasted 2 years.

Skin testing

This was carried out as reported elsewhere [9]. In all cases skin testing was conducted using 0.02 mL of solution prepared daily. The reagents were BPO-PLL (Allergopharma Merck, Reinbeck, Germany) (5×10^{-5} M), MDM (Allergopharma) (2×10^{-2} M), AX (SB Smithkline Beecham, Madrid, Spain) (20 mg/mL) and ampicillin (AMP) (Normon, Madrid, Spain) (20 mg/mL). In the skin prick tests, a weal larger than 2 mm with a negative response to the control saline was considered positive. In the intradermal tests the weal area was marked initially and 20 min after testing, and an increase in diameter greater than 3 mm was considered positive.

Specific human immunoglobulin E antibodies

These were carried out by RAST as described [10]. Briefly, BP and AX were conjugated to PLL (Sigma, St. Louis, MO, USA) and then to the solid phase using cyanogen bromide-activated cellulose discs. The RAST was carried out by incubating 30 μ L of patient sera with the discs with different penicillin-PLL conjugates (BPO-PLL, AXO-PLL, Ampicillin-PLL) for 3 h. After three washes, radiolabelled anti-IgE antibody (Pharmacia diagnostics, Uppsala, Sweden) was added and incubated overnight. The discs were then washed, and their radioactivity was measured in a gamma counter, Cobra II auto-gamma (Packard BioScience Company, Frankfurt, Germany). The results were calculated as a percentage of a maximum (% RAST). Samples were

considered positive if they were higher than 2.5% of the label uptake, which was the mean + 2SD of the negative control group.

Statistical study

The median RAST values were compared at different times between Groups A and B by a non-parametric test (Kruskal-Wallis test). The decreasing trend of the RAST values in each group was evaluated by the Friedman test. The percentage negativization of the skin test in both groups was compared by χ^2 analysis. All reported *P*-values represented two-tailed tests, with values ≤ 0.05 considered statistically significant. The statistical analysis was performed using the SPSS program, version 11.5.

The study was approved by the Hospital Ethics Committee.

Results

The clinical data of the patients, along with the drug involved in the reaction, the dose and the interval between occurrence of the reaction and performance of the study, are shown in Table 1. Group A was composed of seven women and three men, with a mean age of 26 (42-14) years. The mean time between occurrence of the allergic episode and performance of the study was 49 (18-1) months. Group B was composed of six women and four men, with a mean age of 28 (53-17) years. The mean time between occurrence of the allergic episode and performance of the study was 49 (17-2) months. No differences were observed between the groups in the ratio of men to women, the age of the subjects or the interval between the occurrence of the reaction and the study. The results of skin tests at the initial evaluation are shown in Table 2. As stated in the selection criteria, all patients were initially skin test positive to AX and negative to BPO and MDM. Those cases marked with an asterisk were positive by prick test and the others were positive by intradermal testing.

The sequential follow-up of the skin testing in both Groups A and B is shown in Table 2. A variable degree of conversion from positive to negative was observed. In Group A, 80% remained positive at the second evaluation, 70% at the third one and 40% after completing the study, 2 years after the initial evaluation. In Group B, the corresponding values were 80%, 60% and 20% (Table 3, bottom). χ^2 comparison with the percentages of negativization between both groups showed no significant differences at any of the time-points analysed. Regarding the evolution of BPO, in one case, number 10 from Group A, the AX and AMP determinations remained negative and BPO appeared positive despite all previous BPO determinations being negative. When questioned, this patient reported having undergone surgery but denied any antibiotic contact.

The results of the initial RAST values are shown in the first column of Table 3. As occurred with the skin test, all cases were RAST positive to AXO-PLL and negative to BPO-PLL, and only some of the AXO-PLL-positive cases were also RAST positive to AMPO-PLL.

Parallel analysis of the median values of the specific IgE to AXO-PLL is shown in Fig. 1. Friedman analysis showed a significant decrease in each group ($P < 0.001$). However, when

Table 1. Clinical data of patients from Groups A (cases 1–10) and B (cases 11–20)

Patients	Age	Sex	Clinical manifestations and drug involved	Intervals (months)
1	42	F	Generalized pruritus, weals on chest, abdomen and legs 20 min after oral intake of 500 mg of AX	3
2	39	F	Facial erythema, difficult breathing, palm and sole pruritus, hypotension 15 min after oral intake of 500 mg of AX	6
3	28	M	Generalized erythema with palm, leg and genital pruritus after an intramuscular injection of AMP. Previous episode of similar symptoms after oral intake of 500 mg of AX 6 months before	18
4	37	M	Palm and sole pruritus, weals on face, chest and legs, hypotension less than 30 min after oral intake of 500 mg of AX	4
5	14	F	Systemic pruritus, facial angio-oedema with weals on arms, abdomen and legs 30 min after oral intake of 850 mg of AX-CLAV	2
6	17	F	Lip and eye angio-oedema, generalized maculopapulae 30 min after oral intake of 250 mg of AX	1
7	22	F	Generalized pruritus, abdominal pain, diarrhoea, facial erythema and hypotension 20 min after oral intake of 500 mg of AX	4
8	23	F	Maculopapulae with pruritus on abdomen, chest and legs plus facial angio-oedema 30 min after oral intake of 500 mg of AX-CLAV	3
9	18	M	Difficulty breathing, hypotension and generalized erythema 15 min after oral intake of 500 mg of AX	5
10	21	F	Weals on chest, abdomen and legs plus generalized pruritus with transitory faintness less than 15 min after oral intake of 500 mg of AX	3
11	44	F	Weals on abdomen and legs plus upper airway discomfort 20 min after oral intake of 500 mg of AX	2
12	53	M	Palm pruritus, weals on face, chest and legs, faintness less than 30 min after oral intake of 500 mg of AX	4
13	32	F	Abdominal pain, faintness, diarrhoea, facial erythema and hypotension 20 min after oral intake of 500 mg of AX	3
14	31	M	Weals on neck, chest, abdomen and legs plus generalized pruritus less than 15 min after oral intake of 500 mg of AX	2
15	22	M	Facial and neck erythema, palm and sole pruritus, hypotension 15 min after oral intake of 500 mg of AX	2
16	21	F	Systemic pruritus, weals on arms, buttocks and legs after oral intake of 850 mg of AX-CLAV	4
17	26	M	Palm and genital pruritus with erythema after an intramuscular injection of 500 mg of AX	3
18	17	F	Maculopapulae with pruritus on abdomen, chest, buttocks and legs 30 min after oral intake of 500 mg of AX-CLAV	4
19	25	F	Hypotension, pruritus and generalized erythema 15 min after oral intake of 500 mg of AX	17
20	25	F	Facial angio-oedema, generalised maculopapulae 30 min after oral intake of 500 mg of AX	8

AX, amoxicillin; AX-CLAV, amoxicillin-clavulanic acid; AMP, ampicillin.

this trend was compared between the groups, no significant differences were found.

The follow-up of specific IgE antibodies to BPO-PLL and AXO-PLL during the whole period is presented in Table 3. In Group A, 80% of the cases were positive to AXO-PLL at the first evaluation, 40% at the second one, and 10% at the last one. The corresponding values for Group B were 70%, 30% and 10%, respectively. Although the percentage of cases who remained positive at the second and third evaluation was higher in Group A, no significant differences were observed between the groups.

Discussion

The repeated administration of allergens with cross-reactivity may induce a boosting response triggered by the cross-reacting determinant [11]. Furthermore, the possibility also exists of inducing IgE antibodies to new determinants to which a subject was not initially sensitized [12–14].

We have previously shown that in immunotherapy with hymenoptera venoms, in subjects originally sensitized to *Polistes dominulus*, the administration of *Vespa germanica* venom induced a boosting response with IgE antibodies that

recognized common determinants to both venoms, and in some instances specific IgE antibodies recognizing *V. germanica* determinants not originally present in the subject before immunotherapy [11]. To our knowledge, no similar studies have been carried out with the response to drugs.

Resensitization to penicillins in patients with a previous positive history has been estimated to be low (<5%) [15, 16], although the cases were selected on the basis of a previous clinical history rather than prospective studies. One limitation of this type of study is that penicillin-sensitive patients take a long time to become negative and before skin testing can be conducted to assess resensitization [16]. Our study was not designed to examine resensitization, but to determine whether successive administration of PG and PV in selective responders to AX could induce a response to this drug or modify the rate of negativization. This was based on evidence that in the course of a boosting response, subjects can recognize closely related drugs [17, 18]. We were able to perform this study because selective responders to AX may lose sensitivity over a short period of time [18].

In IgE responses, antibodies recognize mainly the original structure with a variable degree of cross-reactivity with other structures [16]. In a boosting response the antibody titres increase with the recognition of closely related structures that were not originally detected, probably because of the

Table 2. Sequential follow-up of skin testing over a period of 2 years at four evaluations (initial and after 12, 18 and 24 months) of patients from Groups A (cases 1–10) and B (cases 11–20)

Patients	Initial evaluation				12 months				18 months				24 months			
	BPO	MDM	AX	AMP	BPO	MDM	AX	AMP	BPO	MDM	AX	AMP	BPO	MDM	AX	AMP
1	–	–	+	+	–	–	+	–	–	–	+	–	–	–	–	–
2	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
3	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–
4	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
5	–	–	+	+	–	–	+	+	–	–	+	+	–	–	+	+
6	–	–	+	+	–	–	+	+	–	–	+	–	–	–	–	–
7	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–
8	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
9	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
10	–	–	+	–	–	–	–	–	–	–	–	–	+	–	–	–
11	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–
12	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
13	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–
14	–	–	+	+	–	–	+	+	–	–	+	–	–	–	–	–
15	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–
16	–	–	+	+	–	–	+	+	–	–	+	–	–	–	+	–
17	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–
18	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–
19	–	–	+	+	–	–	+	+	–	–	+	–	–	–	–	–
20	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
A	10/10 (100%)				8/10 (80%)				7/10 (70%)				4/10 (40%)			
B	10/10 (100%)				8/10 (82%)				6/10 (63.6%)				2/10 (18.2%)			

At the bottom the percentage positivity of skin tests at each evaluation point is shown. BPO, benzylpenicilloyl; MDM, minor determinant mixture; AX, amoxicillin; AMP, ampicillin.

Table 3. Sequential follow-up of RAST values over a period of 2 years at four evaluations of patients from Groups A (cases 1–10) and B (cases 11–20)

Patients	BPO-PLL	AXO-PLL	BPO-PLL	AXO-PLL	BPO-PLL	AXO-PLL	BPO-PLL	AXO-PLL
1	–	26.4	–	5.6	–	–	–	–
2	–	12.9	–	6.47	–	–	–	–
3	–	3.5	–	–	–	–	–	–
4	–	8.6	–	2.6	–	–	–	–
5	–	25	–	11	–	4.3	–	–
6	–	32	–	19	–	5.7	–	3.3
7	–	25	–	–	–	–	–	–
8	–	33	–	12	–	5.9	–	–
9	–	16	–	5	–	3.2	–	–
10	–	19	–	–	–	–	–	–
11	–	23	–	–	–	–	–	–
12	–	18	–	11	–	4.3	–	–
13	–	17	–	–	–	–	–	–
14	–	23	–	6	–	3.8	–	2.8
15	–	18	–	7	–	–	–	–
16	–	17	–	5	–	–	–	–
17	–	23	–	12	–	4	–	–
18	–	32	–	20	–	–	–	–
19	–	12	–	6	–	–	–	–
20	–	13	–	–	–	–	–	–
A	10/10 (100%)		8/10 (80%)		4/10 (40%)		1/10 (10%)	
B	10/10 (100%)		7/10 (70%)		3/10 (30%)		1/10 (10%)	

At the bottom the percentage positivity of the RAST at each evaluation point is shown. BPO-PLL, benzylpenicilloyl-poly-L-lysine; AXO-PLL, amoxicilloyl-poly-L-lysine. RAST values below 2.5% of label uptake are considered to be negative (–).

sensitivity of the detection techniques. This increase in antibody titre may imply a risk of responding to these structures [17]. Thus, in the boosting response to betalactams,

the increasing titre and the affinity of the antibodies could mean that administration of a closely related structure might induce a reaction.

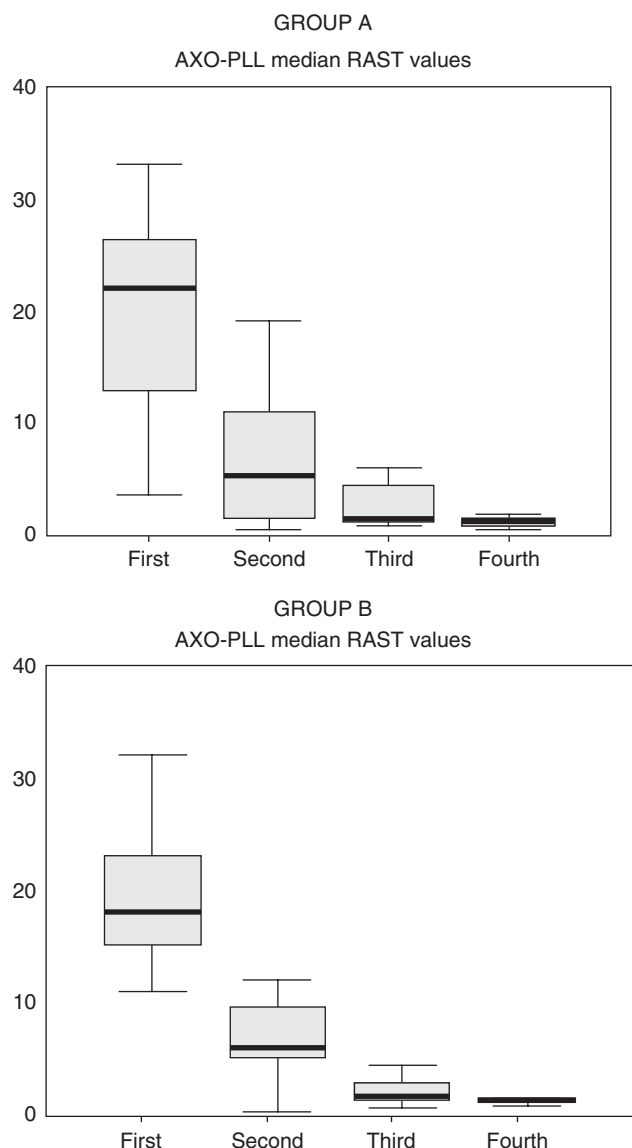


Fig. 1. Box-plot graph presentation of the median RAST values for amoxicilloyl-poly-L-lysine (AXO-PLL) in the two groups of patients studied at the four different determinations.

In our experience, an important percentage of persons who develop a response to AX and cephalosporins often become positive to BPO by skin testing and *in vitro* studies. Because this positivity to BPO accompanies the reaction and falls with time, it is clear that it is induced by a chemically related AX or cephalosporin [7, 19].

With these considerations in mind, our objective was to determine whether the selective response to AX was maintained after subsequent administration of two closely related drugs: BP and PV. The selection of the patients was critical for our study. Accordingly, we only present the results in those cases who showed no response, by either *in vivo* or *in vitro* testing, to other determinants related to BP. Despite being negative to BP determinants, some cases were skin test or RAST positive to AMP. Previous studies indicated that in our series this positivity was because of cross-reactivity between the side chain of AMP and AX [19].

The results of this study indicate that during a follow-up period of 24 months, administration of BP and PV was not followed by any positivization, either in skin test or in the *in vitro* evaluation of specific IgE antibodies. Only one case became positive at the third and final evaluation, and the patient became positive after surgery, although the patient denied the administration of or contact with any betalactam.

Although the patients in Group A tended to remain positive for longer in terms of both skin test and RAST values, there was no significant difference in the rate of negativization. Whether these data represent a tendency in penicillin-stimulated persons to maintain sensitivity cannot be answered because of the limited sample size. Little evidence is available in the literature concerning this problem. Resensitization studies indicate that negative patients may become positive, not only after therapeutic administration [15, 16, 20] but also after non-therapeutic exposure to betalactams [21].

This study may have limitations, as have been reported by some authors [15]. The administration of PG and PV at regular intervals may not be sufficient to induce resensitization, and the possibility exists that a complete therapeutic regime with the drug could better stimulate the resensitization phenomenon. However, previous experience from our group indicates that in subjects with immediate reactions, small therapeutic doses may resensitize [21].

In summary, the selective response to AX seems to be restricted to the chemical structure of the side chain, with no further influence from possible closely related structures in inducing the boosting response. Extrapolating these results to other not so closely related chemical structures, such as cephalosporins, in patients with a selective response, the possibility of conversion to positive is unlikely, although detailed follow-up studies are required to answer this question fully.

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