

Original article

IgE antibodies to betalactams: relationship between the triggering hapten and the specificity of the immune response

Background: In immunoglobulin (Ig)E-mediated responses to betalactams (BL) the antibody is directed to the hapten inducing the response. For benzylpenicillin (BP) the determinant is benzylpenicilloyl (BPO) and for amoxicillin (AX), amoxicilloyl (AXO). Because of cross-reactivity, IgE from some patients reacts to both drugs whereas others have a drug-selective recognition. After an allergic episode, there is an increase in IgE that decreases over time. We analysed the response of patients allergic to BL after penicillin administration, with emphasis on IgE cross-reactivity.

Methods: Subjects who developed an IgE antibody response were studied. Sequential follow-up samples were obtained at different times during the response. Changes in IgE specificity were analysed by competition immunoassays using different penicillin monomeric conjugates.

Results: Two patterns of response were existed: one with IgE directed to the culprit penicillin and another with IgE mainly reactive to BPO. In both, a variable cross-reactivity with the hapten triggering the boosting response was found. This pattern was maintained with no change in specificity over time, even in subjects who experienced one boosting event.

Conclusion: The IgE response can be specific to the drug inducing the reaction or cross-reactive to the classical BPO determinant. This pattern is maintained throughout the whole period of the response, even if re-exposure occurs. The stability of the type of response can be explained by the phenomenon of original antigenic sin: in the presence of antibodies, memory B cells are more easily triggered than naive B cells.

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Immunoglobulin (Ig)E antibodies to betalactams (BL) are induced by a hapten-carrier conjugate and are subject to strict T–B cell cooperation (1). The final structure of the major epitope is thought to be formed by the penicilloyl structure and the amino group of the carrier (2). Although the amino acids surrounding the hapten were initially reported to have a marked contribution (2), no recent study has been undertaken in this direction. In the secondary antibody response to antigens and allergens, IgG or IgE antibodies are primarily induced against a specific epitope and these antibodies then have different degrees of cross-reactivity with structures related to the primary inducer (3, 4). During the ongoing course of the immune response, slight variations in the antigenic

structure induce changes in the specificity and affinity of the antibody, which adapts to the slight conformational changes of the antigen (5). In viral infections, these changes contribute to continuous adaptation of the immunological systems by modifying antibody specificity, in order to adapt to variation in the antigenic structure (6, 7). However, the state of immunity may influence the subsequent immunological response to structurally related antigens, a phenomenon known as the original antigenic sin (8).

In immediate reactions to BL, IgE mainly recognize a penicillin determinant, usually benzylpenicilloyl (BPO), with a variable degree of cross-reactivity with other determinants (9, 10). In other cases, the main structure recognized is the amoxicilloyl (AXO) determinant, again with a variable degree of cross-reactivity with other structures (11). Thus, according to the degree of cross-reactivity, subjects may develop anaphylactic responses to one particular BL but have good tolerance to other BL.

Abbreviations: BL, betalactams; BPO, benzylpenicilloyl; AXO, amoxicilloyl; BP, benzylpenicillin; MDM, minor determinant mixture; AX, amoxicillin; AMP, ampicillin; CUL, culprit drug; RAST, radioallergosorbent test; AMPO, ampicilloyl; CEPHO, cephalosporoyl; PLL, poly-L-lysine; BA, butylamine; CEF, cefixime.

We studied the IgE antibody response in subjects who had an increase in IgE level after developing an allergic reaction. We showed that in some instances specificity was mainly directed to the BL inducing the reaction, but in other cases specificity was mainly directed to another structure, probably related with previous BL exposure. These results can be explained by the theory of original antigenic sin antibody response (12) and are relevant for understanding the problems of IgE hypersensitivity to BL and the different responses seen in different populations.

Material and methods

Patients

The study included a selected group of patients who after an allergic reaction presented an increase in specific IgE antibodies over time. Details of the study group are presented in Table 1. All patients underwent skin tests to major determinants of benzylpenicillin (BP), the BPO, and minor determinant mixture (MDM), amoxicillin (AX) and ampicillin (AMP) and, if necessary, to the culprit drug (CUL), as described by Torres et al. (13). The study was approved by the institutional review board, and informed consent for all the diagnostic procedures was obtained from the patients.

Patient sera were obtained at regular intervals after the episode, ranging from 6 months to 1 year, over a total period of 2 years.

Specific human IgE antibodies

These were determined by radioallergosorbent test (RAST) as described (10). Briefly, BP, AX, AMP or CEF were conjugated to poly-L-lysine (PLL; Sigma, St Louis, MO, USA) and then to the solid phase using cyanogen bromide-activated cellulose discs. The RAST was made by incubating 30 µl of patient sera with the discs with different BL-PLL conjugates 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 Cobra II auto-gamma counter (Packard BioScience Company, Frankfurt, Germany). Results were calculated as a percentage of the maximum (% RAST). Samples were considered positive if they were higher than 2.5% of label uptake, which was the mean $\pm$ 2 SD of the negative control group.

Preparation of monomeric antibiotic conjugates

Monomeric conjugates were used in the solid phase to ensure the accuracy of the hapten inhibition. Conjugates of the antibiotics (AX and BP) to butylamine (BA; Sigma) were prepared as described elsewhere (11). The conjugate BPO-BA was prepared by dissolving 1.03 g of BP in 1 ml of water at room temperature. To this solution 1.4 ml of BA was added and the mixture was shaken for 1 h. After the addition of 20 ml water, 30 ml of HCl was added slowly. The white pellet was washed and filtered with water. The AXO-BA, AMPO-BA and cephalosporoyl (CEPHO)-BA conjugates were prepared by slowly dissolving 1.23 g of the AX, AMP or CEF, respectively, in a solution of BA (14 M) at room temperature. The solution was stirred for 1 h and then lyophilized. The resulting solids were crystallized from acetonitrile ether.

RAST Inhibition

Cross-inhibition studies were made in the solid phase with AXO, BPO, ampicilloyl (AMPO) or cephalosporoyl (CEPHO) conjugated to PLL. This was carried out as reported (11) by incubating sera from patients allergic to BL (with values higher than 10% label uptake) with different monomeric conjugates of BL to BA, at three 10-fold concentrations, ranging from 100 to 1 mM. Comparison of the inhibition capacity of the monomeric conjugates was made at 50% inhibition.

Statistical studies

The coefficient of variation of each measurement was made with a pool of sera in order to establish the minimum variation accepted as different cross-reactivity. Values higher than 15% were considered as different and the coefficient of variation of each sample was within 10% of the variation.

Results

Table 1 shows a group of nine patients who experienced an increase in antibodies to a penicillin derivative after an allergic episode. All of them were nonatopic patients with normal total IgE values at the beginning of the study. The serum number in the figures corresponds to

Table 1. Clinical and immunological characteristics of the patients included in the study

Patient	Drug	Reaction	Time interval (days)	Total IgE (kU/l)	Skin test					RAST			
					BPO	MDM	AX	AMP	CUL	BPO	AXO	AMPO	CUL
1	AX	Anaphylaxis	30	230	—	—	+	+		0.3	20	12	
2	AX	Anaphylaxis	3	120	—	—	+	+		2.8	14	7	
3	AX	Urticaria	120	63	+	+	+	—		7.8	6	5	
4	AX	Anaphylaxis	20	34	+	+	+	—		12.3	9	4	
5	BP	Anaphylaxis	30	87	—	+	+	+		10	3	7	
6	AMP	Anaphylaxis	20	46	+	+	—	+		5.3	0.7	2.3	
7	AX	Anaphylaxis	15	97	+	—	—	—		5.6	1.3	1.4	
8	CEF	Anaphylaxis	45	131	—	—	—	—	—	16	1.2	1.3	1.4
9*	AX	Anaphylaxis	32	265	+	—	+	—		8.3	12	3	

BP, benzylpenicillin; AX, amoxicillin; AMP, ampicillin; CEF, cefixime; BPO, benzylpenicilloyl; MDM, minor determinant mixture; CUL, culprit drug; AXO, amoxicilloyl; AMPO, ampicilloyl; Ig, immunoglobulin; RAST, radioallergosorbent test.

*Case 9 developed a boosting response.

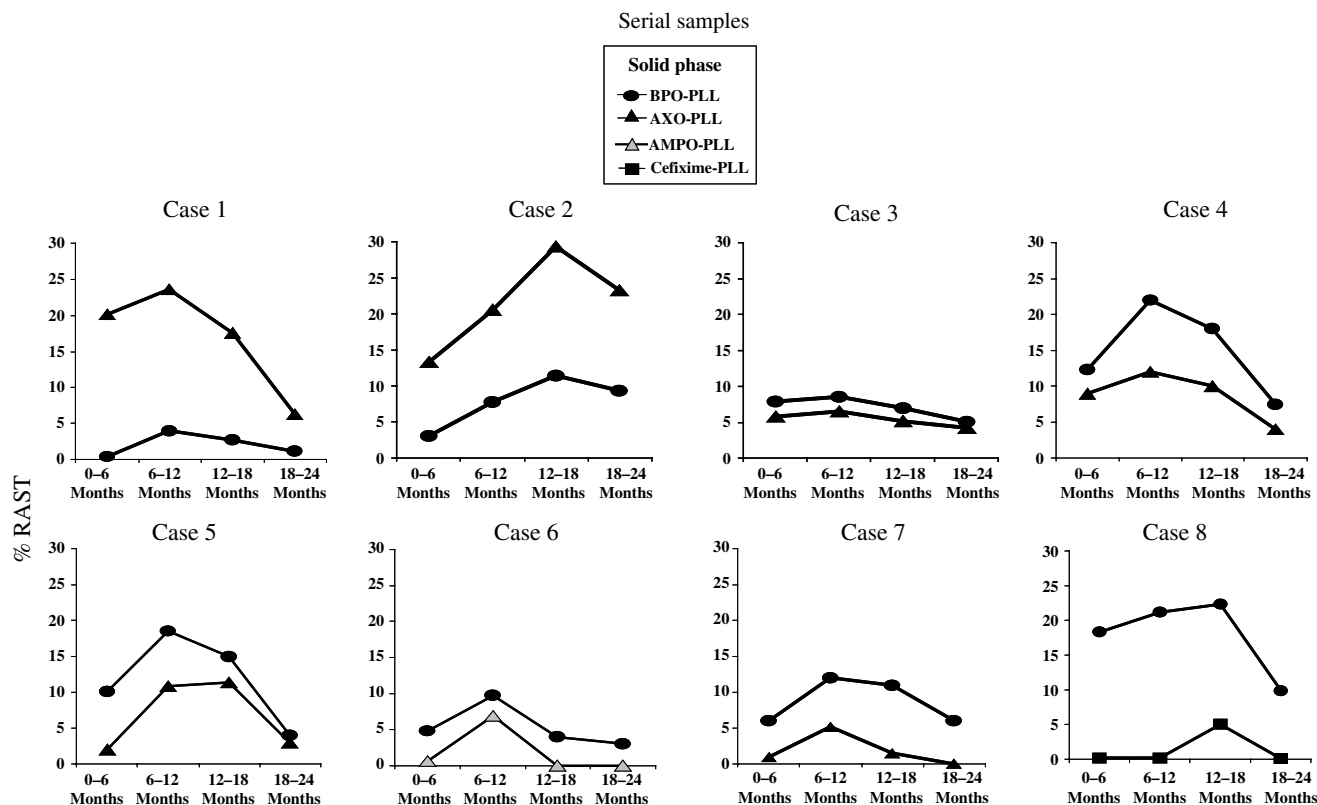


Figure 1. Sequential radioallergosorbent test (RAST) analysis of (all) cases 1–8 included in the study.

the patient number is presented in Table 1. Figure 1 shows the sequential values of the sera of cases 1–8 represented in Table 1. In case 1 an increase in IgE-specific values to AXO was observed, with the appearance of positivity to BPO in the second sequential sample, with a simultaneous parallel increase and decrease during the whole assay period. In case 2, initially positive to both AXO and BPO, a parallel increase was observed with higher values for AXO during the whole period. Case 3 shown in Table 1 was a patient who responded with similar RAST values to both BPO and AXO. During the whole follow-up period, positive IgE values were obtained in the sera at the first determination with a parallel decrease over the period of all the sequential samples. Case 4 was a patient who developed an anaphylactic response to AX but had a greater IgE antibody response to BPO. In case 5, where BP was the trigger, a typical increase in BPO was observed with a parallel increase in AXO. The initial RAST value to AXO was negative, becoming positive in the subsequent measurements in parallel with the BPO-specific IgE values. In patient 6, AMP was the trigger and, as with the previous case, RAST with AMP was initially negative, only appearing positive during one of the following sequential sera. All values to BPO-PLL, however, were positive during the whole follow-up period. In case 7, AX was the trigger but it induced a

positive response to BPO and AX, though the RAST value was initially negative to AXO-PLL. In the case 8, the inducing drug was cefixime (CEF) and we observed an original positive value to BPO that increased during the follow-up period but only appeared positive to CEF in the third sample. It is of note that cases 4 and 6 received in their childhood BP with good tolerance.

Figure 2 shows the cross-inhibition studies of sera belonging to cases 2 and 3 represented in Table 1. Cross-inhibitions with two sequential samples from case 2, using three different 10-fold concentrations in the fluid phase and the discs conjugated to BPO-PLL and AXO-PLL are illustrated in Fig. 2(A–D). With AXO-PLL in the solid phase, a molar concentration of BPO-BA over 10 times higher was required for 50% inhibition (Fig. 2B). When the BPO-PLL solid phase was used, AXO-BA was also the stronger inhibitor but with a similar molar concentration (Fig. 2A). We conclude that the primary specificity of the antibody was directed to AXO-BA with cross-reactivity to BPO-BA. The results of the values of sera sample 3 from the same patient (case 2), showed that AXO-BA was also the stronger inhibitor and the maximum concentration of BPO-BA did not approach 50% inhibition (Fig. 2D). The pattern of response using BPO-PLL discs also indicated that AXO-BA was the stronger inhibitor (Fig. 2C). The two sera of case 3, serial samples 2 and 3, had a similar inhibition pattern to case 2,

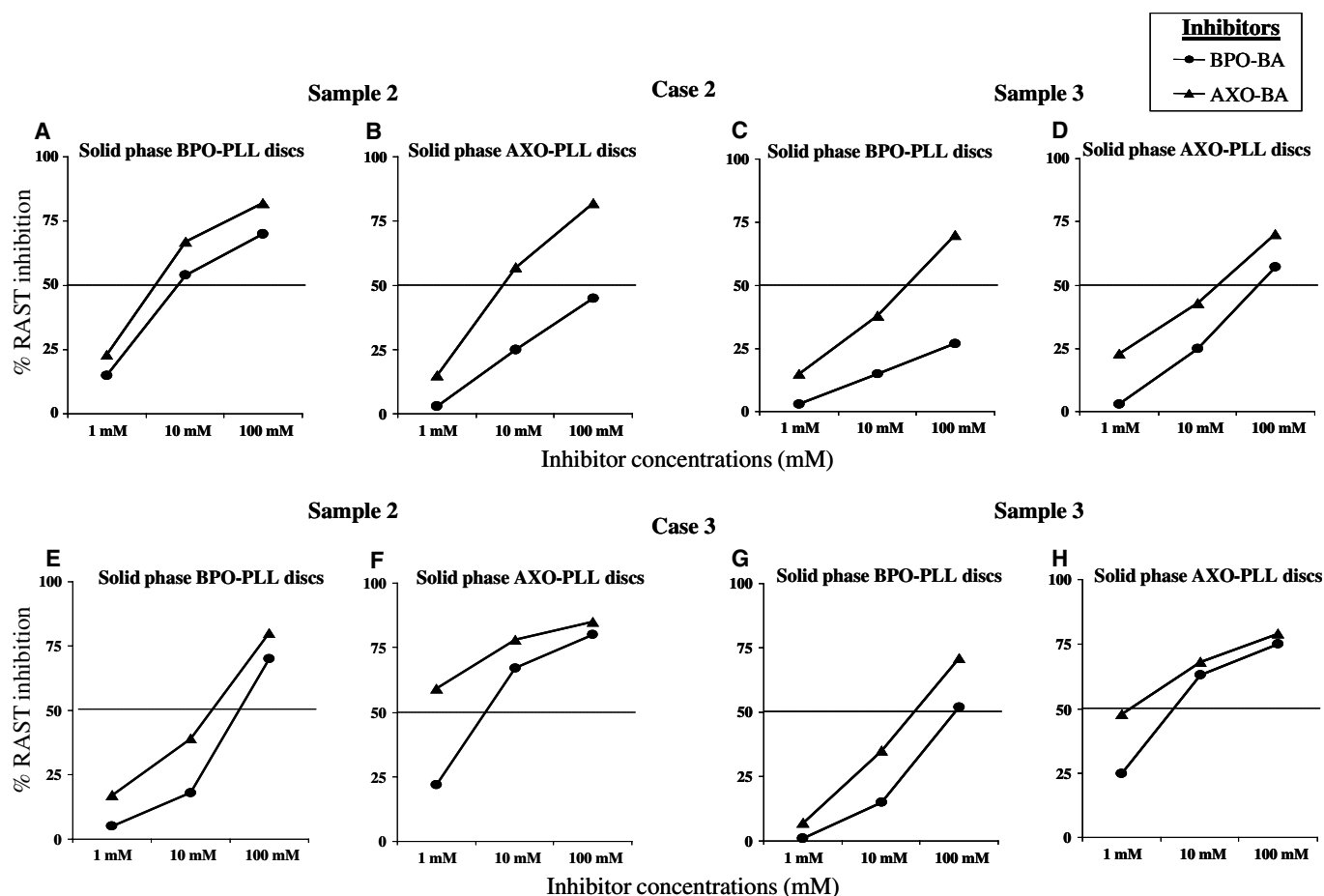


Figure 2. Inhibition studies with two sequential samples from cases 2 and 3, specific to amoxicillin (AX).

indicating that the IgE antibodies mainly recognized AXO (Fig. 2E–H).

Case 1 shown in Table 1 was also inhibited, but only with AXO–PLL discs because the RAST values were either very low or negative for BPO–PLL discs. Specificity to AX was also confirmed. Results of case 4 presented in Table 1 showed a very high degree of cross-reactivity between BPO and AXO (data not shown).

The inhibition with the highest positive serum of each episode from cases 5 to 8 is illustrated in Fig. 3. In case 5 (Fig. 3A), BPO–BA was the main inhibitor using BPO–PLL as the solid phase, requiring an AXO–BA concentration >100 mM to achieve 50% inhibition, whereas this inhibition was obtained with 10 mM of BPO–BA. When the AXO–PLL discs were used (Fig. 3B), over 50% inhibition was obtained with 1 mM of BPO–BA and the inhibition with AXO–BA at the same concentration was of the order of 25%. The results of case 6, where AMP was the trigger, are presented in Fig. 3C,D. Using BPO–PLL discs, although inhibitions were parallel, comparisons at 50% inhibition required lower concentrations of BPO–BA than with AMPO–BA. The use of AMPO–PLL

discs showed a similar behaviour, with 10-fold lower concentration needed for inhibition of BPO–BA compared with AMPO–BA.

Figure 3(E,F) shows case 7, in which AX was the inducing drug. Concentrations of the order of 10 times lower of BPO–BA were required for 50% inhibition, but these differences were higher when AXO–PLL discs were used and AXO–BA concentrations of the order of 100 times greater were required to obtain 50% inhibition.

Case 8 (Fig. 3G,H) corresponds to the patient who developed anaphylaxis to CEF. In this case serum 3 was used. Using BPO–PLL discs, 100 mM of CEPHO–BA conjugate was unable to produce 50% inhibition; higher concentrations were not used because of low drug solubility. The use of the CEPHO–PLL discs showed that BPO–BA was also the stronger inhibitor.

Figure 4(A–D) refers to case 9, who developed a boosting response. The RAST results showed that despite the fact that AX was the trigger on two occasions, BPO values were always higher in all the samples tested (Fig. 4A,B). The inhibition with the highest positive serum of each episode is illustrated in Fig. 4(C,D). As occurred with the cases presented above, in two instances

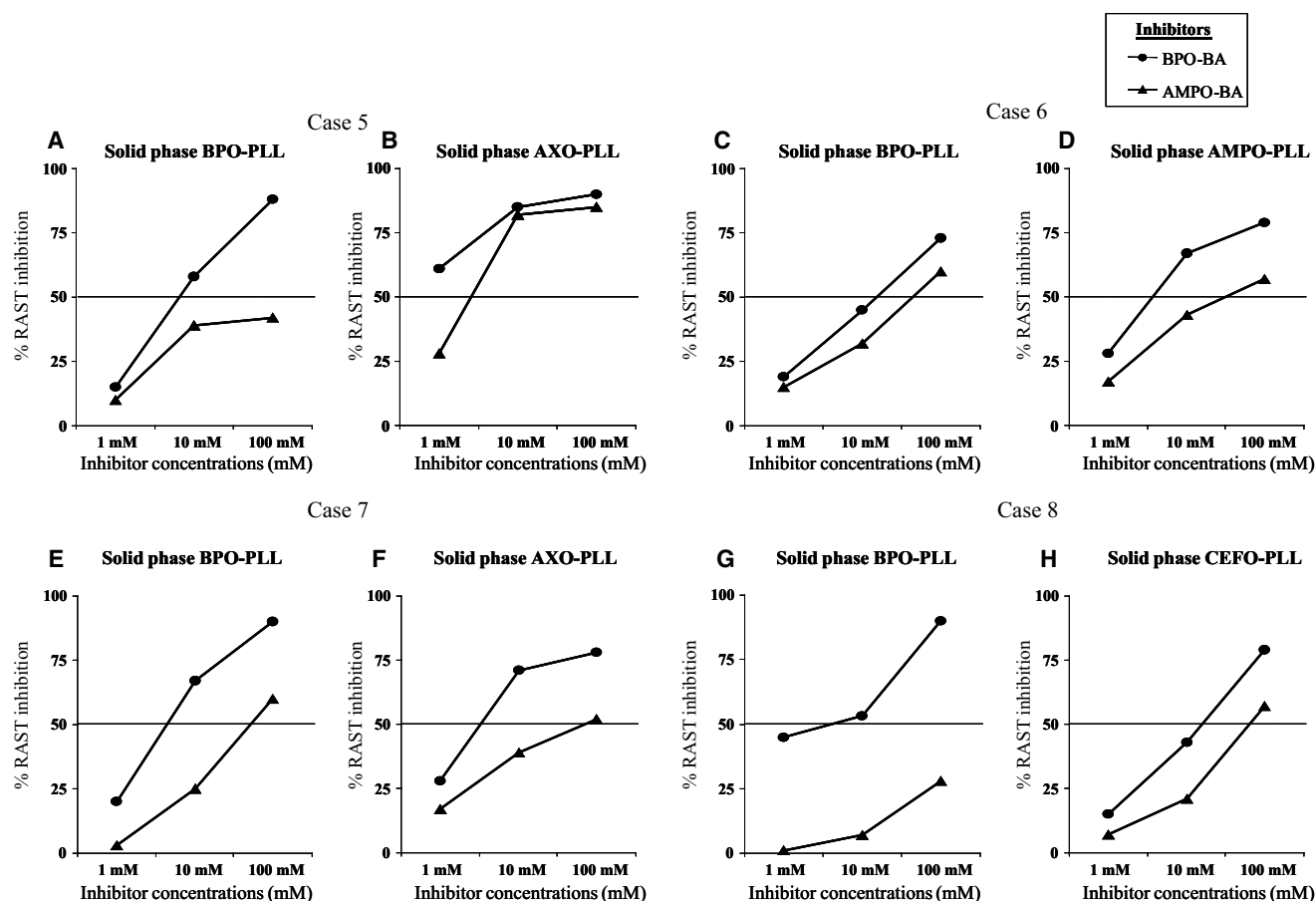


Figure 3. Radioallergosorbent test (RAST) inhibition studies with the highest positive serum of each episode from cases 5, 6, 7 and 8, specific to BPO.

the IgE response was formed by antibodies that recognized mainly the BPO structure with a variable degree of cross-reactivity with AXO.

Discussion

One of the most striking findings in previous studies concerning IgE responses to BL has been that in over half the patients who develop immediate reactions to AX the specificity of the antibodies was mainly directed to BP determinants, showing a variable degree of cross-reactivity with AX. In the remaining patients, IgE mainly recognized the AX determinants (9–11). The question that emerged from these results was why the most commonly prescribed BL and the most common inducer of the allergic responses in our patients, AX, induced heteroclitic IgE antibodies, i.e. antibodies that preferentially recognize related structures rather than the inducer drug (11, 12, 14). Furthermore, in cases of anaphylactic response to cephalosporin, independently of the chemical structure that elicited the response, the specificity was to BPO in a number of cases, which varied depending on the

population studied (15, 16). A possible explanation is that specificity is related to previous exposure of the immunological system to other BL (2). The different patterns of BL consumption may result in different phenomena. This would explain the observation that after taking the same drug, the IgE response varied in different countries in relation with the predominant BL consumed by the relevant population (14–20).

A good approach to understand this phenomenon in more detail is to study the specificity of the IgE antibodies in subjects who develop an IgE response after an immediate reaction to any BL derivative and who produce typical IgE curves, with an increase and a decrease in IgE antibody levels. The results of our study showed that IgE antibodies with different specificities could be found in each patient.

In the first group (cases 1 and 2), AX induced the IgE response and the stronger inhibitor was the AXO-BA conjugate, independently of the penicillin used in the solid phase. This was observed in all sequential sera studied in this group, and cross-inhibition studies showed that no coexisting antibodies were observed. The next group (cases 3 and 4) is formed by patients who had similar binding to

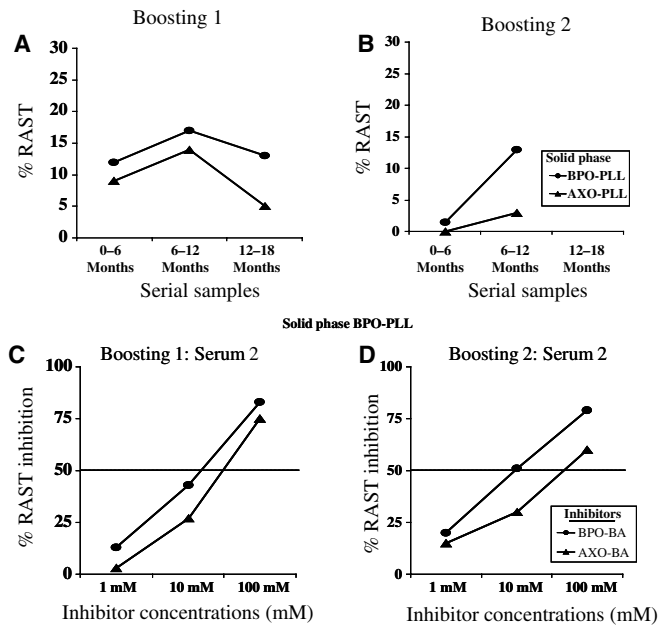


Figure 4. Radioallergosorbent test (RAST) and RAST inhibition assays of the boosting response in the same patient (case 9 from Table 1) with specific immunoglobulin (Ig)E response to amoxicillin (AX).

BPO and AXO. Inhibition studies showed that cross-reacting IgE antibodies were present. The parallel increase in AXO-BA and BPO-BA indicated that the curve was induced by a single haptenic stimulus. The third group is represented by sera from three cases in which, although the trigger was different to BP, the main specificity recognized was BPO-BA. These findings explain the results of the *in vivo* and *in vitro* tests presented in Table 1. The follow up reflected no change in the specificity of the IgE antibodies. These results indicate that only part of the structure induced an antibody response to the original structure and that this was not the trigger of the IgE response.

In B-cell responses, antibodies are subjected to continuous variation according to the structure of the inducer. This phenomenon produces an IgG switching with slight changes in antibody specificity, in order to

adapt to the antigenic shift. It is reasonable to suppose that this phenomenon also applies to IgE (5, 6). However, we detected no change in antibody specificity in the sera of a subset of patients, although in others the specificity was primarily directed to the inducer agent.

Immunoglobulin E antibodies are subject to changes and over time they tend to convert to negative (21). Thus, in those cases where resensitization occurred, we had the opportunity to determine the specificity. For example, after negativization of case 9 (Table 1), a rapid increase again induced positivity to the BPO determinant. All these results explain why in previous studies we observed a high number of positive responses to BPO despite the fact that AX was the original trigger of the reaction (10).

Although the term antigenic sin has been used for describing strain-specific serological responses to viral infections, this concept could be applied to our study. The antibody response to a given antigen may be strongly influenced by the antibody structures that have been generated during previous immune responses to other antigens (12). However, one of the difficulties in this study is that no clinical evidence of a previous IgE antibody response occurred in the patients, unless a transitory IgE antibody response had existed. The other possibility is that in primary antibody responses, subjects may have developed an IgM and IgG response to BPO specificity that is maintained after isotype switching for IgE production. To this extent, though, we are unaware of any previous data regarding this phenomenon.

In conclusion, IgE antibodies to BL seem to be related to previous exposure, even if no previous reaction occurred. This may be related with the ability of the immune system to mount an anamnestic response. In subsequent responses, specificities may be more related to previous exposure than to the inducing agent.

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