

Differences in serum IgE antibody activity to benzylpenicillin and amoxicillin measured by RAST in a group of penicillin allergic patients

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To determine the influence of the acyl side chain on the IgE antibody specificity of the two most common penicillins inducing allergic reactions in our community, benzylpenicillin (BP) and amoxicillin (AX), IgE positive sera from a group of 24 patients allergic to penicillin were studied. RAST was determined in parallel to benzyl penicilloyl-polylysine (BPO-PLL) and amoxicilloyl-polylysine (AX-PLL) in each serum, and values greater than an established coefficient of variation of 15 % were considered as different for either of the haptens used (BP and AX). 16 sera proved to be more positive to BP (Group A), six to AX (Group B) and in two IgE was positive to both haptens with a similar value (Group C). RAST titration concentration effect curves and RAST inhibition studies with a pool of sera from each group (A, B, C) and individual sera showed that most of them were more specific for either BPO or AX, a minority being of similar value to both. These findings indicate that the side chain structure of penicillins is relevant in the constitution of the antigenic determinant, although in some instances the common chemical structure of betalactam is recognised mainly by the IgE antibodies. The clinical relevance of these data needs to be established.

Key words: amoxicillin; benzylpenicillin; IgE antibodies; side chain.

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Penicillin and cephalosporin antibiotics conjugate with body proteins by opening the betalactam ring and forming a covalent linkage with the amino group of proteins (15, 16). These conjugates induce both antibody and cellular mediated immunological reactions (22). Different degrees of cross-reactivity have been reported between penicillins and cephalosporins. Using antibodies raised in animals some authors have found a high degree of cross-reactivity (7, 11). The cause of this has been attributed to the common epitope shared by all these compounds resulting from the conjugation of the carbonyl of the betalactam ring with the amino group of the carrier (9). Other authors

have found that the cross-reactivity is related to the variations in the acyl side chain structure (2, 21). Most studies have been performed using antisera raised in animals. Less work has been done in humans concerning cross-reactivity between penicillins and cephalosporins (4, 22) and between benzylpenicillin and semisynthetic penicillins (7). Although some authors indicate that the side chain structure has little influence in allergic reactions in man (22, 24), others have reported that the use of different betalactams in addition to major and minor determinants will improve the diagnostic efficiency of IgE antibody tests (18, 23). More recently, our group have reported cases where patients could tolerate

benzylpenicillin but had an anaphylactic response after taking amoxicillin (3, 5).

In this study we present the results of RAST and RAST inhibition to BP and AX determined in sera from patients allergic to different penicillins. Data are provided to support the role of the acyl side chain structure in the specificity of the antigen antibody reaction.

MATERIAL AND METHODS

Patients. Sera from 24 patients with allergic reactions to penicillins were studied. In all samples RAST was known to be positive to either AX or BP or both. All the sera with sufficient volume available in our files were used for this study.

PBO-PLL and AX-PLL conjugates We followed a modification of the method described by Edwards et al. (10). For the preparation of poly-L-lysine (PLL) derivatised discs 1 g of cyanogen bromide activated cellulose discs were treated with 20 mg of PLL in 20 ml of 0.1 M sodium bicarbonate, 0.5 M sodium chloride buffer pH 8 (buffer A). The discs were maintained for 2 h at room temperature by gently mixing and then treated with 20 ml of 1 M beta-ethanolamine in water at pH 8. After 2 h at room temperature

discs were washed in 0.5 M buffer A and conjugated in 20 ml of different molar concentrations of BP and AX dissolved in carbonate/bicarbonate buffer 0.05 M pH 10.5. The mixture was maintained at 37°C for 48 h and then treated in alternate washes of 0.1 M sodium bicarbonate and 0.05 M sodium acetate-acetic buffer 0.05 M pH 4.5. A final wash was made in PBS and the discs maintained at 4°C till use.

RAST. Specific IgE antibodies were determined to BP and AX as described (8). Each determination was made in duplicate and in the same serum for BPO-PLL, AX-PLL and PLL sensitized discs. The label uptake produced by the PLL discs was subtracted from BPO-PLL and AX-PLL conjugates and results expressed as percentage of label uptake of maximum counts added. Radiolabelled anti-IgE was obtained from Pharmacia, Uppsala, Sweden. Concentration effect curves were made with variable quantities of PLL, BP and AX to determine the optimal concentration of these reagents. PLL (P-0879), BP (PEN-NA) and AX (A-8523) were obtained from Sigma Chemical Co. Sera were grouped in three categories: Group A with more positive RAST value to BP, Group B with more positive value to AX, and Group C with similar

Table 1
RAST results in the 24 sera studied

Group A			Group B			Group C		
Sera	BPO	AX	Sera	BPO	AX	Sera	BPO	AX
1	30.2	6	17	17.4	31	23	23.3	25
2	26	3	18	1.7	5	24	17.5	16
3	7	2.4	19	0.8	10			
4	10	0.3	20	2	3.5			
5	10.3	0.2	21	2.5	4			
6	6.5	2.4	22	12	16			
7	7.5	3.8						
8	12.5	0.35						
9	3.4	0.4						
10	4.6	2.7						
11	4	0.3						
12	6	1.5						
13	12.5	5						
14	5	2.7						
15	7.8	0.35						
16	3	0.7						

Group A comprises sera more positive to benzyl penicillin (BP) and Group B more positive to amoxicillin (AX). Group C contains those with similar RAST values to both BP and AX (CV lower than 15%).

RAST values to both BP and AX ($<$ the established coefficient of variation (CV)). A control group of 31 sera from subjects with good tolerance to penicillins and with IgE values ranging between 11 and 1300 IU was used for establishing the cut-off point between positive and negative.

RAST inhibition was carried out following a modification of the method previously described (4). Univalent haptens of BP and AX conjugated to EACA (2) were employed in the fluid phase. BP was conjugated with a 10% excess of EACA at pH 11.5 for 1 h. This material was used without further purification.

Five 10-fold decreasing concentrations (100, 10, 1, 0.1 and 0.01 mM/l) of BP and AX were used. Results were expressed as percentage inhibition.

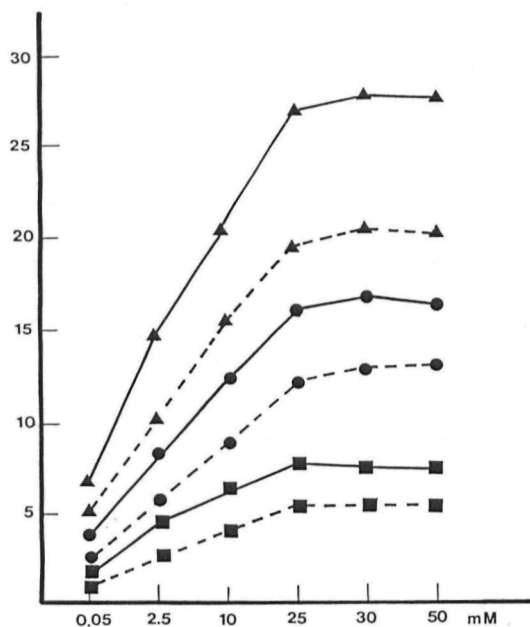


Fig. 1. Concentration effect curves using two concentrations of PLL, 10 mg/ml (continuous line) and 1 mg/ml (dashed line) with three pools of sera positive to BPO-PLL. Pool 1 ($\blacktriangle$) was made from sera with RAST values ranging from 35 to 25% of label uptake (lu). Pool 2 ($\bullet$) from sera with values ranging from 24 to 10 lu and Pool 3 ($\blacksquare$) from sera with a value ranging from 7.5 to 2.5 lu. A similar plateau was obtained with the pools at the concentration of 25 mM/l of hapten. With the concentration of 10 mg PLL a higher value was obtained than with 1 mg PLL. Ordinate presents the molar concentration of benzyl penicillin.

hibition. Pools of sera from Groups A, B and C, as well as individual samples with more binding to BP and AX, were used for inhibition.

Coefficient of variation. This was determined as described (12). Ten determinations with a pool positive to BPO-PLL discs and another positive to AX-PLL were estimated. Values over the established CV to both conjugates were considered as different for each of the sera studied.

RESULTS

Concentration effect curves showed that when using discs derivatized with PLL at the concentration of 10 and 1 mg/ml of PLL, BP concentration of 25 mM/l produced the optimal uptake of specific IgE antibodies, no higher binding being given with values over this concentration. This concentration was similar for three pools of positive sera to benzyl penicillin with different binding. The same experiment was carried out with pools of sera positive to AX, producing similar results. Thus concentrations of 10 mg/ml of PLL and 25 mM/l of BPO and AX were used for running all the experiments including RAST inhibition studies. The CV value was 14% for BPO-PLL discs and 13% for AX-PLL discs. The mean and SD in the negative control group was 0.88 and 0.25 for BPO-PLL and 0.23 and 0.12 for AX-PLL. Values over the mean plus 2 SD were considered as RAST positive (1.18 for BPO-PLL and 0.47 for AX-PLL). Results of the positive sera are presented in Table 1. Sixteen proved to be more positive to BP (Group A). The highest value in RAST binding was 30.2 for sera No. 1 and the lowest was 3 for sera No. 16. Sera Nos. 1, 2, 3, 6, 7, 10, 12, 13, 14 and 16 were also RAST positive to AX. Sera numbered from 17 to 22 were more positive to AX (Group B). The maximum value was 31 and minimum 3.5. Cases 17, 18, 20, 21 and 22 were also positive to BP. Sera numbered 23 and 24 had similar binding to both BP and AX with a CV lower than 15% (Group C).

For comparative studies pools of sera more positive to BPO-PLL (Pool A), AX-PLL (Pool B) or with similar binding (Pool C) and also individual sera were run in parallel in the same assay making concentration effect curves using

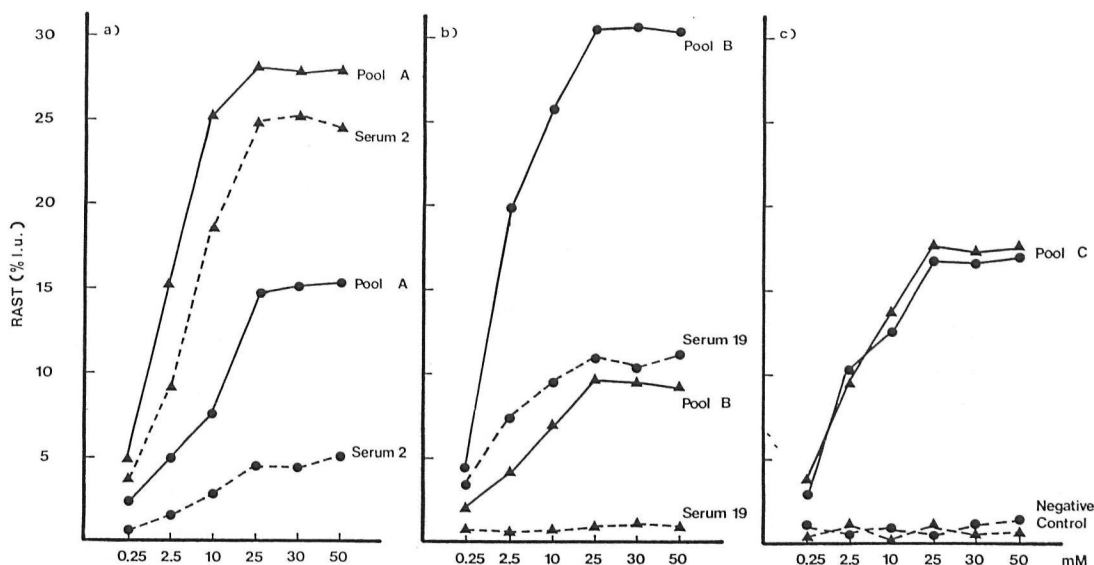


Fig. 2. Titration curves using pools of sera from Group A (more positive to BP), pool B (more positive to AX) and C (similar binding to BP and AX). Individual sera are also used. The Y axis shows the RAST results as percentage label uptake (% Iu). In Fig. 2a the concentration effect curves with pool A and serum 2 are presented. With discs sensitized with BPO-PLL more binding is obtained than with those sensitized with AX-PLL. In Fig. 2b a pool more positive to AX and a serum RAST positive to AX only is used. When serum 19 was assayed with BPO-PLL discs no significant binding was obtained. Using a pool of the sera from Group C (Fig. 2c), similar binding was obtained using either BPO or AX-PLL discs. Binding of the negative control pool is shown at the bottom of Fig. 2c. With all the sera the optimal uptake was obtained at the hapten concentration of 25 mM. ▲ BPO-PLL discs, ● AX-PLL discs.

the same concentration of either BP or AX ranging from 0.25 to 50 mM/l with each sample. Results are presented in Fig. 2. With pool A a maximum binding of 27.3% was obtained at 25 mM/l with no higher binding with hapten concentrations over this value. When discs of AX were used with the same pool, a plateau was also reached with 25 mM/l but with a value of 14.4%. When a serum from Group A was used (sample No. 2), a maximum binding of 24.9% was obtained using BPO-PLL discs and 4.8 with AX-PLL. The results with Pool B can be seen in part (b) of Fig. 2. A maximum RAST value of 31% was obtained with AX at 25 mM/l and with BP the value was 9.5%. When the concentration effect curve was made with serum No. 19 from Group B, a maximum of 10.4% was obtained, giving no positive value with BP-PLL discs at any of the concentrations used. When the Group C pool (sera of similar RAST value) was used, similar parallel curves were obtained (part (c) of Fig. 2).

RAST inhibition results are presented in Fig. 3. With pool A a maximum inhibition of 82% was obtained using 100 mM/l of BP-EACA and 40% with the same concentration of AX-EACA. Inhibition with serum No. 2 from Group A gave 59% of the maximum inhibition for BP and 38% for AX. When the same experiment was made with pool B (part (b) of Fig. 3) 85% inhibition was obtained with 100 mM/l of AX-EAC and 42% with 100 mM/l of BP-EACA. The use of an individual serum from this group (No. 20) gave a value of 78% for AX and 39% for BP. When pool C, formed by sera 23 and 24, was used it gave similar inhibition values with both BPO-EAC and AX-EAC conjugates whichever discs were used in the solid phase (BPO-PLL or AX-PLL).

DISCUSSION

When monoclonal antibodies have been raised to the BPO group at least three epitopes have been found (12). Although different studies sug-

gest that the chemical structure located in the conjugation of the betalactam ring with the amino group of the carrier is the relevant structure (22, 24, 25) others emphasize the role of the acyl side chain (1, 2, 20). In our study the determination of specific IgE antibodies in parallel to both BPO-PLL and AX-PLL using the same concentration of both derivatives gave differences in binding higher than the CV of the assay, suggesting that these are due to differences in the chemical structure of these compounds. When concentration effect curves with pools of more positive sera to either BPO or AX were made, a plateau was reached at the concentration of 25 mM/l of the penicillin derivative and a parallel curve of lower binding was obtained with the other hapten. This indicates that the antibody is raised to either BP or AX and cross-reacts with the other derivative. These results were also confirmed by RAST inhibition studies.

Different results have been reported regarding the influence of the acyl side chain in patients allergic to penicillins. In one study the

addition of ampicillin, cloxacillin, methicillin and cephalothin failed to increase diagnostic efficiency (25) but other authors have found that the use of semisynthetic penicillins for skin tests resulted in a greater number of positive cases (18, 23). These differences can be related to the consumption of the different penicillin derivatives in the populations where these studies were carried out. In a recent study made by our group it was found that the consumption of AX in the population studied was 50% and of BP 25% (6). The involvement of the acyl side chain structure in immunologic cross-reactivity between the different penicillins has been emphasized (21). The phenyl group of benzyl penicillin seemed indistinguishable from the thiopene ring in inhibition studies, but when the amino group was introduced into the benzene ring the specificity of the immunologic reaction decreased. In the group of sera presented in this work we demonstrate that the addition of an amino and an hydroxy group to benzyl penicillin gives differences in binding that change

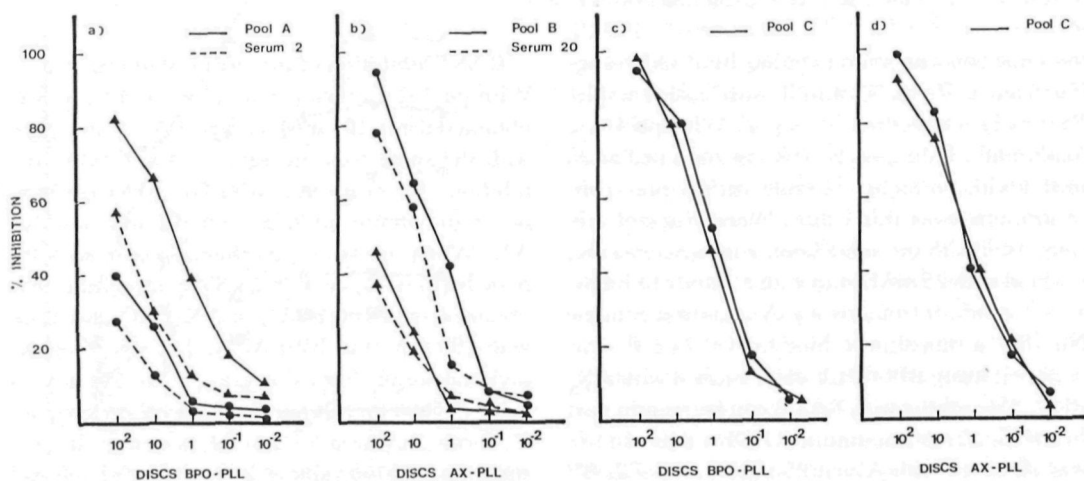


Fig. 3. Results of RAST inhibition studies with pools A, B, C and individual sera more positive to BP (serum No. 12) and AX (serum No. 20). Results are presented as percentage RAST inhibition in the y axis. Five 10-fold dilutions of BPO-EAC and AX-EAC used in parallel at the same molar concentration were employed for inhibition. In Fig. 3a discs were sensitized with BPO-PLL. BPO-EAC conjugates inhibited 82% with 100 mM of BPO in pool A and 59% in serum 2. When the conjugate AX-EAC was used the inhibition was 40% for pool A and 27% for serum 2. In Fig. 3b the same procedure was made using pool B (more positive to AX) and serum 20. 90% inhibition was obtained with 100 mM/l AX-EAC with pool B and 79% with serum 20. When conjugates of BP were used, 43% was obtained for 100 mM/l of BPO-EAC for pool B and 38% for serum 20. In the two graphs on the right half of the figure pool C was used giving a similar inhibition pattern using discs conjugated with either BPO-PLL or AX-PLL. BPO-EAC in fluid phase $\blacktriangle$, AX-PLL in fluid phase $\bullet$.

from sera giving important individual variability. In pools of more positive sera to either BPO or to AX a stronger inhibition was made with the same drug employed in both the solid and fluid phase, giving a weak inhibition with the other penicillin. Interestingly, a pool of two sera presenting similar binding to both BPO-PLL and AX-PLL showed the same degree of inhibition with BPO-EACA or AX-EACA used in the fluid phase and independently of the conjugate coupled to the disc. In this case the main antigenic determinant recognised could be located in the union of the carbonyl group of penicillin with the amino group of the carrier (12).

These results provide important evidence of the role of the acyl side chain in the specificity of IgE antibodies raised to penicillins. We conclude that in populations similar to ours when the consumption of penicillins is also similar both haptens should be considered for RAST testing. The clinical implications of these findings are being investigated by our group.

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