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Immunochemical Analysis of the Specificities of IgE Antibodies Directed to Benzylpenicilloyl

Abstract

The contribution that the different parts of the benzylpenicillin (BP) molecule made to the antigen-binding site was studied using human IgE sera specific to the benzylpenicilloyl (BPO) determinant. Radioallergosorbent test inhibition studies were made using in the solid phase BPO and in the fluid phase BP butyl-amine, 6-amino-penicillanic acid-butylamine, thiazolidine, and phenylacetic acid. The results indicate that the whole BPO structure was required for optimal inhibition in all sera studied and that important differences were obtained comparing these results with those obtained with the different parts of BP. These structures contributed to a different degree to the constitution of the antigenic determinant.

Introduction

Penicillins conjugate to body proteins, inducing both cellular and humoral immune responses. Different isotypes of antibodies are produced to these hapten carrier conjugates [1]. Three relevant structures have been identified as contributing to the antigen-binding site: the side chain (SC), the thiazolidine (THIAZ) ring, and the structure resulting from the combination of the carbonyl of the betalactam to the amino group of the carrier, the new antigenic determinant (NAD) [2]. Preliminary studies of our group [3] indicated that when variations in the side chain of penicillins are considered, an important change in the capture of IgE antibodies by a radioallergosorbent test (RAST) is produced supporting the relevance of this structure. In addition, it has been shown that NAD, SC, and THIAZ were recognized in a different way by specific IgE antibodies [4]. The contribution of the different parts

of the benzylpenicillin (BP) molecule to the binding of the antibody was studied. The specificity of sera with IgE to benzylpenicilloyl (BPO) was analyzed using monomeric conjugates of the PG molecule and different parts of this chemical structure. The results indicate that the whole BPO-butylamine structure was required for optimal inhibition and that the use of separated parts of the molecule decreased to a different extent the capacity of the inhibition.

Methods

Radioallergosorbent Test

Cyanogen bromide activated cellulose discs were conjugated to polylysine, and then BP was fixed to the solid-phase derived discs as described elsewhere [3, 5]. Optimal concentrations of reagents were determined by concentration-effect curves using a pool of positive control sera [6].

RAST Inhibition

Pools of positive and individual sera were used. The following monomers were used during the fluid phase: BP butylamine (BP-BA), 6-amino-penicillanic acid-butylamine (6-APA-BA), THIAZ, and phenylacetic acid (PHA). THIAZ and the monomeric conjugate were prepared as described earlier [7, 8]. Tenfold molar concentrations ranging from 100 to 0.01 mM were used, and the inhibition was made in parallel with all monomers.

Results

In figure 1 the RAST inhibition results are shown with the pool of positive sera. Optimal inhibition was obtained with 100 mM of BP-BA, but with any of the monomers used no more than 50% inhibition was obtained. These data indicate that the whole BP structure was required for optimal inhibition with the pool of sera. Figure 2 shows the patterns of inhibition of two individual sera (2 and 4). As occurred with the pool of positive sera, an optimal degree of inhibition was also obtained with the monomeric conjugate BPO-BA, but when the different parts of the molecule were used, there were differences from serum to serum. In serum 2, the main inhibitor after BP-BA was PHA, and 6-APA-BA inhibited very poorly, and THIAZ did not inhibit. In serum 4 all BP structures (SC, NAD and THIAZ) contributed over 50% inhibition, and PHA was stronger (73%). Figure 3 shows all sera studied at the maximum inhibition used in the assay (100 mM of each chemical structure). It can be seen that considering

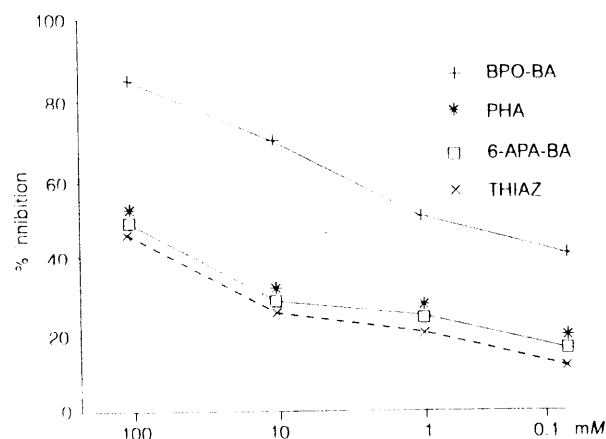


Fig. 1. RAST inhibition study using a pool of sera with specific IgE to BPO. Different monomers were used during the fluid phase, ranging from 100 to 0.1 mM. Results are presented as percentage of inhibition.

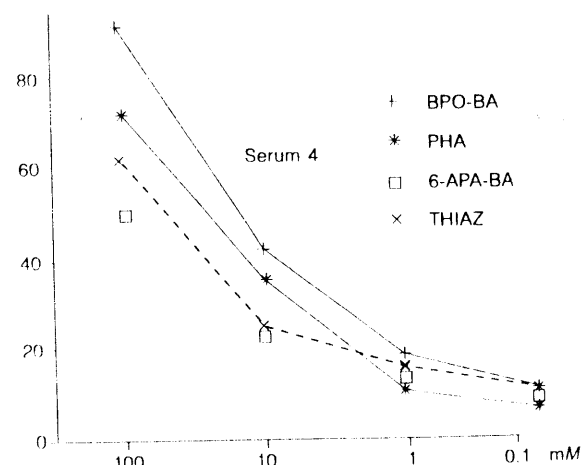
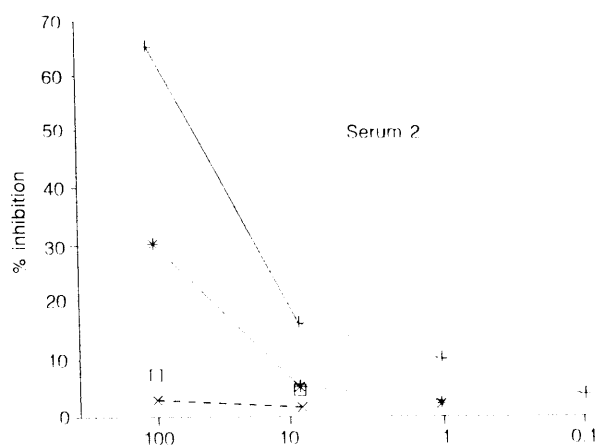
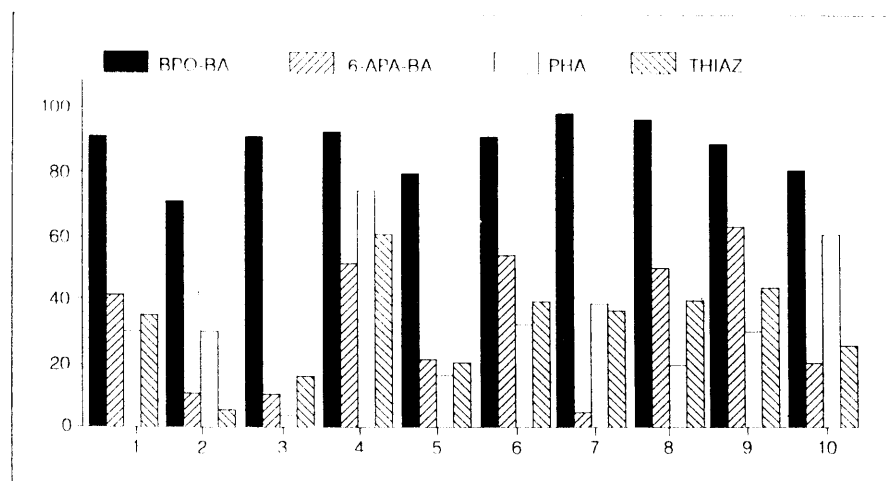


Fig. 2. RAST inhibition using two sera with IgE to BPO. Inhibitions were made as presented in figure 1. Different patterns of inhibition were observed.

Fig. 3. RAST inhibition results comparing ten positive sera to BPO at optimal concentrations of all monomers during the fluid phase (100 mM). The stronger inhibitor was the BPO-BA monomer, and different degrees of inhibition were observed with the others.



the inhibition produced by BPO-BA not any of the structure used achieved this inhibition. In serum No. 4, the higher inhibition was induced by PHA (73%) and in serum No. 9 by 6-APA-BA (62%). The contribution that any of this chemical structures made in the inhibition studies indicates that the best inhibitor in 4 cases was 6-APA-BA and in other 4 PHA. In six sera the inhibition with each part of the BP molecule was below 50% inhibition. In most samples inhibition with THIAZ. was similar to that induced by 6-APA-BA.

Discussion

Levine and Ovary [9] reported that when BP binds to a protein, the whole molecule is recognized by the antibody on the antigen-binding site. Later studies [10] indicated that different populations of antibodies could be identified, NAD and the SC were the better recognized. The use of monoclonal antibodies indicated that three relevant parts could be recognized by different clones: NAD, SC, and THIAZ. [2]. The occurrence of selective reactions to amoxicillin with presence of IgE antibodies that seemed to recognize mainly the SC raised questions about what were the relevant structures of penicillins involved in eliciting the allergic reactions [11]. These data may help understanding the cross-reactivity between penicillins and cephalosporins and between different cephalosporins. In this study, the penicillin molecule coupled to an amino monomer was used, in order to have a structure with the three epitopes identified. In addition, the SC of PG (PHA), THIAZ., and the combination of NAD plus

THIAZ. (6-APA-BA) were used in order to know which were the relevant parts recognized by specific IgE antibodies. Using pool of sera, the results indicated that the best inhibitor was the whole structure and that with any of the structures used no more than 50% was obtained. When individual sera were studied, a great variability was seen from serum to serum, but in any case a similar inhibition produced by the whole molecule (BPO-BA) was not obtained. In some sera the most relevant structure was the SC, but in others seemed to be the combination of NAD and THIAZ. A variability from serum to serum was observed, and in some cases the different parts contributed a very low inhibition pattern. This study with specific IgE human sera provides important data to the study of the antigen-binding site in sera from patients allergic to penicillins. Further studies using a large number of sera and a structure with only NAD are being carried out in order to establish the relevance to the specificity of IgE antibodies presented in sera from subjects allergic to penicillins.

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