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Studies of the Epitopes of Amoxicillin by Monoclonal Antibodies

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Words

Amoxicillin
Ampicillin
Benzylpenicillin

Abstract

Four different mice monoclonal antibodies to amoxicillin (AX) were studied (AO 25.2, AO 6.2, AO 21.1, and AO 3.2). The specificity was determined by enzyme-linked immunosorbent assay inhibition using in the fluid phase free and conjugated monomers of AX, ampicillin, and benzylpenicillin with ϵ -aminocaproic acid. The monoclonal AO 25.2 (IgM isotype) recognized a chemical structure common to all penicillins used, presumably the thiazolidine ring. AO 6.2 (IgE) and AO 3.2 (IgG2a) recognized mainly the side chain of AX and the clone AO 21.1 (IgG3) required for optimal inhibition of the conjugated monomer of AX, indicating that both the side chain and the new antigenic determinant were required for optimal inhibition. These results indicate that there are differences in the specificities of these antibodies and that the different parts of the AX molecule contribute to a different extent to the antigen-binding site.

Introduction

An important proportion of subjects with allergic reactions to penicillins develop a selective response to AX with tolerance to other penicillins including benzylpenicillin (BP). Both cellular and humoral immediate reactions have been reported [1, 2]. According to these results the most relevant structure of the antigenic determinant is to be located in the side chain. The use of monoclonal antibodies has been an important approach for defining the nature of the different epitopes recognized [3]. In this study we present the results of four monoclonal antibodies produced against AX, and the relevant parts of different penicillins recognized by these antibodies are de-

termined by enzyme-linked immunosorbent assay (ELISA) inhibition using monomeric conjugates of AX and two closely related penicillins, BP and ampicillin (AMP).

Methods

Balb/c mice were immunized with AX-HSA conjugates as described [5]. Hybridomas were obtained by the fusion of the mice spleen cells with X63 Ag8.653 mouse myeloma cells. Antibodies of the supernatants were purified by affinity chromatography [5]. The specificity of the different clones was studied by ELISA and ELISA inhibition. The free haptens of AX, AM, and BP and the monomeric conjugates AX-EACA, AMP-EACA, and BP-EACA (EACA = ϵ -aminocaproic acid) were used during the fluid phase at concentrations ranging from 128 to $4^{10^{-1}}$ mM.

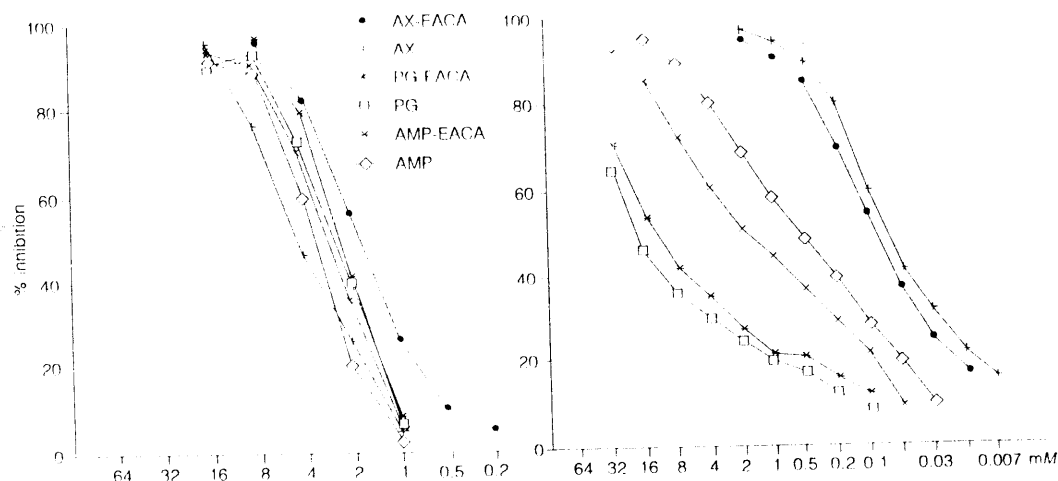


Fig. 1. ELISA inhibition studies of the clones AO 25.2 (left, IgM isotype) and AO 6.2 (right, IgE isotype). Evidence for different patterns of inhibition with the penicillins used. For further explanations see text.

Results

The clones produced were as follows: AO 25.2 (IgM isotype), AO 6.2 (IgE isotype), AO 21.1 (IgG3 isotype), and AO 3.2 (IgG2a isotype). The results of ELISA inhibition with clone AO 25.2 are presented in figure 1 (left). Considering the data obtained by the inhibitions of the other antibodies, there were no important differences using either the free or conjugated monomeric forms of the three penicillins used. Clone AO 6.2 (fig. 1, right) recognized mainly the free AX followed by the conjugated form, AX-EACA. A different degree of cross-reactivity existed with the other penicillins used, but they recognized mainly the structure of the side chain. In all cases the conjugate monomers did not inhibit more than the free compounds. Clone AO 21.1 (fig. 2, left) recognized mainly the monomer AX-EACA followed by free AX. Since there are important differences in the inhibition between conjugated and free AX, both new antigenic determinant (NAD) and side chain were required for optimal inhibition. It was inhibited very poorly with BP and BP-EACA. Clone AO 3.2 had a pattern of inhibition similar to AO 21.1 and recognized mainly free AX followed by the conjugated form AX-EACA (fig. 2, right).

Discussion

The contribution that the different parts of the penicillin molecule make to the antibody-binding site is relevant for understanding the phenomenon of cross-reactivity between penicillins and cephalosporins and between different side-chain penicillins [1, 6-8]. Different populations of IgG antibodies were identified to the major determinant benzylpenicilloyl [7]. With the use of monoclonal antibodies, de Haan et al. [3] reported that three structures were relevant: side chain, NAD, and the thiazolidine ring [3]. Fukushima et al. [4] produced a clone that recognized mainly the side chain of BP, although thiazolidine was also recognized by the antibody, indicating that this structure also contributed to the antigen-binding site. In this study the results with four different monoclonal antibodies are presented. In two (AO 6.2 and AO 3.2), the side chain seemed to be the main structure contributing to the antigen determinant, since the conjugated form AX-EACA did not improve the inhibition capacity. The cross-reactivity with the closely related penicillins also supported the relevance of the side-chain structure, since free AMP inhibition was higher than that of the conjugated form. As AX inhibited more than AMP both the amino and the hydroxy groups of the side chain were relevant parts of the antigenic determinant. In another monoclonal antibody (AO 21.1), the conjugated

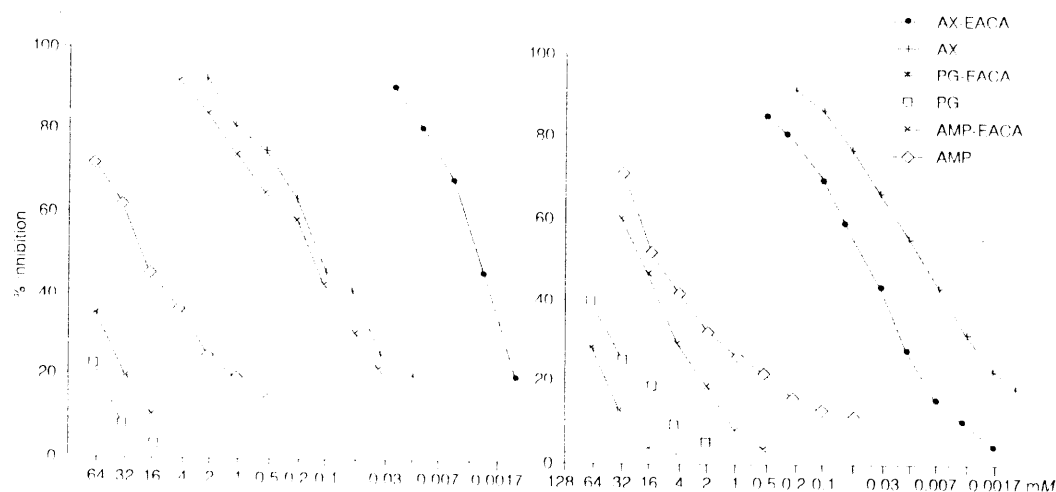


Fig. 2. ELISA inhibition studies of the clones AO 2L1 (left, IgG1 isotype) and AO 3.2 (right, IgG2a isotype). AO 3.2 recognized mainly the side chain and AO 2L1 required the presence of NAD and side chain for optimal inhibition. For further explanations see text.

monomer AX-EACA was the strongest inhibitor, indicating that both the side chain and the NAD were required for optimal inhibition. In this case the conjugate AMP-EACA was also stronger than AMP. Another clone (AO 3.2) recognized a structure common to all, since free and conjugated monomers of all penicillins studied showed no important differences in the inhibition pattern. Since the structure present in all these forms was thiazolidine we conclude that this can be the most relevant part involved. Similar results have been obtained by Nagakura et al. [9]

with a monoclonal antibody to AMP. A wide cross-reactivity to different betalactams was shown, indicating that thiazolidine was the relevant structure. Our results indicate that the monoclonals produced to AX-HSA conjugates showed differences in the pattern of specificity to the AX conjugate and that the three different parts of the molecule contributed to a different extent to the antigen-binding site. Similar results have been obtained by analyzing the specificities of human IgE antibodies to AX [10].

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