

Specific reactions and cross reactions of anti-penicilloyl antibodies

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In human sera the specific reactions and cross reactions of antibodies directed against the penicilloyl groups were determined using different penicilloyl derivatives as antigen. The penicilloyl group providing the highest titre in one Ig class was said to define the specific reaction for this Ig class. This criterium was justified by the fact that antibodies evoked by long-term treatment with benzylpenicillin showed specific reaction to the benzylpenicilloyl group. Moreover, specific reactions of antibodies of different Ig classes in one serum were the same. Both the side chain and the thiazolidine ring of the penicilloyl group can serve as antigenic determinants.

Key words: penicilloyl-protein conjugates; reactions; cross specific.

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In antibiotic therapy a wide range of penicillins is currently in use, for example: benzylpenicillin, pheneticillin, ampicillin and amoxycillin. Drug hypersensitivity after use of these penicillins is a well-known phenomenon (7). Immunological phenomena of humoral (antibodies) and/or cellular (T lymphocytes) nature play a role in penicillin hypersensitivity (2, 4, 5). Little is known about specific reactions and cross reactions of antibodies directed against the penicilloyl group. Specific and cross reactions of IgE antibodies were determined with the RAST (8, 9) using benzylpenicilloyl and pheneticilloyl protein conjugates. However, no data about specific and cross reactions of IgG or IgM antibodies are available. Although, nowadays, aminopenicillins are widely used, nothing is known about the occurrence and role of anti-aminopenicilloyl antibodies. Using monoclonal antibodies we were able to prove that besides the side chain also the thiazolidine ring, present in all penicillins, or the new antigenic determinant (NAD) (12) generated by coupling penicillin to protein (Fig. 1), can serve as an

antigenic determinant (6). The aim of the present study was to determine the specific reactions and cross reactions of anti-penicilloyl antibodies present in human sera.

MATERIAL AND METHODS

Five hundred and seventy-five sera from patients suspected of penicillin hypersensitivity or from patients who had recently undergone a long-term course of benzylpenicillin (5) were investigated.

Penicilloyl-protein conjugates were prepared according to Nishida et al. (10). Human transferrin served as carrier protein. The penicillin derivatives (Fig. 2) used were benzylpenicillin, ampicillin, amoxycillin and pheneticillin (Mycofarm, The Netherlands). Purification of the conjugates was done according to de Haan et al. (3). The penicilloyl load of the protein was determined by the penamaldate method (11). The following conjugates were prepared: (benzylpenicilloyl)₁₀-transferrin, (ampicilloyl)₂₀-transferrin, (amoxycilloyl)₂₀-transferrin and (pheneticilloyl)₁₈-transferrin.

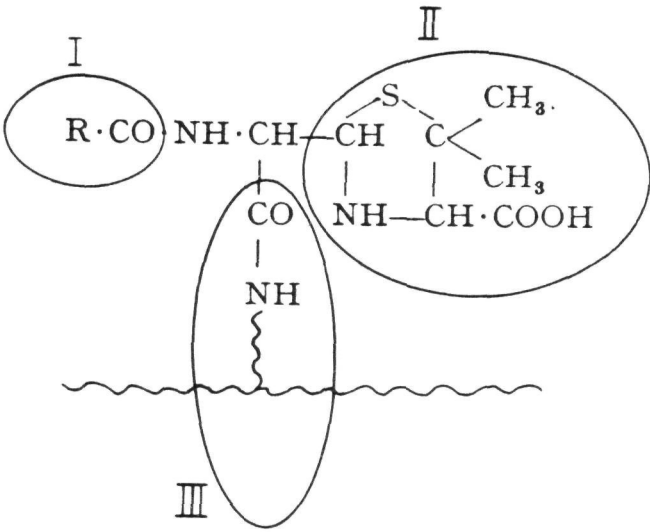


Fig. 1. Three possible determinants of the penicilloyl group. I side chain, II thiazolidine ring, III NAD

ELISA was performed as described earlier (3, 4). The antigens used in the ELISA were the conjugates of human transferrin and different penicillins. Human transferrin was used as negative control. The titre for each Ig-class was determined using the different penicilloyl-transferrin conjugates as antigen.

The penicilloyl group providing the highest titre in one Ig-class was said to define the specific reaction for this Ig-class. The reactions on other penicilloyl protein conjugates resulting in a lower titre were considered as cross reactions.

RESULTS

Positive sera

Anti-penicilloyl antibodies were detected in 142 of the 575 sera investigated (Table 1). It

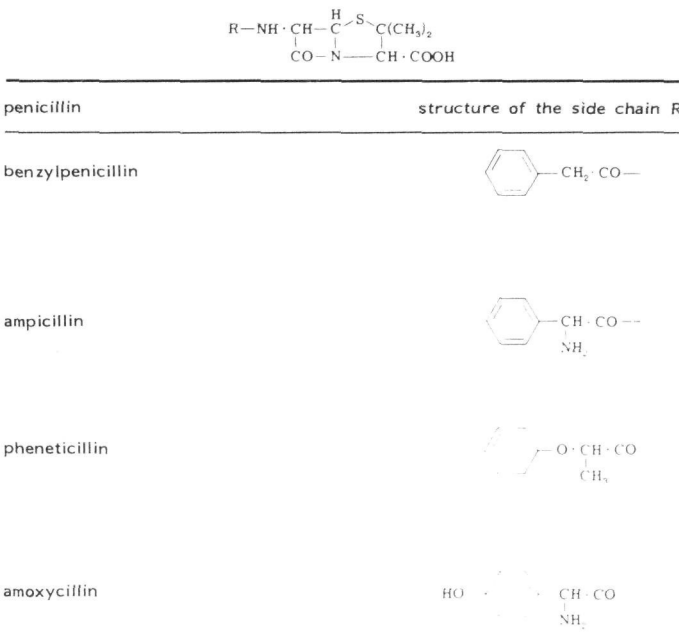


Fig. 2. Penicillin derivatives used.

is remarkable that IgM-class anti-penicilloyl antibodies were found in 137 of 142 sera. Anti-penicilloyl antibodies of the IgE class were always found in combination with those of other Ig-classes.

Specific reactions of the different Ig-classes in one serum

The highest titre of two or more Ig-classes in one serum was always found by using the same penicilloyl-protein conjugate as antigen (Table 1).

The specific reactions of antibodies in sera from patients on a long-term course of benzylpenicillin corresponded with those expected.

Sera with or without anti-penicilloyl anti-

Table 1

Relation between Ig-class or combination of Ig-classes and the specific reactions on the penicilloyl derivates in 142 sera

Specific reaction	Ig-class(es)								Total
	G	M	A + M	M + E	G + E	A + M + E	G + M + E	G + A + M + E	
BPO	2	43	1	6	2	2	1	6	63
FNO	-	12	1	3	-	1	-	2	19
APO	1	31	1	5	-	-	-	-	38
AMO	-	21	-	-	-	-	-	1	22
Total	3	107	3	14	2	3	1	9	142

BPO: benzylpenicilloyl; FNO: pheneticilloyl; APO: ampicilloyl; AMO: amoxycilloyl.

bodies were always negative in control tests on human transferrin.

Cross reactions

Only two of the 15 sera containing IgG class anti-penicilloyl antibodies gave cross reactions (Table 2). Of 15 sera with IgA class anti-penicilloyl antibodies, seven showed cross

reactions with at least one penicilloyl derivate (Table 3). Cross reactions of anti-penicilloyl antibodies of the IgM class were observed in 55 of 137 sera (Table 4): in 28 sera with one penicilloyl derivate, in 17 sera with two and in 10 sera with the other three derivates. In the IgE class, anti-penicilloyl antibody cross reactions were demonstrated in nine out of 29 sera. Also IgE class anti-penicilloyl antibodies demonstrated cross reactions with one (3 sera), two (4 sera) and three (2 sera) penicilloyl derivatives (Table 5).

Table 2
Relation between specific reactions and cross reactions of IgG antibodies (15 sera)

Specific reactions	Cross reactions with			
	BPO	PNO	APO	AMO
BPO	9	-	2	-
PNO	-	2	-	-
APO	-	-	1	-
AMO	-	-	-	1

Abbreviations see Table 1.

DISCUSSION

Using monoclonal antibodies directed against the penicilloyl group, at least three different determinants in the penicilloyl group (Fig. 1) could be detected (6). As different penicilloyl moieties have groups in common, e.g. the thiazolidine ring and the NAD, antibodies

Table 3
Relation between specific reactions and cross reactions of IgA antibodies (15 sera)

Specific reactions	Cross reactions with							
	BPO	PNO	APO	AMO	BPO PNO APO	BPO PNO AMO	BPO APO AMO	PNO APO AMO
BPO	3	-	1	-	2	-	-	3
PNO	1	3	-	-	-	-	-	-
APO	-	-	1	-	-	-	-	-
AMO	-	-	-	1	-	-	-	-

Abbreviations see Table 1.

Table 4
Relation between specific reactions and cross reactions of IgM antibodies (137 sera)

Specific reactions	Cross reactions with							
	BPO	PNO	APO	AMO	BPO PNO APO	BPO PNO AMO	BPO APO AMO	PNO APO AMO
BPO	30	2	7	6	-	-	7	7
FNO	1	9	-	-	3	2	3	1
APO	2	2	29	2	-	-	1	1
AMO	2	3	1	14	-	1	1	-

Abbreviations See Table 1.

directed against these groups may react with all penicilloyl groups. However, penicilloyl moieties differ mutually in the side chain. Anti-penicilloyl antibodies in human sera may be directed against the side chain and against the other determinants. Reactions against the penicilloyl group in a serum containing anti-penicilloyl antibodies are the result of the summation of reactivity of the various antibodies in the total population, each reacting with a part of the penicilloyl group. The highest summation of reactions on a penicilloyl-protein, serum conjugate, as determined by the titre, was considered as the specific reaction.

This could be justified as follows:

- 1. the specific reaction of antibodies evoked in patients with a humoral immune response after a long-term course of benzylpenicillin (4, 5) was found on the benzylpenicilloyl group.
- 2. in a serum the specific reactions of antibodies of different Ig-classes were on the same penicilloyl-protein conjugate.

From Table 1 it can be concluded that the highest titres were most frequently found with benzylpenicilloyl - or ampicilloyl - protein conjugates. This is presumably because benzylpenicillin or ampicillin is prescribed in most cases during the time of investigation.

Cross reactions were demonstrated for IgA class and IgM class penicilloyl-specific antibodies in high percentages. This can be explained by the fact that these Ig-classes are

the first expression of the immune response and probably do have a rather wide specificity. The low degree of cross reactions of IgG class anti-penicilloyl antibodies can be because these antibodies are synthesized during the late phase of the immune response and thus have a narrower specificity.

In the penicilloyl group three separate antigenic determinants can be shown: 1) the side chain, 2) the thiazolidine ring and 3) a new antigenic determinant (6). In sera with antibodies which react with only one penicilloyl group, the antibodies are directed against the side chain, the only difference being that the penicilloyl derivates are mutual. In sera with cross-reacting antibody common determinants, the NAD and/or the thiazolidine ring play an antigenic determinant role. Nishida et al. (1972) and Bird (10) found that only the side chain was an antigenic determinant. This may be because they used benzylpenicilloyl-protein conjugates with a high penicilloyl load, resulting in the synthesis of antibodies directed against the side chain. In cases of a lower load with penicilloyl groups in human beings, it is more probable that the side chain, the thiazolidine ring and the NAD play a role (6, 12, 13).

Cross reactions are more likely to occur with the thiazolidine ring as the antigenic determinant rather than with the NAD (6). As the thiazolidine ring is common in all penicillins one can conclude that patients with cross-reacting antibodies should avoid any contact with penicillins, if possible.

Table 5
Relation between specific reactions and cross reactions of IgE antibodies (29 sera)

Specific reactions	Cross reactions with							
	BPO	PNO	APO	AMO	BPO PNO APO	BPO PNO AMO	BPO APO AMO	PNO APO AMO
BPO	11	-	2	-	-	-	2	2
PNO	-	3	-	1	1	1	-	-
APO	-	-	5	-	-	-	-	-
AMO	-	-	-	1	-	-	-	-

Abbreviations see Table 1.

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