IgE ANTIBODIES FOR PENICILLINS AND CEPHALOSPORINS IN RATS. II

ANTIGENIC SPECIFICITY OF RAT ANTI-PENICILLIN-OvA IgE SERA

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Sprague Dawley (SD) rats were immunized with various penicillin-ovalbumin (OvA) in combination with aluminum hydroxide (alum) and thimerosal-killed *Bordetella pertussis* for the purpose of obtaining rat anti-penicillin IgE sera. In the rat 60-hour passive cutaneous anaphylaxis (PCA) reaction and the hapten inhibition test, a weak cross reaction between penicillin G (PCG) and ampicillin (ABPC) was observed, but no cross reaction was observed between sulbenicillin (SBPC) and other penicillins. Rat anti-6-formamidopenicillanic acid (FPC) IgE serum reacted with PCG-bovine gamma globulin (BGG), ABPC-BGG and SBPC-BGG, but FPC-BGG did not react with rat anti-PCG, anti-ABPC and anti-SBPC IgE sera and the PCA reaction between anti-FPC IgE sera and FPC-BGG was inhibited by FPC, PCG, ABPC and SBPC. These results indicate that the antigenic active sites of PCG, ABPC and SBPC are limited to the acyl side chain moiety of penicillins, while the antigenic active site of FPC is confined to the penicilloyl moiety of the penicillin.

Many clinical reports indicate that penicillins and cephalosporins are capable of inducing diverse types of allergic reactions defined by COOMBS and GELL[1]. The antigenic specificities of penicillins and cephalosporins in the IgG antibodies have been reported.[2~9] The production of IgE antibodies for penicillin was observed in guinea pigs and rabbits[10,11], but the antigenic specificities of penicillins and cephalosporins in IgE antibodies have not been adequately clarified. It was reported in the previous paper[12] that the rat 60-hour PCA reaction with rat anti-penicillin serum, obtained from the rat immunized with penicillin-ovalbumin (OvA) conjugate in combination with aluminum hydroxide (alum) and thimerosal-killed *Bordetella pertussis*, was thought to be mediate by rat anti-penicillin IgE antibodies. This paper describes the antigenic specificities of various penicillins in rat IgE antibodies.

Materials and Methods

Animals

Male JCL: Sprague Dawley (SD, CLEA Japan Inc.) rats were used at 5 weeks of age for the immunization and at 8~10 weeks of age for the passive cutaneous anaphylaxis (PCA) reaction.

Chemicals

Penicillin G (PCG), ampicillin (ABPC) and sulbenicillin (SBPC) were obtained from commercial sources of Takeda Chemical Industries, Ltd., Osaka, Japan. 6-Formamidopenicillanic acid (FPC) was synthesized in Takeda Chemical Industries, Ltd. 6-Aminopenicillanic acid (6-APA) was supplied by Royal Netherland Fermentation Industries, Holland. Ovalbumin (OvA) and bovine gamma globulin (BGG) were purchased from Nutritional Biochemical Corporation, Cleveland, Ohio, U.S.A. Saline suspension of thimerosal-killed *Bordetella pertusis* containing 2×10^{10} cells per ml was prepared in Takeda Chemical Industries, Ltd.

Hapten-protein conjugates

One hundred mg of OvA or BGG was dissolved in 10 ml of physiological saline and penicillin was added at the ratio of 50 to 2,000 M *vs.* 1 M protein. The reaction mixture was adjusted at pH 10~10.5

with 1 N NaOH. After 24 hours incubation at 37°C, the reaction mixture was dialysed at 4°C for 3 days continuously against 5 liters of physiological saline and the penicillin-protein conjugate was chromatographed on Sephadex G-25 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (3 × 100 cm). The final concentration of hapten-protein conjugates was adjusted to about 10 mg protein per ml. The epitope densities of penicillins in penicillin-protein conjugates were measured by the penamaldate method[10]. Penicillin-protein conjugates used in this study were PCG_{20}-OvA, $ABPC_{12}$-OvA, $SBPC_{12}$-OvA, FPC_{13}-OvA, PCG_{50}-BGG, $ABPC_{56}$-BGG, $SBPC_{37}$-BGG and FPC_{51}-BGG.

Immunization

Following the method of TADA[13], rats were initially injected intramuscularly with a mixture of 5 mg of penicillin-OvA and 5 mg of aluminum hydroxide (alum) and intraperitoneally with 1 ml of thimerosal-killed *B. pertussis* saline suspension. At day 5, secondary immunization of penicillin-OvA and alum. Blood specimens are obtained from the abdominal aorta on day 13 after the first immunization.

Passive cutaneous anaphylaxis (PCA) test.

Assay of anti-hapten IgE antibodies was performed by the rat 60-hour PCA reaction in SD rats. One-tenth ml of serial two-fold dilutions of antisera was intradermally injected in the back of rats, and 1 mg of hapten-BGG in 2 ml of 1% Evans blue saline solution was intravenously injected to the rats 60 hours later. Rats were killed 30 minutes after the challenge and the degree of reaction was estimated by measuring two perpendicular diameters of the blue spots on the underside of the skin. Blue spots with an average diameter of more than 5 mm were regarded as a positive PCA reaction. The test were carried out in duplicate for each sample, and the PCA titers were expressed as the highest dilution which gave a positive PCA reaction.

Hapten inhibition test on the rat 60-hour PCA reaction

Two ml of various concentrations of hapten saline solution was intravenously injected to rats sensitized by intradermal injection of 4 units of antisera (antisera so diluted as to show the PCA titer of 4) 60 hours before. The rats were immediately challenged with 1 mg of hapten-BGG conjugates in 2 ml of 1% Evans blue saline solution, and the PCA reactions were measured as described above.

Results

Cross Reactivities among the Rat Anti-penicillin IgE Sera

Each rat anti-penicillin IgE serum reacted most strongly with each corresponding penicillin-BGG conjugate. PCG and ABPC cross-reacted with each other and neither rat anti-PCG nor anti-ABPC IgE sera reacted with SBPC-BGG and FPC-BGG. Rat anti-SBPC IgE serum only reacted with SBPC-BGG. Rat anti-FPC IgE serum cross-reacted with PCG-, ABPC- and SBPC-BGG (Table 1).

Table 1. Cross reactivity between rat anti-penicillin IgE sera and penicillin-BGG in the rat 60-hour PCA reaction.[a]

Antiserum[b]	Challenge antigen			
	PCG-BGG	ABPC-BGG	SBPC-BGG	FPC-BGG
PCG-OvA	256[c]	64	<1	<1
ABPC-OvA	64	128	<1	<1
SBPC-OvA	<1	<1	512	<1
FPC-OvA	32	64	32	128

[a] Rats sensitized with 0.1 ml of serial two-fold diluted antisera were challenged with 1 mg of various penicillin-BGG.

[b] Rat anti-penicillin-OvA sera obtained on day 13 after the first immunization.

[c] Mean rat 60-hour PCA titers of 3 recipients.

Table 2. Cross reactivity between anti-penicillin IgE sera and penicillin-BGG in the hapten inhibition test of the rat 60-hour PCA reaction.[a)]

System[b)]	Inhibited by				
	Saline	PCG	ABPC	SBPC	6-APA
PCG	10.8±1.0[c)]	0	0	8.3±0.5	10.0±0.8
ABPC	10.0±0.8	0	0	8.0±1.4	9.5±1.3
SBPC	10.5±0.6	9.3±0.5	10.5±0.6	0	8.8±1.7
FPC	10.3±1.7	0	0	0	0

a) Rats sensitized with 4 units of antisera were intravenously injected with 2 ml of 0.125M of penicillin at the same time of antigen challenge.

b) Rat 60-hour PCA reactions induced by rat anti-penicillin-OvA sera and corresponding penicillin-BGG.

c) Mean diameter±S.D. of 2 spots in 2 recipients.

Hapten Inhibition Test

Rats were sensitized with 4 units of rat antipenicillin IgE sera and injected with 2 ml of 0.125 M of hapten saline solutions just before the antigen challenge. The PCA reaction between rat anti-PCG serum and PCG-BGG was inhibited by PCG and ABPC. The PCA reaction between rat anti-ABPC IgE serum and ABPC-BGG was also inhibited by PCG and ABPC. The PCA reaction between rat anti-SBPC serum and SBPC-BGG was inhibited only by SBPC. The PCA reaction between rat anti-FPC IgE serum and FPC-BGG was inhibited by PCG, ABPC, SBPC and FPC (Table 2).

Fig. 1. Hapten inhibition test on the rat 60-hour PCA reaction induced by rat anti-SBPC-OvA IgE serum and SBPC-BGG.

Rats sensitized with 4 units of anti-SBPC serum were injected with 2 ml of various concentrations of SBPC, α-sulfophenyl acetate and 6-APA just before antigen challenge. Percent inhibition against the PCA reactions without the injection of hapten was represented. Each point represents mean diameter of 3 spots in 3 recipients.

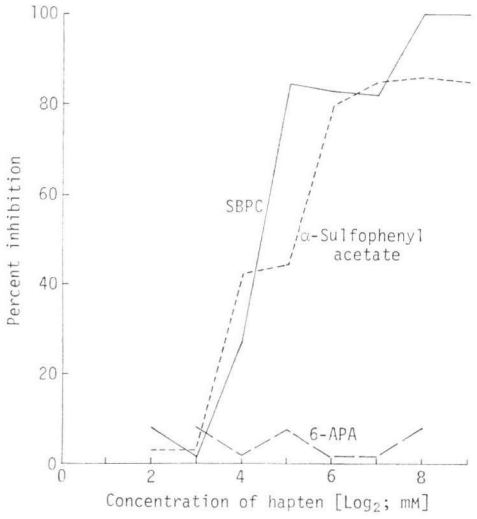
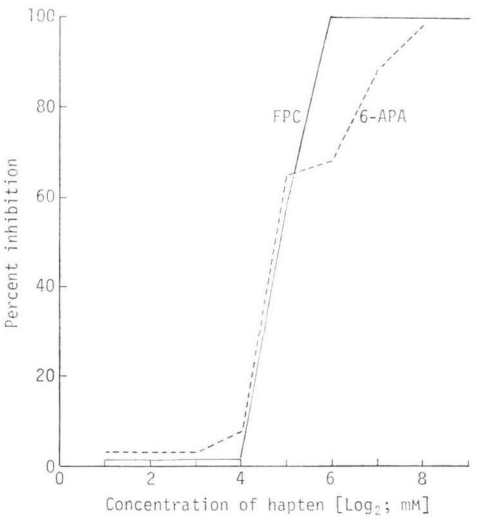


Fig. 2. Hapten inhibition test on the rat 60-hour PCA reaction induced by rat anti-FPC-OvA IgE serum and FPC-BGG.

Rats sensitized with 4 units of anti-FPC serum were injected with 2 ml of various concentrations of FPC and 6-APA just before antigen challenge. Percent inhibition against PCA reactions without injection of hapten was represented. Each point represents mean diameter of 3 spots in 3 recipients.



Antigenic Specificity

The quantitative hapten inhibition test was performed in order to investigate the antigenic specificities of the antisera, using 4 units of rat anti-SBPC and anti-FPC IgE sera. The PCA reaction between rat anti-SBPC IgE serum and SBPC-BGG was completely inhibited by the injection of 2 ml of 8 mM of SBPC or sodium α-sulfophenyl acetate, but hardly inhibited by the injection of 6-APA (Fig. 1). The PCA reaction between rat anti-FPC IgE serum and FPC-BGG completely inhibited by the injection of 2 ml of 16 mM of FPC or 6-APA (Fig. 2).

Discussion

The cross-reactions among various penicillins in the IgG antibodies have been well documented.[6~10] According to these authors, the cross reactivity among penicillins in the IgG antibodies have been attributed to the common penicilloyl moiety and the acyl side chain moieties. In other words, the IgG antibodies are considered to bind to both the acyl side chain moiety and the penicilloyl moiety.

In the present cross reactivity test and the hapten inhibition test of various penicillins in the rat IgE antibodies, SBPC did not react with PCG, ABPC and FPC. In the quantitative hapten inhibition test, the anti-SBPC IgE serum bound to the acyl side chain moiety. From these findings, it was concluded that, firstly common penicilloyl moiety plays little role in the cross-reactions of the rat anti-penicillin IgE antibodies, and secondly, the α-sulfobenzyl group does not cross-react with the benzyl group and the α-aminobenzyl group, while the benzyl group and the α-aminobenzyl group cross-reacted with each other. These antigenic specificities of penicillins in the rat IgE antibodies are clearly different from that in the rabbit IgG antibodies. This difference is believed to be caused by the facts that the rat anti-penicillin IgE sera contain the IgE antibodies for either the acyl side chain moiety or the penicilloyl moiety and that the rabbit anti-penicillin IgG sera contain the IgG antibodies for both moieties.

There would be three cases where rats produce the IgE antibodies for either the acyl side chain moiety or the penicilloyl moiety. First, the immunogen, penicillin-OvA, may not have penicilloyl moiety, but this possibility is unlikely because rat anti-FPC IgE serum can cross react with penicillin-BGG which was prepared by the same method. Second, the penicilloyl moieties may be placed where the antibody producing cells hardly recognized them, but this would be also unlikely because IgG antibodies for the penam moiety were produced, using the same antigen. It is possible, however that the IgE antibody producing system can recognize the minor determinants more readily than the IgG antibody producing system. Third, the immuno-system for one antigenic determinant on a carrier may regulate the immuno-response for another antigenic determinant present on the same carrier.

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References

1) COOMBS, R. R. A. & P. G. H. GELL: The classification of allergic reactions underlying disease. *In* P. G. N. GELL & R. R. A. COOMBS (*ed.*): Clinical aspects of immunology. pp. 317~337, Blackwell Scientific Publications, Oxford, 1963
2) DE WECK, A. L.: Studies on penicillin hypersensitivity. I. The specificity of rabbits anti-penicillin antibodies. Int. Arch Allergy Appl. Immunol. 21: 20~37, 1962
3) MINE, Y. & M. NISHIDA: Cefazolin, a new semisynthetic cephalosporin antibiotic. IV. Antigenicity of cefazolin and its cross reactivity with benzylpenicillin, ampicillin and cephaloridine. J. Antibiotics 23: 195~203, 1970
4) PETERSEN, B. H. & J. GRAHAM: Immunologic cross reactivity of cephalexin and penicillin. J. Lab. Clin. Med. 83: 860~870, 1974

5) SCHNEIDER, C. H. & A. L. DE WECK: Evaluation of intrinsic and extrinsic penicilloyl specific immunogenicity of penicillins. The use of penicillin hydrolysis catalysed by 3,6-bis-(dimethylaminomethyl) catechol. Immunochemistry 7: 157~166, 1970
6) SHIHO, O. & K. TSUCHIYA: Passive hemagllutination test by sheep erythrocyte coated with penicillin-bovine gamma globulin (BGG) conjugate. Jpn. J. Allergol. 9: 645~656, 1977
7) TSUCHIYA, K.; M. KONDO, T. OISHI & K. ISHIFUJI: Immunological cross reactions of sulfobenzylpenicillin. J. Antibiotics 26: 458~491, 1973
8) TSUCHIYA, K.; O. SHIHO & M. KONDO: Immunological specificity of mecillinam. Chemotherapy (Tokyo) 25: 100~104, 1977
9) TSUCHIYA, K.; O. SHIHO & M. KONDO: Immunological cross-reactivities of sulfocephalosporins. J. Antibiotics 32: 488~495, 1979
10) LEVINE, B. B.; H. CHANG, Jr. & N. M. VAZ: The production of hapten-specific reaginic antibodies in the guinea pigs. J. Immunol. 106: 29~33, 1971
11) RODELL, A. K.; S. AHLSTEDT, T. K. OLSSON & P. O. SVÄRD: Formation of homocytropic antibodies with penicilloyl specificity in the rabbits. Int. Arch Allergy Appl. Immunol. 51: 156~167, 1976
12) SHIHO, O. & K. TSUCHIYA: IgE antibodies for penicillins and cephalosporins in rats. I. Characteristics of the IgE antibodies for penicillins and cephalosporins in rats. J. Antibiotics 34: 72~78, 1981
13) TADA, T. & K. OKUMURA: Regulation of homocytropic antibody formation in the rat. I. Feed back regulation by passive administered antibody. J. Immunol. 106: 1002~1011, 1971
14) DE WECK, A. L.: Studies on penicillin hypersensitivity. II. The role of the side chain in penicillin antigenicity. Int. Arch. Allergy Appl. Immunol. 21: 38~50, 1962
15) KONDO, M.; O. SHIHO, T. OISHI & K. TSUCHIYA: Immunological specificity of cephacetrile. Chemotherapy (Tokyo) 24: 86~93, 1976