IgE ANTIBODIES FOR PENICILLINS AND CEPHALOSPORINS IN RATS. III

ANTIGENIC SPECIFICITY OF RAT ANTI-CEPHALOSPORIN-OvA IgE SERA

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Sprague Dawley (SD) rats were immunized with various cephalosporin- and penicillin-ovalbumin (OvA) in combination with aluminum hydroxide (alum) and thimerosal-killed *Bordetella pertussis*. Anti-cephalosporin IgE antibody production was inferior to anti-penicillin IgE antibody production. Cefsulodin (CFS), sulbenicillin (SBPC) and α-sulfophenyl acetic acid (SPAA) cross-reacted with each other but did not react with cephaloridine (CER), cefazolin (CEZ) and penicillin G (PCG). CER and PCG slightly cross-reacted with each other but did not cross-react with the others tested. Anti-CFS and anti-SBPC IgE sera were related specifically to the SPAA moiety.

The role of the IgE antibodies in drug allergy has been believed to be highly important in recent years[1,2]. There are three possibilities for the mechanism of the drug allergy to be elicited; first, the denatured serum protein conjugate with drug or its degradation products[3~5], second, the polymers derived from the drug[6,7] and third, the contaminants in the drug[8,9]. The antigenic specificity of the IgG antibodies for penicillins and cephalosporins has been clarified according to the first possibility[9~14]. It was reported in the previous paper[15] that the antigenic specificity of rat anti-penicillin IgE sera depended on the acyl side chain moiety. This paper describes the antigenic specificities of various cephalosporins in rat IgE antibodies in comparison with the antigenic specificities of penicillins.

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 to 10 weeks of age for the passive cutaneous anaphylaxis (PCA) test.

Chemicals

Cefsulodin (CFS) and α-sulfophenyl acetic acid (SPAA) were prepared in Takeda Chemical Industries, Ltd., Osaka, Japan. Sulbenicillin (SBPC) and penicillin G (PCG) were obtained from commercial sources of Takeda Chemical Industries, Ltd., Osaka, Japan. Cephaloridine (CER) and cefazolin (CEZ) were purchased from Shionogi & Co., Ltd., Osaka, Japan and Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, respectively. 7-Aminocephalosporanic acid (7-ACA) was supplied by CIBA-GEIGY Ltd., Basel, Switzerland. Isonicotinic acid was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Ovalbumin (OvA) and bovine gamma globulin (BGG) were purchased from Nutrional Biochemical Corporation, Cleveland, Ohio, U.S.A. Saline suspension of thimerosal-killed *Bordetella pertussis* containing 2×10^{10} cells per ml was prepared in Takeda Chemical Industries, Ltd., Osaka, Japan.

Hapten-protein conjugates

One hundred mg of OvA or BGG was dissolved in 10 ml physiological saline and cephalosporin or penicillin was added in a ratio of 2,000 M *vs.* 1 M protein. The reaction mixture was adjusted to pH 10 to 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 then hapten-protein conjugates were 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 haptens in cephalosporin- and penicillin-protein conjugates were measured by the amino acid analysis method[16] and the penamaldate method[17], respectively. Hapten-protein conjugates used in this study were CFS_{20}-OvA, $SBPC_{38}$-OvA, $SPAA_{42}$-OvA, CEZ_{19}-OvA, CER_{21}-OvA, PCG_{20}-OvA, CFS_{59}-BGG, $SBPC_{38}$-BGG, $SPAA_{42}$-BGG, CEZ_{79}-BGG, CER_{86}-BGG and PCG_{63}-BGG.

Immunization

Following the method of TADA[18], rats were initially injected intramuscularly with a mixture of 5 mg of hapten-OvA and 5 mg of aluminum hydroxide (alum) and intraperitoneally with 1 ml of thimerosal-killed *B. pertussis* saline suspension. At day 5, a secondary immunization was made by the intraperitoneal injection of the same dose of the mixture of hapten-OvA and alum. Blood specimens were obtained from the retro orbital plexus at appropriate intervals or the abdomal aorta on day 13 after the first immunization.

Passive cutaneous anaphylaxis (PCA) test

Assay of anti-hapten IgE antibodies were 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 blue spots on the under side of the skin. Blue spot with an average diameter of more than 5 mm was regarded as a posivite PCA reaction. The tests were carried out in duplicate for each sample, and the PCA titers were expressed as the highest dilution giving a positive PCA reaction.

Hapten inhibition test of the rat 60-hour PCA reaction

The rats were sensitized by the intradermal injection with 4 units of antisera (antisera so diluted as to show a PCA titer of 4). The rats were challenged with 1 mg of hapten-BGG conjugates in 2 ml of 1 % Evans blue saline solutions immediately after the injection of hapten saline solutions, and the PCA reactions were measured as described above.

Results

Production of the IgE Antibodies for Cephalosporins and Penicillins

Anti-CFS IgE antibodies were obtained in small amounts on day 7 and reached the maximum on day 13. Anti-SBPC IgE antibodies were observed on day 7 and reached the maximum on day 10 to 13 as well as anti-SPAA IgE antibodies. The anti-CFS IgE titers on day 13 was less than the anti-SBPC

Table 1. Production of anti-hapten-OvA antibodies.

| Antigen	Diameter of dye leakage (mm)[a] (Mean±S.D.)			PCA titer[c]
	7 days[b]	10 days	13 days	
CFS-OvA	3.9±6.9	5.6±7.3	12.0±6.0	11
SBPC-OvA	9.4±7.8	16.2±2.5	16.5±2.0	97
SPAA-OvA	9.8±7.4	15.7±1.5	16.1±1.2	111
CEZ-OvA	0	0	13.9±3.3	28
CER-OvA	0	4.9±5.4	14.9±1.5	21
PCG-OvA	6.0±6.9	15.8±1.2	15.7±1.5	147

[a] Rats were sensitized with 0.1 ml of antisera, and challenged with 1 mg of hapten-BGG 60 hours after sensitization.

[b] Days after the first immunization.

[c] PCA titers were measured on sera obtained on day 13 after the first immunization. Mean titers of 5 rats were represented.

Fig. 1. Effect of pretreatment with carrier on production of anti-PCG IgE antibodies.

Rats were pretreated with mixtures of OvA plus alum, CFS-OvA plus alum or SBPC-OvA plus alum or with saline, and simultaneously injected with thimerosal-killed *B. pertussis*. Five days after pretreatment, rats were immunized with a mixture of PCG-OvA and alum. Each point and vertical bar represents mean and standard deviation of 8 rats.
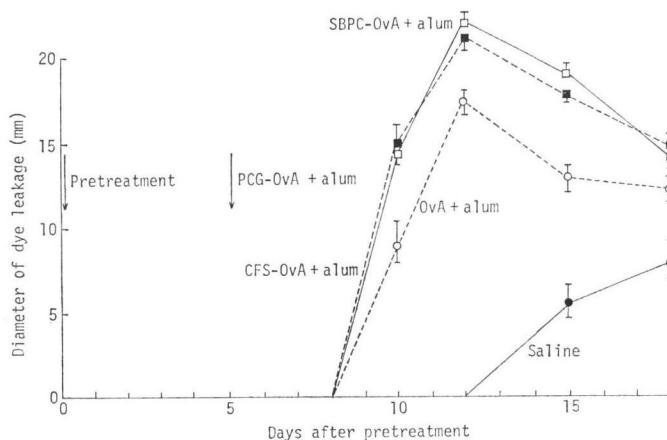
and anti-SPAA IgE titers. The production of anti-CER IgE antibodies was weak in comparison with that of anti-PCG IgE antibodies (Table 1). The effect of the pretreatment with carrier protein on the production of the IgE antibodies was investigated (Fig. 1). Rats were preimmunized with CFS-OvA, SBPC-OvA or native OvA in combination with alum and *B. pertussis* 5 days before the immunization with PCG-OvA and alum. Anti-PCG IgE antibodies were produced much more effective by the preimmunization with CFS-OvA or SBPC-OvA and more effective by the preimmunization with native OvA than the pretreatment with saline.

Cross-reactivities among Cephalosporins
and Penicillins

Anti-CFS-OvA IgE serum cross-reacted with SBPC-BGG and SPAA-BGG but did not cross-react with CEZ-BGG, CER-BGG and PCG-BGG. Anti-SBPC-OvA IgE serum weakly cross-reacted with CFS-BGG and SPAA-BGG but did not cross-react with CEZ-BGG, CER-BGG and PCG-BGG. Anti-SPAA-OvA IgE serum moderately cross-react with CFS-BGG and SBPC-BGG. Anti-CEZ-OvA IgE serum did not cross-react with other cephalosporin- or penicillin-BGG. Anti-CER-OvA IgE serum slightly cross-reacted only with PCG-BGG. Anti-PCG-OvA

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

Rats sensitized with 4 units of anti-CFS serum were injected with 2 ml of various concentrations of CFS, SPPA and 7-ACA plus isonicotinic acid just before antigen challenge. Percent inhibition against the PCA reactions without injection of hapten was represented. Each point represents the mean percent inhibition of 3 spots in 3 recipients.
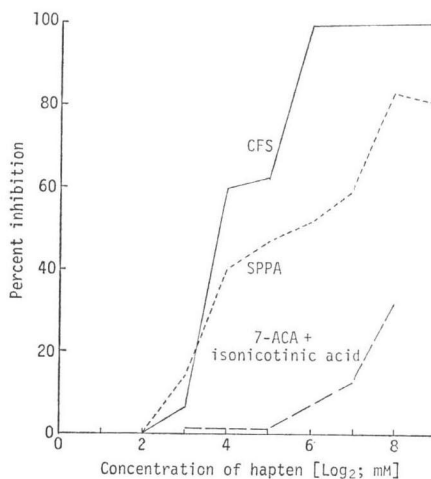
Table 2. Cross reactivity between rat anti-hapten-OvA IgE sera and hapten-BGG in the rat 60-hour PCA reaction.[a]

Antiserum[b]	Challenge antigen					
	CFS-BGG	SBPC-BGG	SPAA-BGG	CEZ-BGG	CER-BGG	PCG-BGG
CFS-OvA	64[e]	64	64	<1	<1	<1
SBPC-OvA	8	128	8	<1	<1	<1
SPAA-OvA	32	32	128	n.t.[d]	n.t.	n.t.
CEZ-OvA	<1	<1	n.t.	64	<1	<1
CER-OvA	<1	<1	n.t.	<1	64	4
PCG-OvA	<1	<1	n.t.	<1	1	128

[a] Rats sensitized with 0.1 ml of serial two-fold diluted antisera were challenged with 1 mg of various hapten-BGGs.
[b] Rat anti-hapten-OvA sera obtained on day 13 after the first immunization.
[e] Mean rat 60-hour PCA titer of 2 recipients.
[d] Not tested.

IgE serum weakly cross-reacted only with CER-BGG (Table 2).

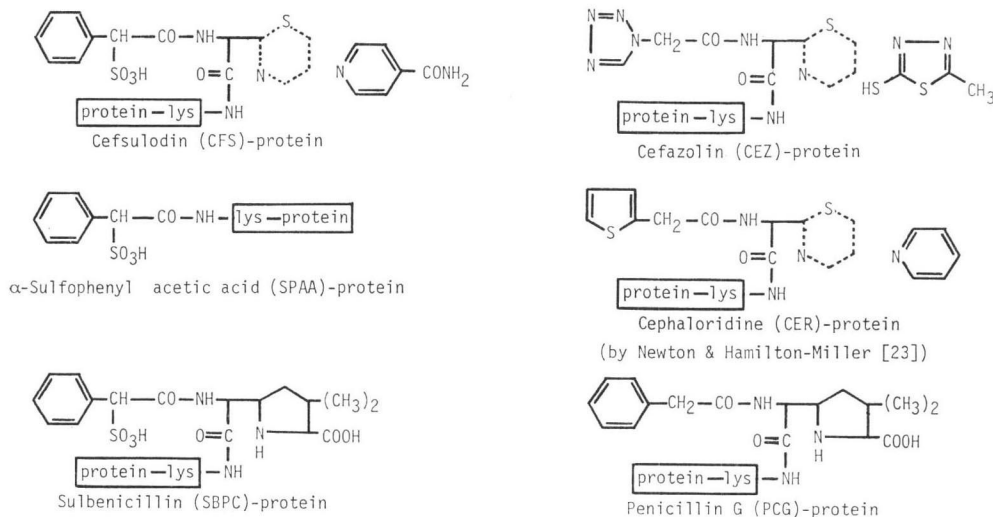### Antigenic Specificity of Anti-CFS-OvA IgE Serum

The antigenic specificities of anti-CFS-OvA IgE serum was tested by the quantitative hapten inhibition test (Fig. 2). Fifty percent inhibitory concentrations by CFS, SPAA and 7-ACA plus isonicotinic acid in the PCA reaction between rat anti-CFS-OvA IgE serum and CFS-BGG were 14 mM, 52 mM and more than 256 mM, respectively.

Discussion

In rats, the production of the IgE antibodies for cephalosporins was inferior to those for penicillins[10]. This was confirmed in this study by using cephalosporins and penicillins having the same acyl side chain or analogues in the acyl side chain. Although CFS-OvA and SBPC-OvA had the same antigenic determinant, the SPAA moiety, the anti-CFS IgE antibody production was much less than the anti-SBPC IgE antibody production. The anti-CER IgE antibody production was also much less than the anti-PCG IgE antibody production, though the thienyl acetic acid moiety at the 7-position of CER resembled the phenyl acetic acid moiety at the 6-position of PCG. In the IgE antibody production against hapten or in the PCA reactions, the epitope density on hapten-protein conjugates is an important factor[10]. Antigens used in this paper have sufficient epitope densities for antigenicity. Even more, in the IgE antibody production against hapten, the helper activity of carrier protein is thought to be important. Carrier proteins of CFS-OvA and SBPC-OvA showed the sufficient helper activity in the IgE antibody production system for non-cross reactive hapten, PCG.

CFS, SBPC and SPAA cross-reacted with each other, and the reactivities of IgE antisera against these compounds were specific for SPAA. The reactivity of anti-SBPC-OvA IgE serum with SPAA-BGG, however, was inferior to that with SBPC-BGG. This fact might be accounted for by the length of the α-sulfophenyl groups on both antigen[21]. SBPC-protein conjugates seem to be have longer spacer arms than SPAA-protein conjugates. The chemical structures of PCG-proteins presented by LEVINE and OVARY[17] demonstrate that the penicilloyl groups are bound to the ε-amino groups of lysine moiety in protein and this has been proved on a few penicillin-protein conjugates in our laboratory (unpublished data). The chemical structures of cephalosporins bound to the protein have not been adequately clarified. From preliminary studies, it was found that the acyl side chain was bound to the protein and another side chain at the 3-position was lost from the cephalosporin under the conjugation conditions (unpublished data). As shown by NEWTON and HAMILTON-MILLER[21], the chemical structure of CFS

Fig. 3. Chemical structures of penicillin- and cephalosporin-protein conjugates.

Cefsulodin (CFS)-protein

Cefazolin (CEZ)-protein

α-Sulfophenyl acetic acid (SPAA)-protein

Cephaloridine (CER)-protein
(by Newton & Hamilton-Miller [23])

Sulbenicillin (SBPC)-protein

Penicillin G (PCG)-protein

and CEZ bound to protein may be postulated as shown in Fig. 3. The difference between CFS and SBPC in the sensitizing activity and the cross-reactivity was thought to be dependent on their chemical structures bound to the protein. The penicilloyl moiety of penicillin is stable under the condition of the conjugation process to the protein but the cephalosporyl moiety of cephalosporin is highly destructive. The degradation product of the cleavage of the cephalosporyl moiety of CFS was thought to disturb the antigenicity of the sulfophenyl moiety of CFS and the same reasons may apply to the case of CER and CEZ. The immunological cross-reactivities of cephalosporins in the IgE antibodies seem to depend on the resemblance of the acyl side chain moiety as those in the IgG antibodies[11,13,14]. The cross-reactivities between CFS and SBPC or between CER and PCG in the IgE antibodies, however, are still inferior to that in the IgG antibodies. This fact also indicates that the antigenicity of the acyl side chain moiety of cephalosporin is modified by the degradation of cephalosporyl moiety.

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