Cephalothin and Penicillin G Polymers as Elicitors of Rat PCA Mediated by Mouse IgE Antibodies

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Abstract—PCA-eliciting activities of cephalothin- and penicillin G-polymers were examined in rats sensitized with homologous IgE antibodies of mouse origin. Cephalothin-polymers elicited PCA regardless of the source of antibodies and the methods to raise them, the minimal effective dose being 2 to 20 μ g/animal. Penicillin G-polymers provoked PCA only when anti-benzylpenicilloyl IgE antibody of C57BL/6J mouse raised early after immunization was used. The minimal effective dose in this case was 5 μ g/animal, being comparable to that of cephalothin polymers.

β -Lactam antibiotics cause allergic side effects in some patients, even though these agents have low molecular weights and are therefore considered to be non-antigenic. Some workers have postulated that polymers of antibiotic molecules are one group of materials responsible for allergic reactions (reviewed by Dewdney, see reference 1). However, the information available is insufficient for systematic understanding of the role of polymers of antibiotics in the antibody response and in the elicitation of hypersensitivity in presensitized animals. Thus, we started a series of experiments to examine the immunogenicity and hypersensitivity-eliciting activity of some antibiotic polymers. The present study examined the activity of penicillin G (PCG) and cephalothin (CET) polymers in provoking passive cutaneous anaphylaxis (PCA) in rats locally sensitized with homologous IgE antibodies of mouse origin. PCA-eliciting activity of antibiotic polymers has mostly been investigated using IgG antibodies of rabbit origin (2–6), and only a few publications (7, 8) concern the reactivity of polymerized materials with antibodies of different origin and class. Studies on IgE antibody-mediated reactions are particularly important because this class of antibodies

plays the main role in human allergic reactions.

Female C57BL/6JShi and C3H/HeNShi mice, 7 to 8 weeks of age, were immunized with CET₂₂-BGG (BGG: bovine γ -globulin, Sigma, St. Louis, MO, U.S.A.) or BPO₃₁-BGG (BPO: benzylpenicilloyl). These conjugates were prepared according to Levine and Ovary (9), and their epitope densities were determined by the technique of Ebata et al. (10). Immunization was done either by three intraperitoneal injections at one-week intervals of 1 μ g of an immunogen adsorbed to 1 mg of aluminum hydroxide gel or by one or two intraperitoneal injections at 2 weeks apart of 1 mg of an immunogen emulsified with Freund's complete adjuvant (FCA, Difco, Detroit, MI, U.S.A.). Mice were bled 6 or 7 days after the last injection, and the sera of five mice were pooled.

Aqueous solutions of CET (250 mg/ml; Shionogi, Osaka, Japan) and PCG (240 mg/ml; Meiji Seika, Tokyo, Japan) were left standing at room temperature for 7 and 15 days, respectively, and the polymeric materials formed were fractionated by Sephadex G 25 gel filtration (Fig. 1a and 1b). CET polymers were eluted with pure water at 4°C and then lyophilized. PCG polymers were first fractionated by gel filtration using

Fig. 1. Sephadex G₂₆ gel filtration of aged aqueous solutions of CET and PCG. a: CET solution (250 mg/ml, for 7 days at room temperature, eluted with pure water). b: PCG solution (240 mg/ml, for 15 days at room temperature, eluted with 0.5% sodium chloride solution).

Table 1. PCA-eliciting activity of CET and PCG polymers in rats

Exp.	Immunized to	Antiserum used		Pool No.	IgE Ab titer against		Minimal effective polymer dose (μ g/animal)
		Origin	Adjuvant employed		OVA-conjugate ^b	Polymers ^c	
I	CET ₂₂ -BGG	C3H/HeNShi	Alum ($\times 3$) ^a	1	1:128	1:8	2
			FCA ($\times 2$) ^a	2	1:128	1:16	5
				3	1:256	1:8	20
		C57BL/6JShi	FCA ($\times 1$) ^a	4	1:128	1:16	5
II	BPO ₃₁ -BGG	C3H/HeNShi	Alum ($\times 3$) ^a	5	1:256	Negative	
			FCA ($\times 2$) ^a	6	1:256	Negative	
				7	1:64	Negative	
		C57BL/6JShi	Alum ($\times 3$) ^a	8	Negative	Negative	
			FCA ($\times 1$) ^a	9	1:128	1:64	5
				10	1:256	1:64	10
			FCA ($\times 2$) ^a	11	1:16	Negative	
				12	1:16	Negative	

^a Number of injections. ^b CET₄-OVA (Exp. I) and BPO₄-OVA (Exp. II), 1 mg/animal. ^c CET polymers (Exp. I), 100 μ g/animal and PCG polymers (Exp. II), 300 μ g/animal.

0.5% sodium chloride solution as eluant, then made salt-free by the second gel filtration with pure water, and finally lyophilized.

PCA tests were performed according to Mota and Wong (11) using Wistar/Shi rats (female, 7 to 9 weeks of age) as recipients. Rats were intradermally injected with 0.05 ml at two-fold serial dilution of each antiserum pool at several sites of the dorsal skin, and

then they were intravenously challenged 18 hr later with various amounts of polymeric materials together with 10 mg of Evans blue. As the polyvalent elicitors, BPO₄-OVA (OVA: ovalbumin, Seikagaku Kogyo, Tokyo, Japan) and CET₄-OVA conjugates were employed. One hour later, the animals were sacrificed, and the highest dilution of antiserum to produce minimal bluing (5 mm in diameter) was recorded as the IgE antibody titer. Every

test was performed in duplicate using two rats.

As shown in Table 1, IgE antibody titers of all the antisera except for those obtained from C57BL/6JShi mice after a booster injection showed similar antibody titers when an adequate dose (1 mg) of the homologous OVA conjugate was given; the titer ranged from 1:64 to 1:256. When CET polymers were intravenously injected, all the anti-CET sera produced obvious PCA. In this case, the PCA titers ranged from 1:8 to 1:16. The minimal dose of CET polymers necessary to elicit PCA did not vary much, being 2 to 20 μ g per animal. The CET monomer fraction failed to provoke PCA even at a high dose of 1 mg per animal. In contrast, the PCA-eliciting activity of PCG polymers markedly differed depending on the antibody preparation used. Potent activity was observed only when the anti-BPO antiserum obtained from C57BL/6JShi mice shortly after one injection of FCA emulsion containing BPO₃₁-BGG was employed. In this case, IgE antibody titer was estimated to be as high as 1:64, and the minimal effective dose of the polymers was approximately 5 μ g per animal. Therefore, as far as this antibody preparation is concerned, PCG polymers were nearly equally active as antigens as CET polymers. However, PCA was not provoked by PCG polymers when other antisera were used (Table 1). Note that PCG polymers failed to produce PCA when rats were sensitized with antisera obtained from C57BL/6JShi mice which had been given a booster injection. Sera obtained from this strain of mice, which had been immunized by repeated injections of minute amounts of BPO₃₁-BGG adsorbed to aluminum hydroxide gel, did not induce rat PCA when either the BPO₄-OVA or PCG polymer fraction was used as an eliciting antigen. This seems to be reasonable because this strain is a low-responder to BGG (12).

Previous studies (3, 5) on the rabbit IgG antibody system have demonstrated that PCG polymers are far less active than polymers of other β -lactam antibiotics in eliciting guinea pig PCA. Our finding that PCG polymers in contrast with CET polymers are usually incapable of eliciting rat PCA seems to be along the same line. The potent activity

observed in the rat sensitized with antibodies obtained from C57BL/6JShi mice soon after an immunizing injection may be an exceptional case. The reason that only this type of IgE antibodies was capable of reacting with PCG polymers is now being investigated.

References

- 1 Dewdney, J.M.: Immunology of the antibiotics. In *The Antigen*, Edited by Sela, M., Vol. IV, p. 73-244, Academic Press, New York, San Francisco and London (1977)
- 2 Dewdney, J.M., Smith, H. and Wheeler, A.W.: The formation of antigenic polymers in aqueous solutions of β -lactam antibiotics. *Immunology* **21**, 517-525 (1971)
- 3 Munro, A.C., Dewdney, J.M., Smith, H. and Wheeler, A.W.: Antigenic properties of polymers formed by β -lactam antibiotics. *Intern. Arch. Allergy Appl. Immunol.* **50**, 192-205 (1976)
- 4 Ahlstedt, S., Kristofferson, A., Svärd, P.O. and Strannegård, Ö.: Immunological properties of ampicillin polymers. *Intern. Arch. Allergy Appl. Immunol.* **53**, 247-253 (1977)
- 5 Ahlstedt, S., Kristofferson, A., Svärd, P.O., Thor, L. and Örtengren, B.: Ampicillin polymers as elicitors of passive cutaneous anaphylaxis. *Intern. Arch. Allergy Appl. Immunol.* **51**, 131-139 (1976)
- 6 Smith, H., Dewdney, J.M. and Wheeler, A.W.: A Comparison of the amounts and the antigenicity of polymeric materials formed in aqueous solution by some β -lactam antibiotics. *Immunology* **21**, 527-533 (1971)
- 7 Takeuchi, Y., Nishimura, Y., Yamazi, Y. and Kimura, Y.: Fundamental studies on penicillin allergy. (1) Immune response to PCG polymers in the rats. *Japan. J. Allergol.* **26**, 10-16 (1977) (in Japanese)
- 8 Iwata, M., Tokiwa, H. and Matuhashi, T.: Detection and characterization of polymers in cephalothin by passive cutaneous anaphylaxis in mice. *Intern. Arch. Allergy Appl. Immunol.* **70**, 132-137 (1983)
- 9 Levine, B.B. and Ovary, Z.: Studies on the mechanism of the formation of the penicillin antigen. III. The N-(α -benzylpenicilloyl) group as an antigenic determinant responsible for hypersensitivity to penicillin G. *J. Exp. Med.* **114**, 875-904 (1961)
- 10 Ebata, M., Miyake, Y. and Uchida, J.: A quantitative assay of covalently bound cephalosporin derivatives in cephalosporin-protein conjugates. *J. Antibiotics* **29**, 655-666 (1976)
- 11 Mota, I. and Wong, D.: Homologous and

- heterologous passive cutaneous anaphylaxis activity of mouse antisera during the course of immunization. *Life Sci.* **8**, 813–820 (1969)
- 12 Levine, B.B. and Vaz, N.M.:** Effect of combination of inbred strain, antigen, and antigen dose on immune responsiveness and reagin production in the mouse. *Intern. Arch. Allergy Appl. Immunol.* **39**, 165–171 (1970)