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Induction of IgG_1 and IgE responses to protein-conjugated and unconjugated β-lactam antibiotics in the mouse – efficacy of Freund's complete adjuvant

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Summary. One or two injections two weeks apart of protein-conjugated penicillin G, cephalothin or cefmetazole emulsified with Freund's complete adjuvant were quite effective in producing anti-antibiotic antibodies of the IgE as well as of the IgG_1 class in mice. Long-lasting and boostable production of both antibody classes was also obtained against unconjugated cephalothin or cefmetazole, though the positivity depended on the mouse strain.
Key words. Freund's complete adjuvant; IgE response; IgG_1 response; β-lactam antibiotics; strain difference.

To analyze the allergic side-effects of β-lactam antibiotics in man, we first need to know the immunogenicity of these drugs in animals. Especially important is the ability to produce antibodies which mediate anaphylactic reactions, because the most prevalent and severe side effects belong to Type I of immediate hypersensitivity. Therefore, we examined the immunogenicity of some antibiotic agents in the mouse and found that not only IgG_1 but also IgE responses to both protein-conjugated and unconjugated antibiotics could be obtained in animals immunized using Freund's complete adjuvant.

Materials and methods. 1) Animals. C_3H/HeNShi and $C_{57}BL$/6JShi mice (female, 7–8 weeks old) were used as antibody producers, and DS/Shi mice (female, 7–8 weeks old) and Wistar/Shi rats (female, 8–9 weeks old) were employed as recipients in the PCA test.

2) Antibiotics. Cephalothin (CET, Shionogi), penicillin G (PCG, Meiji Seika) and cefmetazole (CMZ, Sankyo) were used.

3) Antibiotic-protein conjugates. The antibiotics were conjugated with bovine γ-globulin (BGG, Sigma) or guinea pig serum albumin (GpSA, Sigma) according to Levine et al.[1] and the epitope densities of the conjugates prepared were determined by the technique of Ebata et al.[6]. The conjugates used as immunogens were CET_{17}-BGG, BPO_{31}-BGG (BPO: benzylpenicilloyl, the main haptenic form of PCG) and CMZ_{17}-BGG, and those employed as PCA-elicitors were CET_{13}-GpSA, BPO_{17}-GpSA and CMZ_{16}-GpSA.

4) Immunization. A) Immunization with antibiotic-BGG conjugates: C_3H/HeNShi and $C_{57}BL$/6JShi mice were i.p. injected with 0.2 ml of an emulsion of Freund's complete adjuvant (FCA) containing 1 mg of an antibiotic-BGG conjugate once or twice at 2 weeks apart. C_3H/HeNShi mice were also immunized by three biweekly i.p. injections of alum-precipitated immunogen (1 µg of an immunogen plus 1 mg of aluminum hydroxide gel) prepared according to Katsura[4]. As negative controls, both strains of mouse were immunized to plain BGG in the same way. The immunized animals were bled by heart puncture 1 or 2 weeks later, and antisera of a group of five mice were pooled for the determination of antibody activity. B) Immunization with unconjugated antibiotics: C_3H/HeNShi and $C_{56}BL$/6JShi mice were i.p. injected with 0.2 ml of FCA emulsion containing 1 mg of each antibiotic agent three times a week for 2 weeks (a total of 6 times), and antisera were prepared 4 weeks after the last injection. Controls were injected with an emulsion of FCA and physiological saline solution.

Table 1. Production of anti-hapten IgE and IgG_1 antibodies after immunization with antibiotic-BGG conjugates

Mouse strain	Immunizing injection		Time of bleeding*	Elicitor	Ab titer** IgE	IgG_1
C_{57}BL/6JShi	BPO_{31}–BGG	1 mg + FCA, × 1	7	BPO_{17}–GpSA	1:256	1:128
	CET_{17}–BGG	1 mg + FCA, × 1	7	CET_{13}–GpSA	1:64	1:8
	CMZ_{17}–BGG	1 mg + FCA, × 1	7	CMZ_{16}–GpSA	1:128	1:128
	BGG	1 mg + FCA, × 1	7	BGG	1:4	1:4
				BPO_{17}–GpSA	Neg.	Neg.
				CET_{13}–GpSA	Neg.	Neg.
				CMZ_{16}–GpSA	Neg.	Neg.
C_3H/HeNShi	BPO_{31}–BGG	1 mg + FCA, × 1	7	BPO_{17}–GpSA	1:16	1:16
		× 2	14		1:128	1:256
	CET_{17}–BGG	1 mg + FCA, × 2	14	CET_{13}–GpSA	1:256	1:512
	CMZ_{17}–BGG	1 mg + FCA, × 2	14	CMZ_{16}–GpSA	1:256	1:4096
	BGG	1 mg + FCA, × 2	14	BGG	1:128	1:2048
				BPO_{17}–GpSA	Neg.	Neg.
				CET_{13}–GpSA	Neg.	Neg.
				CMZ_{16}–GpSA	Neg.	Neg.
	BPO_{31}–BGG	1 μg + Alum, × 3	14	BPO_{17}–GpSA	1:128	1:1024
	CET_{17}–BGG	1 μg + Alum, × 3	14	CET_{13}–GpSA	1:256	1:8
	CMZ_{17}–BGG	1 μg + Alum, × 3	14	CMZ_{16}–GpSA	1:256	1:512

* Days after the last immunizing injection. ** Antibody titration was done with the pooled antisera of 5 mice using 1 mg of each elicitor.

5) Determination of antibody activities. IgE and IgG_1 antibody activities were assayed by means of 24-h rat PCA[2] and 1-h mouse PCA[3], respectively. PCA was elicited by i.v. injection of 1 mg of the homologous GpSA conjugate with 5 mg (rat) or 1 mg (mouse) of Evans blue. Dye leakage into the skin of more than 5 mm was taken as a positive reaction. Tests were performed in duplicate using two recipients for each antiserum, but little difference was observed between the recipients.

Results. From table 1, it is evident that CET_{17}-BGG, BPO_{31}-BGG and CMZ_{17}-BGG emulsified with FCA produced not only IgG_1 but also IgE anti-hapten antibodies in both C_3H/HeNShi and C_{57}BL/6JShi strains, because antisera to the carrier protein, BGG, did not react with any antibiotic-GpSA conjugate. To obtain the maximal level of IgE response, one injection only was enough with C_{57}BL/6JShi mice, whereas a booster injection was necessary with C_3H/HeNShi mice. The IgE antibody titers thus obtained were comparable to those of antisera of C_3H/HeNShi mice which had been immunized with multiple injections of minute amounts of the corresponding BGG-conjugates adsorbed on aluminum hydroxide gel.

As seen from table 2, both IgE and IgG_1 antibodies were also produced when the animals were immunized by repeated injections of FCA emulsion containing unconjugated CET or CMZ. The anti-CET IgE antibody response was observed in half of 42 C_{57}BL/6JShi mice but in only 1 of 16 C_3H/HeNShi mice at 4 weeks after the 6th injection of the emulsion. In contrast, anti-CMZ IgE antibody was detected in almost all of the C_3H/HeNShi mice and less than half of the C_{57}BL/6JShi mice. Similar strain differences were observed with anti-CET and anti-CMZ IgG_1 responses, though they were less obvious than those with the IgE responses.

Discussion. FCA is known to be an excellent adjuvant for the IgG response but not for the IgE response[5,6,8,9]. However, the present study showed that this is not the case in mice. Obvious production of IgE antibodies as well as IgG_1 antibodies occurred not only in response to protein-conjugated but also to unconjugated antibiotics. Even three intermittent injections of FCA emulsion of unconjugated CMZ raised IgE antibodies in most of the C_3H/HeNShi mice, though a single injection failed to induce the response (data not shown). Our preliminary experiments showed that Freund's incomplete adjuvant (FIA) was as effective as FCA, which indicates that tubercle baccilli contained in FCA are not essential for raising these antibodies. The possibility that some high molecular weight impurities in these antibiotic preparations are responsible for the antibody formation cannot be ruled out. However, no high molecular weight materials were found by Sephadex G 25 gel filtration (data not shown),

Table 2. Production of IgE and IgG_1 antibodies to unconjugated antibiotic preparations

Mouse strain	Immunizing injection		Elicitor	Ab positive/total IgE	IgG_1
C_{57}BL/6JShi	CET 1 mg	+ FCA, × 6	CET_{13}–GpSA	21/42	19/42
	CMZ 1 mg	+ FCA, × 6	CMZ_{16}–GpSA	8/20	5/20
	Saline	+ FCA, × 6	CET_{13}–GpSA	0/10	0/10
			GMZ_{16}–GpSA	0/10	0/10
C_3H/HeNShi	CET 1 mg	+ FCA, × 6	CET_{13}–GpSA	1/16	6/16
	CMZ 1 mg	+ FCA, × 6	CMZ_{16}–GpSA	14/16	15/16
	Saline	+ FCA, × 6	CET_{13}–GpSA	0/10	0/10
	Saline	+ FCA, × 6	CMZ_{16}–GpSA	0/10	0/10

which suggests an alternative mechanism, e.g., that the antibiotic molecules or their metabolites acquire immunogenicity by binding with some endogenous protein carriers. If this is the case, FCA or FIA emulsion would prolong the in vivo retention of antibiotic molecules and therefore allow the continuous production of immunogenic protein conjugates. Whatever the mechanism mitht be, it is clear that FCA induced a long-lasting and boostable IgE response to some protein-conjugated and unconjugated antibiotic preparations in the mouse. Another interesting finding is that the production of antibodies, particularly of IgE antibodies, to unconjugated antibiotics differed markedly with the antibiotic agent and mouse strain. Biochemical and genetic analyses of the cause of such strain differences should help in establishing an animal model for human atopic drug hypersensitivity.

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