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Polyethylene Glycol Reactive Antibodies in Man: Titer Distribution in Allergic Patients Treated with Monomethoxy Polyethylene Glycol Modified Allergens or Placebo, and in Healthy Blood Donors

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Abstract. Antibodies to polyethylene glycol (PEG) were analyzed in patients with various allergies and in healthy blood donors employing passive hemagglutination. In untreated allergic patients and in healthy blood donors, naturally occurring anti-PEG antibody titers between 32 and 512 were seen in 3.3 and 0.2%, respectively. During hyposensitization with monomethoxy polyethylene glycol modified ragweed extract and honey bee venom, respectively, the patients showed an anti-PEG antibody response. Titers of 32–512 were found in 50% of the patients directly after the first treatment course. After 2 years of treatment the percentage of patients with such titers declined to 28.5%. Mercaptoethanol treatment of sera indicated that the anti-PEG antibodies predominantly were of the IgM isotype. The weak IgM response found in treated patients is considered to be of no clinical significance.

Introduction

Polyethylene glycol (PEG) has been widely used in formulating pharmaceuticals and cosmetics for several decades [2]. Recently, PEG has found new applications after covalent coupling to proteins, e.g. as PEG modified allergens for hyposensitization of allergic patients [4, 6] and as PEG modified enzymes intended for clinical use. The latter showed decreased antigenicity in animal experiments as well as prolonged persistence in the circulation [8]. We have found that PEG itself is poorly immunogenic in animals, whereas PEG covalently coupled to proteins elicits a humoral antibody response, especially in the presence of Freund's complete adjuvant (FCA) [9]. Since PEG modified allergens are being introduced clinically as potentially safer and effective agents for hyposensitization, it was considered important to evaluate whether such therapy would result in anti-PEG antibodies induced by these agents in humans. Sera of patients from three clinical studies were analysed. In two of the studies, subcutaneous treatment was performed with monomethoxy polyethylene glycol (mPEG) modified ragweed extract, and in one with mPEG modified honey bee venom. Sera were assayed by passive hemagglutination, as described recently [9]. Selected sera were also treated with mercaptoethanol for differentiation of anti-PEG antibodies into IgM and non-IgM isotypes.

Patients, Materials and Methods

Patients

Patients sensitive to ragweed and honey bee venom, respectively, were treated with mPEG modified allergens according to the protocol shown in table I. The clinical data are reported elsewhere [4, 7, *Norman* et al., to be published]. The control group (n = 92) comprised patients before onset of hyposensitization (n = 58), patients before treatment with an aqueous ragweed extract (n = 11) and reagweed-sensitive allergic patients before placebo treatment (n = 23). We are obliged to the doctors performing the clinical trials for kindly providing us with serum samples for the antibody determinations.

Blood Donors

Serum samples of healthy blood donors of both sexes were obtained from three blood centers in Japan (n = 142), Germany (n = 151) and Italy (n = 160).

Materials

Proteins Modified with mPEG. The mPEG modified ragweed allergen extract was prepared by coupling mPEG succinate of molecular weight 3,000 daltons to a ragweed allergen extract (*Ambrosia elatior*) using the mixed anhydride method [10]. The product contained 58% mPEG and 14% protein and was comparable to com-

Table I. Clinical studies with mPEG modified allergens

Study	Immunotherapy with	Number of patients		Dose schedule		Medium cumulative dose, μg protein	
		1st year	2nd year	1st year	2nd year	1st year	2nd year
Norman et al. [to be published]	mPEG modified ragweed allergen extract	13	5	start April 1981, injections were given every 2 weeks until October 1981	start December 1981, injections were given every 2 weeks until October 1982	170 (45–1,055)	4630 (1,580–20,600)
Juniper et al. [4]	mPEG modified ragweed allergen extract	31	23	start May, 1981, injections were given every week (6 or 10 visits)	start May 1982, injections were given every week (10 visits)	47 (3–2,089)	490 (10–12,066)
Öhman et al. [7]	mPEG modified honey bee venom	14		start January 1981, injections were given every week 5 times and then with 2 weeks interval (9–11 visits)		1605	

Table II. Distribution of titers of PEG-reactive antibodies in sera of allergic patients treated with mPEG modified allergens (A, B), untreated allergic patients (C), and blood donors (D): PEG reactive antibodies were estimated by homologous passive hemagglutination (figures in the body of the table are percentages for each titer group)

Population group	Titer groups										
	0	2	4	8	16	32	64	128	256	512	32–512
A Allergic patients (n=58) treated for 1 year[1]	22.5	3.4	5.1	6.9	12.1	20.7	15.5	5.2	5.2	3.4	50.0
B Allergic patients (n=28) treated for 2 years[2]	14.4	21.4	7.1	17.9	10.7	7.1	14.3	7.1	0	0	28.5
C Allergic patients (n=92) not treated	79.4	5.4	4.3	6.5	1.1	3.3	0	0	0	0	3.3
D Healthy blood donors (n=453)	95.1	2.6	1.5	0.4	0.2	0.2	0	0	0	0	0.2

[1] Sera taken directly after termination of 1st treatment course of mPEG modified allergen.
[2] Sera taken directly after termination of 2nd treatment course of mPEG modified allergen.

pound IIIb described in *Richter and Akerblom* [9]. The mPEG modified honey bee venom was similarly prepared by coupling mMPEG succinate of molecular weight 5,700 to honey bee venom. The product contained 80% mPEG and 15% protein and was identical to that described recently [1].

Methods

Passive Hemagglutination. The coupling of mPEG to red cells for passive hemagglutination has been described recently [9]. In this study, freshly collected human red cells of blood group O (Rh^+) were used. 50 μl of serial twofold dilutions of sera were mixed in plastic microtiter plates with 50-μl aliquots of 2% sensitized red cell suspension in 0.9% saline. The antibody titers are given as reciprocals of the highest serum dilution producing hemagglutination.

Treatment of Sera with Mercaptoethanol [5]. Serum (0.10 ml) was mixed with 0.3 ml of 0.133 *M* mercaptoethanol dissolved in Tris buffer-saline of pH 8.2 (Tris-hydroxymethylamine 0.02 *M*, and sodium chloride 0.15 *M*). The mixture was incubated at room temperature for 1–2 h. Directly thereafter the hemagglutination assay was performed.

Table III. Kinetics of the humoral anti-PEG response in allergic patients during hyposensitization [4][1]

Patient No.	A (April 81)	B (Juli 81)	C (Sept. 81)	D (Jan. 82)	E (July 82)	F (Sept. 82)
1	0	256	8	0	8	4
2	0	256	32	4	64	32
3	4	64	16	16	64	32
4	0	64	4	32	64	32
5	0	32	4	2	32	32
6	0	32	8	2	8	4
7	0	32	8	2	8	8
8	0	32	64	8	128	128
9	0	32	8	0	64	32
10	0	32	16	4	64, 128	64, 128
11	0	32	4	4	4	4
12	0	16	4	2	32	8
13	0	16	32	16	16	64, 128
14	0	8	4	0	8	16
15	0	4	0	0	16	4

Titers of PEG-reactive antibodies estimated by passive hemagglutination in sera of patients before (A), directly after (B), 2 months (C) and 6 months (D) after the 1st treatment course and directly after (E) and 2½ months (F) after a 2nd treatment course 1 year later with a mPEG modified ragweed allergen extract (for treatment scheme see table I).

[1] 8 patients with titers 0–4 throughout the treatment courses were considered as nonreactors and were not included in the table.

Table IV. Treatment of sera with mercaptoethanol shows that anti-PEG antibodies from patients treated with mPEG modified allergens are predominantly of IGM isotype

Patient No.	Titers of PEG-reactive antibodies estimated by passive hemagglutination[1]	
	non-mercaptoethanol-treated serum	mercaptoethanol-treated serum
1	128	0
2	128	0
3	64	8
4	512	0
5	128	4
6	256	32
7	256	32
8	16	0
9	512	16
9	512	4
Rabbit anti-PEG serum	32,768	8,192

Patients 1–5 were treated with mPEG modified honey bee venom, and patients 6–9 with mPEG modified ragweed allergen extract.

[1] Sera were collected after first treatment period, excepting patient 9, which was obtained later (after first season).

Results

As shown in tables II and III, PEG reactive antibodies appeared in patients during treatment with mPEG-modified allergens. PEG reactive antibodies with titers of 32–512 were demonstrable in 50% of allergic patients treated with mPEG modified ragweed extract or honey bee venom directly after the first treatment course. After the second year of treatment there was a marked decline of titers in the patients, titers of 32–512 comprising only 28.5%. In contrast, only a small percentage of individuals of untreated allergic patients and healthy blood donors had PEG-reactive antibodies.

The magnitude of the humoral anti-PEG response elicited by a first and second treatment course with mPEG modified allergens over a 2-year period can be followed in table III. A response was elicited in 15 of 23 patients. The highest titers occurred at the end of the first treatment period, and decreased during the following year. Before the second treatment period, 4 of 23 patients had titers of 8–32. The second treatment period also induced a response in many patients, 14 of 23 showing titers of 8–128 at the end of the period. However, no marked booster effect, characteristic of a secondary response was seen. 2½ months after the

second period the titers had declined in 8 and remained unchanged in 5 patients.

Most or all of the antibody activity recorded belonged to the IgM isotype since mercaptoethanol treatment of serum samples abolished or markedly reduced the titers (table IV).

Discussion

The anti-PEG antibody response following parenteral injection of PEG modified allergens could be expected from animal experiments conducted with other comparable conjugates [9]. The data are in accordance with earlier reports on conjugates between other repetitive polymers and protein [3]. The antigenic stimulation in man exerted by subcutaneous injection of the soluble PEG allergen conjugates was weak and mainly elicited antibodies of IgM isotype. This may also explain the lack of a typical secondary booster response in connection with the second treatment course after an interval of about 1 year. The conclusion that IgM antibodies are involved is based on the nearly complete abolition of hemagglutinating activity of serum samples after mercaptoethanol treatment. In contrast, the anti-PEG titer of a hyperimmune rabbit antiserum in a previous study was only slightly reduced by mercaptoethanol treatment, indicating that the IgG isotype predominated [9]. The precipitating antibodies in this serum had been induced by strong antigenic stimulation with mPEG modified allergens given intramuscularly in the presence of FCA.

Whether the small percentage of individuals with anti-PEG antibody titers found in normal human population samples (blood donors) is a reflection of environmental contact with PEG or represents the outcome of 'normal' polyclonal stimulation is uncertain. It is of interest, however, that in allergic patients not treated with PEG-modified allergens, the percentage of individuals with demonstrable anti-PEG antibodies is several times higher than in normal individuals. The reason for this difference is unknown.

In conclusion, the present data show that the moderate humoral anti-PEG response elicited in allergic patients following treatment with PEG modified allergens is in accordance with present concepts about the immunobiological properties of this type of compounds. Since the response is only moderate, usually not boosted by repeated treatment, and mainly of IgM isotype, we consider it to be of no clinical significance. Such an antibody response will most probably not interfere with the clinical usefulness of PEG modified allergens in hyposensitization therapy.

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