

Decrease of IgE-dependent platelet activation in Hymenoptera hypersensitivity after specific rush desensitization

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SUMMARY

A receptor for the Fc fragment of IgE on human platelets has been recently described, which mediated an IgE-dependent activation in the presence of specific allergen. We investigated the allergen-induced activation of platelets from patients with Hymenoptera hypersensitivity before and after specific rush desensitization. Nineteen patients with a history of anaphylactic reactions were included (15 sensitive to yellow-jacket and four to honey-bee venom), fourteen/nineteen having experienced severe life-threatening systemic reactions and 5/19 large local reactions. All showed positive skin tests and high values of specific IgE. By comparison to the baseline results obtained before desensitization, a significant decrease of platelet activation (76·8% inhibition) after rush desensitization was observed. In the case of two polysensitized patients, after Hymenoptera venom desensitization alone, platelets not only lost their reactivity to venom but also towards the other allergen.

This modulation of the IgE-dependent platelet reactivity during desensitization offers therefore a new approach for the study of allergen-induced desensitization.

Keywords Platelet desensitization Hymenoptera venom hypersensitivity

INTRODUCTION

Recent studies demonstrated the presence of a specific receptor for the Fc fragment of IgE on the membrane of blood platelets (Cines, Van der Keyl & Levinson, 1986; Joseph *et al.*, 1986). The percentage of platelets bearing IgE receptors varied in a range of 10–20% in normal human donors and increased up to 50% in patients with immediate hypersensitivity. In allergic diseases, platelets could be directly stimulated by the addition of either anti-human IgE antibody or the corresponding allergen, but the precise implication of platelets in allergic diseases still remains to be defined. Because of this apparent modulation of the expression of the FcεR on platelets, which is also found on other FcεR-bearing cells such as macrophages (Joseph *et al.*, 1983) and eosinophils (Capron *et al.*, 1985) but not on basophils and mast cells, we investigated whether efficient desensitization could not result in decreasing the allergen-induced reactivity of platelets, in Hymenoptera venom hypersensitivity.

Our results were compared before and after specific rush desensitization. In addition an attempt was made to correlate our results with biological parameters such as specific IgG and IgE antibodies.

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MATERIALS AND METHODS

Patients

Nineteen patients, age from 16 to 63 years, with a history of anaphylactic reactions after Hymenoptera stings, were included in this study; 15 were sensitive to *Vespula germanica* and 4 to *Apis mellifica*. Fourteen out of 19 experienced life-threatening systemic reactions and five presented large local reactions. Table 1 presents the main characteristics of the patients. Clinical reactions were classified in large local reactions and systemic reactions according to Mueller classification (Mueller, 1966).

Skin tests

Skin tests were performed intradermally by injection of approximately 0·02 ml of serially diluted venoms between 10^{-5} and 10^{-1} µg/ml. Positive responses were defined as the development of a 5 mm wheal, or greater, within 15 min. Allergens were supplied by laboratoire des Stallergenes (Fresnes, France) and were reconstituted in albumin containing buffer.

Desensitization regimen

The method used for treatment was the rush desensitization, as described by Nataf and co-workers (Nataf, Guinépain & Herman, 1984). Briefly, increasing doses of venom were injected

Table 1. Clinical and biological data in 19 patients

Patients	Allergic antecedents	Insect	Clinical score*	Skin test†	Specific IgE (RAST) ‡		Specific IgG (RIP) §		Platelet cytotoxicity (%) ¶	
					A	B	A	B	A	B
1	Penicillin allergy	Honey-bee	1	10 ⁻⁴	4	0	N.D.	2	N.D.	27.1
2	No	Vespula	1	10 ⁻⁵	4	3	N.D.	3	N.D.	15
3	No	Vespula	5	10 ⁻³	3	2	N.D.	2	N.D.	0
4	Atopic dermatitis	Vespula	5	10 ⁻⁵	4	4	N.D.	2	N.D.	22
5	No	Vespula	5	10 ⁻⁵	4	4	1	1	78	18
6	No	Vespula	5	10 ⁻³	2	3	N.D.	3	85	26.5
7	No	Honey-bee	1	10 ⁻²	2	2	0	2	63	16.5
8	No	Vespula	5	10 ⁻⁵	3	2	0	1	61	19.5
9	No	Honey-bee	3	10 ⁻⁵	3	3	0	2	55	3.5
10	No	Vespula	5	10 ⁻²	4	3	3	4	66	20
11	Food allergy	Vespula	5	10 ⁻⁵	3	3	3	4	77.8	12.5
12	No	Vespula	2	10 ⁻³	2	2	2	3	55	10.5
13	No	Vespula	1	10 ⁻¹	2	4	2	3	59.6	18.4
14	Grass pollen rhinitis	Vespula	1	10 ⁻²	4	4	0	3	57	8
15	No	Vespula	3	10 ⁻⁵	2	2	1	2	65	6.5
16	No	Honey-bee	4	10 ⁻⁵	2	2	3	4	71	15
17	No	Vespula	5	10 ⁻⁵	4	3	2	3	60	21
18	Atopic dermatitis	Vespula	3	10 ⁻¹	2	3	2	3	58	16.5
19	Grass pollen asthma	Vespula	2	10 ⁻⁵	3	3	2	3	76.5	14

Platelets from patients 1, 2, 3 and 4 were studied only after desensitization.

* Clinical reactions were classified in large local reactions and systemic reactions as follows: (1) Large local reaction; (2) Slight general reaction: with generalized urticaria; (3) General reaction: any of the above, plus two of the following: angioneurotic oedema, constriction of chest, abdominal pain, nausea, vomiting, diarrhoea; (4) Severe general reaction: any of the above, plus two of the following: dyspnoea, dysphagia, hoarseness; (5) Shock reaction: with fall in blood pressure and/or unconsciousness.

† Venom concentration used to obtain a positive skin test (wheal ≥ 5 mm).

‡ Specific IgE were expressed as score 0–4.

§ Specific IgG were expressed as score 0–4 as follows: percentage less than 5% = Negative; percentage between 5 and 10% = Score 1; percentage between 10 and 40% = Score 2; percentage between 40 and 70% = Score 3; percentage more than 70% = Score 4.

¶ The percentage of cytotoxicity was expressed as explained in Fig. 2. (A) Before desensitization; (B) After desensitization. Biological data were studied 6 weeks after rush desensitization except for patients 1, 2, 3 and 4 who were investigated several years later after the initiation of rush desensitization.

each hour for 3–4 days. No systemic reaction was observed during desensitization. The maintenance dose was 100 μ g monthly.

Laboratory tests

Total serum IgE (PRIST-Pharmacia) and specific IgE antibody (RAST-Pharmacia) were measured successively before and after rush desensitization. Measurements of venom-specific IgG were performed by radioimmunoprecipitation in liquid phase according to a method previously described (Sobotka *et al.*, 1976) and results were expressed as percentage of bound radioactivity. IgG-circulating immune complexes were measured according to Stevens method (Stevens & Bridts, 1984) 6 weeks after rush desensitization, i.e., 3 or 4 weeks after the former injection of venom, corresponding to the day of platelet testing.

Platelet preparation

Forty ml venous blood were collected on ACD-C (Caen *et al.*, 1976) (ACD-C 1 vol/blood 6 vol). Platelet rich plasma was obtained by centrifugation (120 g 15 min) and the platelets washed 3 times by repeated centrifugation (2,000 g 15 min) in saline supplemented with citric acid (36 mM), glucose (5 mM), calcium (2 mM), magnesium (1 mM) serum albumin (0.35%) and PGE1 (100 nM) (Patscheke, 1981). After counting by phase contrast microscopy, platelets were resuspended and adjusted to the adequate concentration and used within 2–3 h after blood collection. Examination of platelet preparations before and after gentian violet staining showed a leukocyte contamination less than 0.05% as previously demonstrated (Ameisen *et al.*, 1985). Blood was collected before specific immunotherapy and 6 weeks after rush desensitization, i.e., 3 weeks after the last injection of venom.

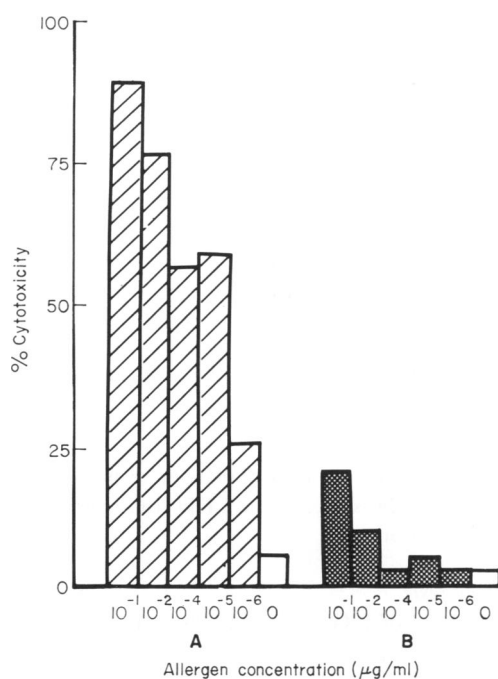


Fig. 1. Allergen-induced dose-dependent anti-parasite cytotoxicity of platelets in one patient studied before (A) and after (B) desensitization. Cytocidal platelet functions were estimated against *S. mansoni* larvae, after overnight incubation, by the percentage of dead larvae. Results were expressed after subtracting the control test of allergen without platelets.

Tests of platelet activation

Antiparasite cytotoxicity by platelets. Platelets (15×10^7), suspended in Eagle's Minimum Essential Medium (MEM) were incubated in flat-bottomed microplates (Nunc-Roskilde, Denmark) with 50–100 *Schistosoma mansoni* schistosomula, 10% pooled sera from normal donors, and in the presence of the related allergen (honey-bee or yellow-jacket venom) at concentration varying from 10^{-5} μg – 10^{-1} $\mu\text{g}/\text{ml}$, in a final volume of 200 μl . After 24 h at 37°C and 5% CO_2 in air, motionless and dark dead larvae were clearly distinguished from mobile and refringent living schistosomula by optical microscopy, which allowed to define a percentage of parasite cytotoxicity.

Chemiluminescence assay. The H_2O_2 -dependent generation of chemiluminescence by washed platelets was investigated as previously reported (Joseph *et al.*, 1984, 1986) using the amplification technique described by Whitehead *et al.* (1983), in the presence of luminol, luciferin and horse-radish peroxidase. Results were expressed as millivolts of light at the peak production measured in a Nucleotimeter 107 (Interbio, Le Thillay, France).

Statistical analysis. Data were analysed by the two-way analysis of variance, and results expressed as mean $\pm$ standard error of the mean (s.e.m.).

RESULTS

The biological data of the 19 patients before and after specific rush desensitization are exposed in Table 1.

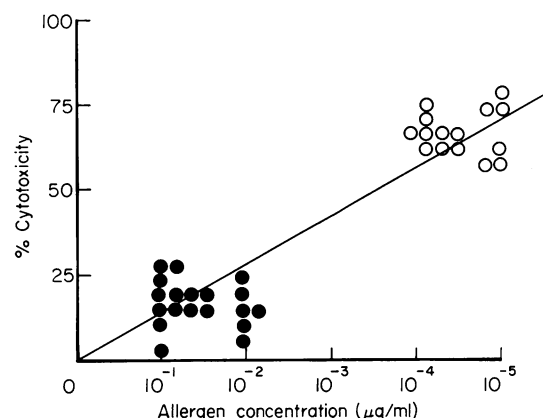


Fig. 2. Allergen specific anti-parasite cytotoxicity of platelets from patients with Hymenoptera hypersensitivity is observed before (O) and after (●) desensitization ($P < 0.001$). The allergen concentration before desensitization corresponds to the lowest concentration inducing at least 50% cytotoxicity, and after desensitization to the concentrations giving the highest cytotoxicity.

Platelet activation tests

Platelet activation tests were performed before and after specific rush desensitization with venoms. In 15 cases, the same patients were studied before and after specific rush desensitization, i.e. after the maintenance dose had been reached for at least 6 weeks. In four additive patients (cases 1–4), tests were only performed *a posteriori* after several months or years of treatment, which demonstrates that fall in platelet activation was persistent after several months or years of desensitization.

The platelet anti-parasite cytotoxicity was dose-dependent (Fig. 1): high levels of cytotoxicity were obtained at 10^{-4} and 10^{-5} $\mu\text{g}/\text{ml}$ of specific allergen before desensitization, while after desensitization, even for high allergen concentration such as 10^{-1} and 10^{-2} $\mu\text{g}/\text{ml}$ cytotoxicity values were constantly low.

The allergen specific antiparasite cytotoxicity significantly decreased after rush desensitization (Fig. 2). The mean percentage of allergen-induced platelet cytotoxicity was $65.9 \pm 2.5\%$ in untreated patients before immunotherapy and $15.3 \pm 1.7\%$ after treatment. In 15 patients, cytotoxicity was studied in parallel before treatment and within 6 weeks after the start of the rush desensitization: the allergen-specific antiparasite cytotoxicity demonstrated a significant reduction (respectively $65.9 \pm 2.5\%$ and $15.1 \pm 1.6\%$ before and after desensitization, $P < 0.001$).

The evaluation of the allergen-induced platelet chemiluminescence showed, similarly, a dramatic reduction in the generation of oxygen-metabolites after rush desensitization (1.82 ± 0.39 mV before treatment and 0.59 ± 0.17 mV after treatment, $P < 0.001$ for $n = 7$). As for platelet anti-parasite cytotoxicity, the allergen-induced platelet chemiluminescence was dose-dependent.

The platelet anti-parasite cytotoxicity was allergen-specific before desensitization. Patients only sensitive to yellow-jacket venom showed no platelet cytotoxicity in presence of honey-bee venom and conversely. In the same way, normal human platelets showed no cytotoxicity in the presence of Hymenoptera venoms at concentrations lower than 10^{-1} $\mu\text{g}/\text{ml}$.

The platelet anti-parasite cytotoxicity was IgE-dependent: when normal human platelets were passively sensitized with sera rich in specific IgE antibodies (obtained from patients before

Table 2. IgE-dependence of platelet cytotoxicity in Hymenoptera venom hypersensitivity*

Normal platelets + Serum from allergic patients	cytotoxicity (%)		Inhibition (%)	P values
	With allergen 10 ⁻⁴ µg/ml	Without allergen		
Native	62.9 ± 2.9	21.2 ± 3.0	—	—
Heated (56°C, 3 h)	29.9 ± 6.9	26.0 ± 5.8	91	<0.05
IgE-depleted	27.6 ± 9	20.3 ± 7.5	84	<0.05

* Normal platelets were passively sensitized with serum from Hymenoptera venom sensitive patients collected before desensitization (mean ± s.e.m. of five experiments).

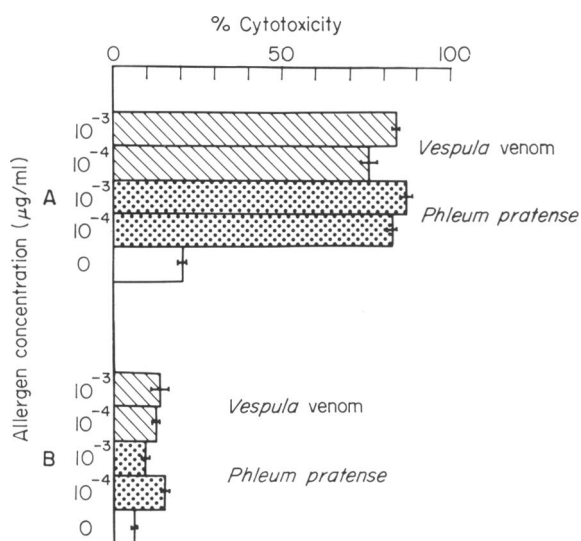


Fig. 3. Evolution of platelet cytotoxicity in one typical patient (n°19) with dual sensitivity. This experiment, performed in duplicate, shows a parallel decrease of platelet cytotoxicity towards *Vesputa* venom and grass pollen (*Phleum pratense*) allergens (A) after exclusive desensitization towards the venom (B).

desensitization), the addition of the specific allergen induced a high level of cytotoxicity. This cytotoxicity was abolished after heating the serum at +56°C for 3 h but not restored after addition of fresh complement (i.e., unheated fresh normal serum). Similarly, after removal of IgE through passage on anti-IgE immunosorbant column, no cytotoxic platelet activity could be elicited (Table 2). (After immunoadsorption, total serum IgE levels were lower than 1.8% of the initial value).

The specificity of the platelet response and particularly its relationship with the allergen was studied: patients 14 and 19 with dual sensitivity to grass pollen and Hymenoptera venom (proved by clinical history, positive skin tests and positive IgE RAST for both allergens) were investigated before and after desensitization conducted for *Vesputa* venom alone. Before desensitization, platelets showed high cytotoxicity in the presence of both allergens; 6 weeks after rush desensitization, platelet cytotoxicity was abolished concurrently towards both Hymenoptera venom and grass pollen allergen (*Phleum pra-*

tense) (Fig. 3). This abolished platelet response was not in relation with cell lysis because no LDH could be detected in the platelet suspension.

Laboratory tests

The mean level of IgG-circulating immune complexes in sera before desensitization was 1.02 ± 0.16 mg/ml (corresponding to a reference curve drawn by several dilutions of heat-aggregated human IgG), and 6 weeks after immunotherapy was 0.97 ± 0.16 mg/ml, at the moment of the depressed platelet activity state. No significant difference was observed ($P = 0.34$ for $n = 9$) (data not shown).

Total seric IgE decreased after rush desensitization with geometric means of 168.9 ± 1.3 vs 251.1 ± 1.4 before treatment.

No significant difference was observed between specific IgE before and after desensitization.

After venom immunotherapy, all, but one, patients demonstrated an increase of specific IgG antibodies. However pretreatment values between patients appeared very different from each other. Comparison of specific IgG levels between pre and post-treatment could not be established in four patients whose desensitization was initiated a long period before the beginning of this study.

DISCUSSION

Recent investigations have allowed the demonstration of a specific receptor for IgE on macrophages (Capron *et al.*, 1975; Joseph *et al.*, 1983), monocytes (Melewick & Spiegelberg, 1980), B and T cells (Spiegelberg, 1981), eosinophils (Capron *et al.*, 1981), and platelets (Joseph *et al.*, 1986) in man. This receptor for IgE differs from the classical receptor on mast cells by a lower affinity and a distinct antigenicity, justifying the individualization of a second class of receptors for IgE (FcεR_{II}) (Capron *et al.*, 1986).

The interaction of specific IgE bound to the platelet membrane with the relevant antigen has been shown to induce a potent and selective activation process. In atopic patients, platelets can be triggered by an IgE-mediated mechanism. It has been suggested therefore that platelets might interfere in immediate type allergic reactions perhaps as secondary cells, triggered at the site of the reaction by mediators released from other inflammatory cells (e.g., platelet-activating factor) (Morley, Page & Sanjar, 1984; Page, Paul & Morley, 1984). However, recent findings also open the possibility that platelets could react

primarily with allergen and initiate a reaction. The activation of platelets by anti-IgE has not yet revealed the release of specific platelet mediators. In our study, platelets were used as marker cells to evaluate desensitization. Three main reasons led us to study the platelet activation in Hymenoptera allergy: first, although the precise role of platelets in immediate hypersensitivity pathogenesis remains to be defined, it was consistent to investigate this cell type in anaphylactic disorders such as Hymenoptera sting hypersensitivity, in which, besides mast cells and basophils, the implication of other circulating blood cells could be logically evoked (Smith *et al.*, 1980). Second, the well-known efficacy of rush desensitization in this model (Hunt *et al.*, 1978) allowed an easy approach of desensitization effects on platelet reactivity. Third, the IgE-mediated Hymenoptera hypersensitivity has been previously recognized (Sobotka *et al.*, 1974).

In our study, all patients lost their allergen-specific platelet reactivity, either after prolonged desensitization or even after some weeks of treatment. In 15 patients studied successively before and after rush desensitization, platelet activation was abolished within 6 weeks, i.e. at 3 or 4 weeks after the last-injection of venom and was kept in the same state several months later.

The results of platelet activation were compared to biological parameters (total serum IgE, specific IgE and IgG antibodies). In our study total serum IgE decreased after rush desensitization, but the most prominent reductions were observed in patients with long-term treatment. Concerning specific IgE levels no significant change could be detected, susceptible to explain the modification in platelet activation. However modulation of the expression of the Fc ϵ R on platelets after desensitization should be studied.

Another hypothesis was the involvement of blocking IgG antibodies (Golden & Valentine 1983). Indeed an increase in specific IgG antibodies was observed after desensitization but some patients with initial relatively high levels of specific IgG showed concurrently a high platelet cytotoxicity and no correlation could be established between absolute amounts of specific IgG antibodies and the percentage of platelet cytotoxicity.

Moreover, the reduced platelet response after desensitization was not allergen specific as shown in patients with dual IgE-mediated sensitivity. This fact might be related to the presence of immune complexes during chronic immunization responsible for this non-specific abolition of the platelet response. However no increase of circulating immune complexes was found in the sera of our patients, 6 weeks after desensitization, as already demonstrated (Clausen & Yanari, 1983).

These observations led to hypothesize the potential role of a lymphokine which may be produced during the desensitization process. Several works have underlined the implication of T lymphocytes in immediate hypersensitivity and particularly in desensitization: Gatien, Merler & Colten (1975) demonstrated a decrease in proliferative T-cell response after specific immunotherapy and Rocklin *et al.* (1980) showed the generation of antigen-specific suppressor T cells during ragweed desensitization. More recently Hsieh (1984) found that hyposensitization depressed the sensitivity to specific allergen of CD4⁺ cells and augmented the proliferative response to allergen of CD8⁺ cells. Interestingly, a platelet activity suppressive lymphokine has recently been described (Pancré *et al.*, 1986). This factor, present in Con-A and antigen-stimulated T-cell supernatants (of man

and rat) was able to inhibit platelet activation. The producing T-lymphocyte subpopulation was identified as CD8⁺. These data are consistent with the hypothesis that the absence of allergen specificity in the response of desensitized platelets may be due to a suppressive lymphokine being produced by antigen-specific T cells. However, this hypothesis needs further investigations, and remains presently speculative.

In conclusion, it appears that modifications in effector cells induced by desensitization are not only restricted to basophils and mast cells. Platelets used here as a marker, seem also to be implicated in desensitization processes. Its exact significance remains to be defined.

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