

Effect of allergen-specific immunotherapy on platelet secretory activity in patients with grass-pollen allergy

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Abstract

Platelet may become activated following antigen challenge to participate then actively in the immune-inflammatory response. Moreover, some evidence proves that specific immunotherapy induces changes in the platelet function. The objective of this study was to determine circulating platelet activity during the early phase of allergen-specific immunotherapy (SIT) in patients with grass pollen-sensitive allergic rhinitis. Twelve grass-pollen allergic patients (seven men and five women) with intermittent allergic rhinitis were treated with specific subcutaneous allergoid preparation. SIT was received by six weekly injections, the vaccine dose increasing until the maintenance level was reached. Blood was sampled at four different time points: before and directly before SIT, 30 min and 24 h after the maximum dose injection of the vaccine. Plasma level of beta-thromboglobulin (beta-TG), marker of platelet activation *in vivo* was measured using ELISA method. Baseline beta-TG level did not differ significantly among the patients and healthy subjects. Moreover, no significant differences were observed in the degree of platelet activity between the different times of this study in the patients group. We failed to detect any significant changes in circulating platelet activity, the measure of plasma level of beta-TG, in patients with grass-pollen induced intermittent rhinitis during the course of the dose increase phase of grass pollen SIT. In particular, it seems that both early (after 30 min) and late (after 24 h) changes in plasma level of this marker do not occur following the maximum dose administration of the allergen vaccine during the early SIT phase.

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1. Introduction

Platelet activation induced during allergic processes could depend not only on the secondary effect of inflammatory cells and mediators, but also on the specific effect of allergens upon platelet. It has been demonstrated that both, low affinity receptor [1,2] and high affinity receptor for the Fc fragment of IgE [3,4], are expressed on human platelet and may thus be involved in the antigen-specific platelet activation. Experimental exposure to aeroallergens by inhalation may contribute to enhanced release of platelet-derived CXC-chemokines into the blood stream of patients suffering from

pollen allergy [5]. CXC-chemokines, such as platelet factor 4 (PF-4) and beta-thromboglobulin (beta-TG) modulate inflammatory events [6,7] and are useful to reveal *in vivo* platelet activation [8].

Subcutaneous allergen-specific immunotherapy (SIT) is a causative treatment of IgE-mediated allergic diseases and affects different systemic aspects of allergic inflammation [9]. Only a few studies have investigated the influence of a specific immunotherapy on platelet function in allergic patients. Decreased IgE-dependent platelet activation following specific rush desensitisation has been observed in patients with *Hymenoptera* hypersensitivity [10].

We demonstrated earlier some significant reduction in platelet aggregation response showed by house dust mite-sensitive patients with persistent allergic rhinitis, which

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improved partially following conventional mite SIT [11]. Taken together, it seems that allergen exposure following subcutaneous injection may affect platelet activity.

The present study went further on to examine the platelet behaviour in SIT course and to contribute more information on the mechanism underlying the therapy as well as platelet function in atopic allergy. Platelet activation, the measure of plasma level of beta-TG, has been investigated in patients sensitized to grass pollens with intermittent allergic rhinitis during the dose increase phase of conventional SIT. We focused especially on both, early (after 30 min) and late (after 24 h) effect of maximum vaccine dose on platelet activity.

2. Material and methods

2.1. Subjects

Twelve monoallergic grass pollen-sensitive patients (five women and seven men; the median age 21 years, range 18–26) with intermittent rhinitis, free of asthma symptoms, were included into the study. All subjects had shown severe nasal symptoms throughout at least two consecutive grass-pollen seasons. None of the subjects suffered from concomitant allergy and none had undergone any SIT treatment prior to the study. They proved positive skin-prick tests to mixed grass pollens as well as negative response to all other common aeroallergens. SIT was performed using Allergovit® (grass pollen allergoid preparation by Allergopharma, Reinbek, Germany). SIT was administered to all patients pursuant to standard administration schedule. A series of once-a-week subcutaneous injections of the increasing concentration of Allergovit® were performed for 6 weeks until the maintenance dose was reached. SIT was accomplished at least 1 month before the expected natural exposure to allergens. We did not observe any episode of systemic side events during the SIT course.

The control group consisted of 22 healthy volunteers showing no signs of atopy, 10 females and 12 males (the median age was 23 years; range 18–27).

Individuals were not enrolled for the study if their personal history had indicated any disorders to affect platelet function. None of the patients or control subjects were smokers nor received any drug known to affect platelet activity throughout the preceding 4 weeks.

The Ethics Committee of the Medical University of Silesia approved of the study and written, informed consent was obtained from all the subjects participating.

2.2. Blood sampling for platelet analysis

Peripheral venous blood was sampled four times during the dose increase phase of pollen SIT: (a) directly before the initial dose of SIT (T_0); (b) directly before the highest dose injection (T_1); and (c) 30 min and 24 h after the maximum

dose (T_2 and T_3 , respectively) was reached. A single blood sample was taken from controls to compare the baseline level of beta-TG between the patients and controls.

Blood samples were obtained in the morning (7.00–8.00 a.m.; in the fasting state) after rest in the supine position, at slight or no stasis from the antecubital vein into Diatube H tubes placed then immediately on ice. The tubes were then centrifuged at $2500 \times g$ for 30 min at 4°C . Plasma was then collected and stored at -60°C until the assay time (no longer than 1 month).

2.3. Beta-TG measurement

Plasma level of beta-TG was measured by the enzyme-linked immunosorbent assay (ELISA), using commercial Asserachrom® kit by Diagnostica Stago, Paris, France, following the manufacturers' instructions.

2.4. Skin-prick tests

Skin-prick tests were performed using a panel of common inhalant allergens (Allergopharma, Reinbek, Germany). Saline and histamine solutions were used as negative and positive controls, respectively. Results were read out after 15 min. The reaction was considered positive if the wheal diameter was 3 mm or larger.

2.5. Immunotherapy protocol

The treatment followed the manufacturers' instructions. Generally, SIT comprised subcutaneous injections of grass pollen allergoid preparation for the period of 6 weeks, starting with 0.1 ml to increase at weekly intervals up to 0.8 ml of Vial A, 1000 TU/ml. The patients were then receiving the up dosing injections, also once-a-week, until the maximum dose was reached (0.6 ml of Vial B, 10,000 TU/ml). According to information supplied by Allergopharma, about 30 µg of the major allergen (Phl p 5) is delivered along with the maximum dose of the vaccine. The early SIT phase was accomplished at least 1 month before the expected natural exposure to allergens.

2.6. Statistical analysis

The Mann–Whitney *U*-test and the Student's *t*-test were performed to compare results between the patients and the controls. Sample data obtained at four different time points of the study were compared using non-parametric Friedman repeated measure analysis of variance. *P*-values below 0.05 were considered significant.

3. Results

The baseline beta-TG level was not significantly different among the patients and healthy subjects. Moreover, in

Table 1

The values of plasma level of beta-TG in control subjects and in the intermittent allergic rhinitis patients, receiving SIT, obtained during consecutive measurements

Plasma beta-TG level (IU/ml)	T_0 ; mean $\pm$ S.D.; median range	T_1 ; mean $\pm$ S.D.; median range	T_2 ; mean $\pm$ S.D.; median range	T_3 ; mean $\pm$ S.D.; median range
Patients ($n = 12$)	24.4 $\pm$ 8.14; 24.3; 12.5–36.0	20.4 $\pm$ 6.99; 19.0; 10.0–32.5	26.7 $\pm$ 11.7; 22.2; 14.5–55.5	22.7 $\pm$ 9.77; 21.4; 8.7–39.4
Controls ($n = 22$)	21.9 $\pm$ 7.24; 21.8; 8.00–35.1			

allergic rhinitis patients treated with the allergen vaccine, plasma level of beta-TG did not differ significantly between different times of the study ($T_0 - T_3$) (Table 1). Only a single case showed plasma level of beta-TG greater than the upper limit of the normal range, 30 min after the vaccine administration.

Also, the baseline platelet number did not differ significantly between the patients and the healthy subjects. No statistically significant differences in platelet count in intermittent allergic rhinitis patients, receiving SIT, were observed between different times of the study (data not included).

4. Discussion

To our knowledge, this has been the first study to evaluate the platelet release reaction assessed by circulating levels of platelet-derived CXC-chemokines throughout the SIT course in patients with atopic allergy. As mentioned above, we had earlier demonstrated changes in platelet function, as measured by platelet aggregation in patients with house dust mite-sensitive persistent allergic rhinitis following mite SIT. However, it has not been recognised if such therapy affected the platelet function directly or indirectly [11]. In two polysensitized cases, following specific *Hymenoptera* desensitization, platelet not only lost its reactivity to venom but also towards other allergens, therefore non-specific effect of SIT could have occurred [10].

Previously, we did not observe any significant differences in plasma levels of PF-4 and beta-TG in patients with IgE-mediated allergic rhinitis undergoing continuous and periodic natural exposure to sensitizing aeroallergens, as compared to healthy non-atopic subjects [12,13]. This is consistent with the present results, demonstrating that the degree of circulating platelet activity (expressed by beta-TG) in pollen-sensitive allergic rhinitis patients, prior to SIT was not significantly different than that of the healthy subjects. Moreover, we were unable to prove any significant changes in circulating level of this chemokine after the dose increase phase of pollen SIT in such patients. As suggested by Kaplan and Owen [8], normal plasma levels of beta-TG and PF-4 in different clinical situations prove well enough that increased platelet activation does not occur in vivo. Therefore, it seems likely that subcutaneous administration of increasing doses of the allergen extracts, during early SIT phase does not trigger circulating platelet hyperactivity, manifested by enhanced plasma level of this chemokine in allergic rhinitis patients, especially after

30 min and overnight the maximum dose administration of the vaccine.

Our study was, however, limited in terms of methodological details that should have been considered. Firstly, it was merely an observational case, comprising a small number of patients, therefore probably not powerful enough to detect minor differences in chemokine plasma level. Secondly, we cannot rule out the possibility of platelet activation during SIT, yet the study protocol and the method used to assess platelet function were insufficient to detect such platelet hyperactivity. Plasma beta-TG was determined only four-fold, both before the initial dose and before the maximum dose administration, and twice after the highest dose injection. For ethical reasons we were not able to examine more precisely the dynamics of platelet activity, using serial measurements of platelet markers. Moreover, some factors conditioning and controlling platelet behaviour should have been taken into consideration. For instance, circulating platelet might develop a state of at least partial unresponsiveness to repeated allergen administration during the course of allergen immunotherapy, due to desensitization of mechanism(s) regulating the platelet release reaction. Such explanation may be supported by the experimental data suggesting that besides its important role in platelet activation and degranulation, the platelet activating factor could induce specific desensitization of platelet activation mechanism in order to limit platelet secretory activity [14].

It is worth-mentioning that the exact mechanism underlying SIT has not been completely understood and the effect of such therapy seems to differ significantly depending on the nature of allergen used, the treatment strategy, the allergen dose, and/or the route of immunisation [9]. In addition, it is known that the type of allergoid preparation used in our study reduced potential systemic reactions, as compared to conventional allergen extracts, preserving however the immunogenicity. For these reasons, it is tempting to speculate that during SIT performed with a rush or ultra-rush regime or with no chemically modified allergens a patient would be exposed an allergen stimulus strong enough to induce systemic platelet degranulation. Further studies should be performed to confirm or exclude such possibility. In addition, our study did not show any episode of systemic side-effect following subcutaneous injection of allergen extracts. It would be interesting to examine whether platelet function is altered upon systemic reactions following SIT, as it has been suggested that platelets play an important role in anaphylaxis (for review [15]).

5. Conclusion

Platelet activity, the measure of plasma level of beta-TG, did not change significantly in patients with grass pollen-sensitive intermittent allergic rhinitis during the dose increase phase of conventional pollen SIT. It seems that both early (after 30 min) and late (after 24 h) significant alterations in the chemokine level do not occur following the maximum dose administration of the allergen vaccine at SIT early phase. However, other methods, such as flow cytometric detection of activated platelet as well as other study protocols may prove more accurate to determine the presence of platelet activation in systemic circulation.

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