

Markers of platelet activation in plasma of patients suffering from persistent allergic rhinitis with or without asthma symptoms

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Summary

Background Increased circulating levels of platelet release products are detected in various types of inflammation. It has been demonstrated that allergen challenge promotes platelet activation and leads to the release of chemokines such as platelet factor 4 (PF-4) and β -thromboglobulin (β -TG).

Objective The aim of this study was to determine whether or not circulating platelets get into an activated state during allergic inflammatory reactions induced by long-term natural exposure to allergens.

Methods Plasma levels of PF-4 and β -TG (established markers of *in vivo* platelet activation) were determined by ELISA method in symptomatic patients with allergy to house dust mites, suffering from persistent allergic rhinitis (PAR) in the absence of asthma symptoms (12 men and 8 women; mean age 22 years) and from PAR with mild asthma (10 men and 6 women; mean age 23 years), as well as in healthy controls (10 men and 10 women; mean age 23 years).

Results No significant differences were found between PAR patients with or without symptoms of asthma and healthy non-atopic subjects with respect to plasma levels of PF-4 and β -TG as well as platelet count.

Conclusions It seems that patients undergoing continuous natural exposure to sensitizing allergens, with subsequent PAR alone or with concomitant mild asthma, have no altered platelet activity *in vivo*, as reflected by plasma levels of the chemokines. These findings, in conjunction with earlier data, indicate that differences may exist in platelet activity, including releasability of platelet products between patients with distinct clinical manifestation of atopy.

Keywords asthma, atopy, β -thromboglobulin, house dust mite, platelet activation, platelet factor 4, rhinitis

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Introduction

Atopic diseases like asthma and rhinitis are inflammatory disorders characterized by the mucosal changes at the site of allergic processes. Moreover, allergic reactions trigger a systemic increase of inflammatory activity [1, 2]. As a variety of cells are activated in the systemic circulation of patients with allergic diseases, it is interesting to show whether circulating platelets are activated in distinct clinical manifestation of atopy. Platelets may be important in the pathogenesis of allergic asthma [3, 4] and atopic eczema/dermatitis syndrome (AEDS) [5]. The degree of systemic involvement of platelets in allergic events may be evaluated by measuring plasma levels of specific proteins released upon such cell activation. Platelet activation may occur during human allergic reactions, both locally and systemically, possibly as a result of the *direct* IgE-dependent process and as a

secondary event caused by other inflammatory or immune stimuli [3, 6]. It has been demonstrated that both the low affinity receptor (Fc ϵ RII) [7, 8] and the high affinity receptor (Fc ϵ RI) for IgE [9, 10] are expressed on platelet membrane. Activated platelets are the most abundant source of platelet factor 4 (PF-4) and β -thromboglobulin (β -TG), which are good markers of platelet activation *in vivo* [11], and play an important role in chemotaxis of inflammatory cells [12, 13]. Earlier studies have indicated that enhanced platelet degranulation may occur in the systemic circulation of patients suffering from chronic allergic inflammatory diseases, such as asthma [14, 15] and AEDS [16]. In contrast, periodic naturally occurring exposure to aeroallergens seems not to be accompanied by increased circulating platelet activity in grass-pollen-allergic patients with intermittent rhinitis [17].

Given the fact that circulating platelets are activated in various inflammatory conditions and that allergen challenge may provoke platelet activation, we aimed to determine whether patients with house dust mite (HDM) allergy manifested as persistent allergic rhinitis (PAR) only and persistent rhinitis with concomitant mild asthma may prove enhanced platelet activity assessed by plasma levels of PF-4 and β -TG.

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Materials and methods

Subjects

36 non-smoking patients with PAR sensitized to HDM were enrolled in the study, 16 of whom also had mild asthma. They were recruited from the Outpatient Department of Allergology. Patients were divided into two groups: patients with HDM-induced PAR in the absence of asthma symptoms (PAR group, $n = 20$) and patients suffering from PAR with mild bronchial asthma symptoms because of HDM allergy (PAR with asthma group, $n = 16$). They had positive skin tests to HDM (*Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*) extracts and positive serology (specific IgE = class 3 or higher) as well as negative response to all other common aeroallergens.

Of the 20 patients with moderate-to-severe symptoms of PAR without any history of asthmatic symptoms, 12 were males and 8 females, and the mean age was 22 ± 4.5 years (range: 18–35 years). The mean time since the PAR diagnosis had been established was 5 years (range: from 2 to 10 years). Topical nasal medications (steroids and antihistamines) were stopped at least 2 weeks prior to the study. The clinical diagnosis of allergic rhinitis (AR) was performed according to criteria reported in the ARIA document [1]. The mean value of total IgE was 127 ± 91 kU/L.

Of the 16 patients with PAR and concomitant mild asthma, 10 were males and 6 females, and the mean age was 23 ± 4 years (age range: 18–31 years). All patients showed forced expiratory volume in 1 s (FEV₁) of 80% or more of the predicted value. Mild asthma was diagnosed according to the Global Initiative for Asthma (GINA) criteria [18]. Mean duration of asthma symptoms was 6 years (range: from 2 to 10 years). Topical medications for nasal (steroids and antihistamines) and bronchial (steroids) use were stopped at least 2 weeks before the study. When required, only short-acting β_2 -mimetics were allowed for symptom relief. The mean value of total IgE was 223 ± 173 kU/L.

None of the examined subjects had taken oral antihistamines or leukotriene antagonists within 1 month and oral corticosteroids within 8 weeks before the study. None of the patients had either received immunotherapy, had diseases which would affect platelet activity such as obesity, hypertension, diabetes or thrombotic disorders, were receiving drugs that affect platelet function, or had suffered from respiratory tract infections throughout the preceding 2 months.

The control group consisted of 20 healthy non-atopic volunteers, 10 males and 10 females, and the mean age was 23 ± 4 years (range: 18–32 years). All the subjects were non-smokers and proved normal by physical examination.

The study was approved by the Ethics Committee. All participants gave informed written consent before entering into the study.

Blood sampling; PF-4 and β -TG measurement

Blood samples were obtained in the morning (07.00 a.m. to 08.00 hours; in the fasting state) after 25-min rest at slight or no stasis from the antecubital vein into Diatube[®] H tubes (Becton Dickinson, Oxford, UK) and then immediately placed in ice/water bath for 20 min. The tubes were then centrifuged at 2500 g for 30 min at 4 °C, and platelet poor

plasma fractions were collected and frozen at -60 °C until assay (not longer than 1 month). Plasma levels of PF-4 and β -TG were measured by ELISA, using the commercial Asserachrom[®] kit by Diagnostica Stago (Paris, France).

Skin prick tests

Skin prick testing was performed using a panel of common inhalant allergens (grass, weed and tree pollens, cat and dog dander, moulds, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) (Allergopharma, Reinbek, Germany). Saline and histamine solutions were used as the negative and positive controls, respectively. Results were read out after 15 min. A weal with a diameter 3 mm greater than the negative control was taken as a positive response.

Other laboratory investigations

Serum total IgE and specific IgE to *D. farinae* and *D. pteronyssinus* were measured by ELISA using a commercial kit (Allergopharma, Reinbek, Germany) according to the manufacturer's instructions. Peripheral blood platelets were calculated by a direct method.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Parameters were tested for normal distribution using the Shapiro–Wilks test. Analysis of variance (ANOVA) was used for screening differences between the three groups. Correlation coefficient was obtained by Spearman's test. *P*-values less than 0.05 were considered significant.

Results

The plasma levels of PF-4 and β -TG in the three groups are expressed as IU/mL and are shown in Table 1. No significant differences were found between PAR patients with or without symptoms of asthma and healthy non-atopic subjects in plasma levels of PF-4 and β -TG, as well as platelet count (Table 1). Moreover, there were no significant differences among males and females with respect to the degree of platelet activity in the groups.

In the PAR group and in the PAR with asthma group, PF-4 or β -TG levels were not significantly correlated with the duration of the diseases ($r = 0.13$, $P = 0.59$ or $r = 0.18$, $P = 0.45$, and $r = 0.32$, $P = 0.22$ or $r = 0.31$, $P = 0.23$, respectively).

Table 1. Levels of PF-4 and β -TG, and platelet count in the blood of the control, PAR and PAR with mild asthma groups

Analysed parameters (unit)	Means $\pm$ SD		
	Control ($n = 20$)	PAR ($n = 20$)	PAR with asthma ($n = 16$)
PF-4 (IU/mL)	5.12 ± 1.78	4.50 ± 2.05	4.39 ± 1.65
β -TG (IU/mL)	27.5 ± 10.2	25.7 ± 9.92	29.8 ± 8.62
Blood platelets ($\times 10^9/L$)	244 ± 45.8	247 ± 51.0	239 ± 58.1

n, number of subjects; PAR, persistent allergic rhinitis; PF-4, platelet factor 4; β -TG; β -thromboglobulin.

Discussion

Altered platelet function has been described in individuals with atopic allergy. Platelet activation as measured by platelet aggregation has been found to be diminished in patients with respiratory atopy [19–21], but not in AEDS patients [22].

It is known that allergen challenge may induce platelet release reaction in patients with IgE-mediated allergic diseases. Knauer et al. [23] have demonstrated significant changes in PF-4 level in the circulation of asthmatic patients after bronchial provocation to ragweed extract, and this was inversely correlated to a decline in FEV₁, and they state that the mean pre-challenge PF-4 level in these patients was similar to the control group. PF-4 and β -TG were also reported to significantly increase in bronchoalveolar lavage fluids of ragweed-allergic asthmatics undergoing antigen challenge; no difference was observed at baseline measurements of the chemokines before antigen bronchoprovocation in these patients [24]. Based on *in vitro* studies, it has been suggested that allergen-specific and IgE-mediated platelet activation may occur in patients sensitized to *D. pteronyssinus* [25].

In the present study we investigated circulating platelet activity in patients with chronic allergic inflammation because of allergy to HDMs. Patients suffering from HDM-sensitive PAR with or without mild asthma as measured by lower airway symptoms did not differ significantly from the normal controls, with respect to the platelet count or levels of PF-4 and β -TG in the peripheral venous blood. This is in keeping with our previous study performed during the grass-pollen season, in which plasma levels of the chemokines in grass-pollen-allergic patients with intermittent rhinitis alone did not differ significantly in comparison to non-atopic healthy subjects [17].

Luzzatto et al. [26] failed to find a significant difference in the level of circulating PF-4 and platelet count between symptomatic patients with asthma and normal controls. Similarly, Moritani et al. [27] did not observe platelet activation in stable asthma using flow cytometric analysis of the resting expression of CD62P. However, it seems that platelets in asthmatics are easily activated upon stimulation [27]. Others have shown that enhanced platelet activation may occur in the circulation of asthmatic patients, during the stable period of the disease, as well as spontaneous asthma exacerbation [14, 15, 28]. In addition, platelet release reaction has been shown to accompany nocturnal asthma and was correlated to maximum reduction in peak expiratory flow, and to maximal increase in bronchial hyperactivity [29]. Yamamoto et al. [15] observed elevated plasma PF-4 and β -TG levels in some symptomatic as well as asymptomatic patients with chronic asthma. Interestingly, in five out of six asthmatics with strongly positive IgE antibody against HDMs, plasma levels of the chemokines did not change during dust mite extracts challenge and remained within normal range [15].

As mentioned, our previous results suggested that increased platelet activity *in vivo* may occur in some AEDS subjects [16]. Taken together, these data lead to some interesting considerations regarding the differential activity of circulating platelets in asthmatic and AEDS patients vs. patients with AR. So far, evidence is lacking to prove that platelet activation manifested by increased plasma levels of PF-4

and β -TG occurs in the case of all allergic disorders developed on genetic background, the so-called atopic diathesis, regardless of their clinical expression. Similarities should be noted between various atopic diseases such as asthma, rhinitis, conjunctivitis, or AEDS, but there are also differences; thus, profiles of inflammatory mediators and cells activity may appear different. Consistent with these data, it seems likely that differences exist in platelet function, including releasability of platelet products between patients with various clinical manifestation of atopy.

In conclusion, this study shows the lack of significant differences in plasma levels of PF-4 and β -TG between HDM-allergic patients and healthy non-atopic subjects. Therefore, it seems that persistent natural exposure to a sensitizing allergen with subsequent AR without any asthma history or with mild lower airway symptoms only is not accompanied by an increased systemic platelet activity as expressed by plasma levels of the markers. Moreover, on the basis of the present study as well as the earlier ones, it appears likely that platelet activity varies among atopic patients. Analysis of circulating PF-4 and β -TG levels indicates that intravascular platelet activation occurs in some cases of atopic allergy, but not in patients with AR. Potential limitations of our study are the small number of subjects evaluated and the method used to detect platelet activation. Assessment of plasma levels of the platelet release products may appear insufficiently sensitive due to dilution effect in plasma. Thus, further studies are necessary to clarify more accurately a functional state of platelets in atopic patients.

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