Enhanced release of platelet factor 4 into the circulation in patients with atopic eczema/dermatitis syndrome

Kasperska-Zając A, Rogala B, Nowakowski M

Chair and Clinical Department of Internal Diseases, Allergology and Clinical Immunology,
Medical University of Silesia, Poland

Abstract

Purpose: The active participation of platelet in IgE-mediated inflammatory response is well documented. Platelet factor 4 (PF4), a platelet-specific protein may play an important role in the development of the atopic eczema/dermatitis syndrome (AEDS).

Objective: The aim of the study was to evaluate the activation state of circulating platelets in patients suffering from AEDS.

Material and methods: In vivo platelet activity was assessed by measuring plasma level of PF4 (enzyme-linked immunoassay method) in 9 males AEDS patients and 11 healthy, nonatopic subjects.

Results: Plasma PF4 was significantly increased ($p < 0.05$) in AEDS (31.88 ± 20.48 IU/ml) patients compared with control subjects (2.95 ± 0.6 IU/ml).

Conclusions: This result suggests that patients with AEDS may have increased in vivo platelet activation expressed by PF4 release. The aim of the study was to evaluate the activation state of circulating platelets in patients suffering from AEDS.

Key words: atopic dermatitis, platelet activation, platelet factor 4.

Introduction

Platelets play an important role in haemostasis and are actively involved in the inflammatory response. Moreover, altered platelet function has been reported in different forms of allergy [1]. There is evidence that platelet participates in the pathogenic mechanism of allergic diseases, including AEDS [2,3].

Platelet factor 4 (PF4) is regarded as a platelet-specific protein secreted when platelet is activated and belongs to the C-X-C chemokine family. Measurements of plasma levels of PF4 have been shown to be marker of platelet degranulation in vivo, can be used to detect activation of the circulating pool of platelets [4]. PF 4 is shown to be an inflammatory response mediator. In particular, by the fact that it can attract inflammatory cells to sites of inflammation [5,6]. Recently, by using an experimental model of AEDS, it has been demonstrated that PF4 may play an important role in the pathophysiology of this disease [7]. Taken together, we investigated whether patients with moderate AEDS exhibit increased platelet activity, assessed by circulating level of PF4. Moreover, AEDS belongs to the atopic triad and is characterised by high serum IgE levels. So we also analysed relationships between platelet activity and total IgE serum levels.

Material and methods

The investigation was carried out on non-smoking patients, treated at the outpatient department of Chair of Internal Disease, Allergology and Clinical Immunology, Zabrze.

The AEDS group consisted of 9 patients (9 males), the median age was 28 years (range: 25-30 years) and the median duration of recognized AEDS was 25 years (range: 18-26 years). The diagnosis of AEDS had been established by the criteria of Hanifin and Rajka [8]. All the patients had an elevated serum IgE levels (>500 kU/l) and positive skin prick tests to three or more different common airborne allergens. The disease activity was measured by assessing erythema, excoriation, cracking, dryness and lichenification. Only patients with moderate AEDS were included in the study. None of the patients suffered from concomitant inhalatory allergy, non had treatment with topical steroid or oral medications for 2-weeks before this study.

ADDRESS FOR CORRESPONDENCE:
Rogala B, MD PhD,
Chair and Clinical Department of Internal Diseases,
Allergology and Clinical Immunology,
Medical University of Silesia, ul. 3-go Maja 13-15,
41-800 Zabrze, Poland,
e-mail: b_rogala@pro.onet.pl

Received 30.04.2003 Accepted 14.07.2003

Table 1. Plasma level of PF4 in patients with AEDS and healthy controls. Values presented as mean ± SD and median.

Analysed parameters	Healthy controls (n=11)	AEDS patients (n=9)	Statistical analysis
PF4 (IU/ml) mean ± SD median	2.95±0.6 2.5	31.88±20.48 32.5	p=0.00017

Control group consisted of 11 healthy non-smoking subjects with no signs of atopy who were matched for age and sex with the analysed group. They were instructed to take no medications (to avoid affecting platelet activation) for at least 10 days before the study.

An informed consent was obtained from the control subjects and patients. Permission of the University Ethics Committee was also obtained.

Blood sampling, measurement of PF4

The blood was obtained in the morning (7.00 to 8.00) after 25-minute rest with slight or no stasis from the antecubital vein into Diatube H tubes (Becton Dickinson) which were immediately placed in the ice/water bath for 20 minutes. Then the tube were centrifuged at 2500 g for 30 minutes at 4°C, and top third the volume of the resultant plasma supernatant were collected and frozen at -20°C for assay (not longer than 1 month).

PF4 was assayed by enzyme-linked immunosorbent assay (ELISA) kit (Diagnostica Stago, France) according to manufacturer's instructions. Samples were assayed in duplicates. PF4 levels were expressed in IU/ml. Precision of the assay was ±0.7 UI/ml in replicate determinations.

Details of the methods as well as references are given in the leaflets included in the sets.

Skin prick tests

Skin prick tests for common airborne allergens (Nexter-Allergopharma, Germany) were performed. A positive immediate skin response: wheal diameter of >3 mm and greater than or equal to that of the histamine control were selected for this study.

Other investigations

Serum total IgE were determined by ELISA kit according to the manufacturer's instructions (Nexter-Allergopharma, Berlin).

Total platelet counts were calculated by a direct method.

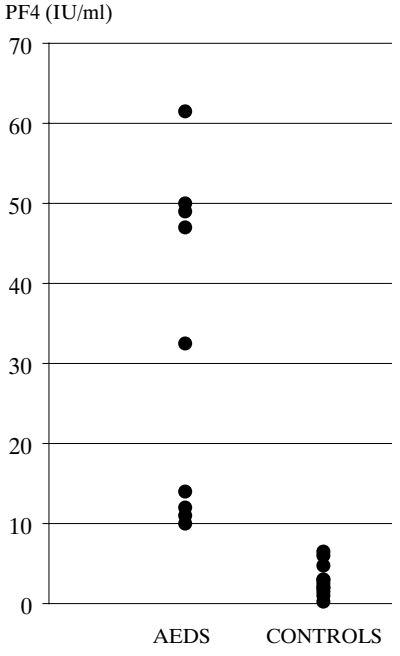
Statistical analysis

Data were compared between groups by Mann-Whitney's unpaired rank sum test. Correlation analyses were performed by Spearman's rank method. The results was presented as mean ±SD and median. P values less than 0.05 were considered significant.

Results

Plasma PF4 was significantly increased ($p < 0.05$) in the AEDS compared with the control group (*Tab. 1*). *Fig. 1* shows individual values of PF4 in patients with AEDS and healthy subjects.

Figure 1. Individual plasma levels of PF4 in patients with AEDS in comparison to the group of healthy individuals.



There were no significant differences in platelet number between the two examined groups (data not shown).

There was no correlation between plasma PF4 and IgE levels ($r = 0.083$, $n = 9$, $P > 0.05$).

As we failed to observe any changes in platelet number in persons who suffered from AEDS, we found it groundless to analyse relationship between the normal platelet counts and total IgE.

Discussion

Blood platelets can be activated by immune and non-immune stimuli. It has been suggested that in allergic disorders, IgE-dependent activation of platelet expressed by the release of mediators can be specifically triggered by the corresponding allergens [9]. The importance of circulating platelets and their mediators in atopic dermatitis is complex and still not completely understood [2,3]. In our previous study, platelet activation as measured by platelet aggregation in response to some aggregating agents in atopic dermatitis patients were not impaired [10]. As mentioned earlier, platelet activity can be

also determined by measurement of plasma levels of platelet-specific proteins, including PF4 [4]. Chemokines related to the beta subfamily, the so-called PF4 superfamily have been shown to modulate allergic inflammatory process and are considered to be important mediators of inflammation [5,6,11,12].

The results of our study revealed that PF4 plasma level was statistically significantly higher in patient with AD than in control group. These data may support the hypothesis of the occurrence of platelet activation in patients with AD. PF4 is a multifunctional protein specific to platelets, which may be involved in the recruitment and activation of inflammatory cells, including eosinophils and thereby contribute to the pathogenesis of skin inflammation associated with AD. It has been previously shown in a mouse model of AEDS that increased expression of PF4 may play an important role in the development of this disease [7]. Bruijnzeel et al. reported that eosinophils from the circulation of patients with atopic dermatitis exhibit potentiated migratory responses toward PF4 compared with eosinophils from normal donors [6]. Thus, the release of PF4 might lead to amplify inflammatory processes associated with atopic dermatitis and contribute to tissue injury.

Also, in other disorders platelets are sometimes present in an activated state in the circulation. There was evidence that platelet activation expressed by in vivo release of PF4 occurred during IgE-mediated airway reactions after allergen challenge in patients with allergic asthma [13]. Enhanced plasma level of PF4 in mast cell-dependent disorders, such as idiopathic cold urticaria has been described [14]. On the other hand, increased in vivo platelet activation has not been found in seasonal allergic rhinitis during natural pollen exposure [15]. The clinical significance of abnormal platelet activity in IgE-mediated human disease is not definitely established.

Conclusions

It seems that individuals with atopic dermatitis may have increased in vivo platelet activity, assessed by measuring plasma PF4. Platelet activity should be investigated further in larger groups of AEDS patients. Moreover, further studies are needed to assess whether this enhanced platelet activity correlates with severity of AEDS.

Acknowledgements

This study was supported by a research grant (NN-2-078/01) from Medical University of Silesia.

References

1. Ameisen JC, Joseph H, Tonnel AB, Capron A. A role for platelets in allergy and asthma. In: Holme G, Morley J, editors. PAF in asthma. London: Academic Press; 1989, p. 169-81.

2. Hilger RA, Neuber K, Konig W. Conversion of leukotriene A4 by neutrophils and platelets from patients with atopic dermatitis. Immunology, 1991; 74: 689-95.

3. Neuber K, Hilger RA, Konig W. Differential increase in 12-HETE release and CD29/CD49f expression of platelets from normal donors and from patients with atopic dermatitis. Int Arch Allergy Immunol, 1992; 98: 339-42.

4. Kaplan KL, Owen J. Plasma levels of β-thromboglobulin and platelet factor 4 as indices of platelet activation in vivo. Blood, 1981; 57: 199-202.

5. Burgers JA, Schweizer RC, Koenderman L, Bruijnzeel PL, Akkerman JW. Human platelets secrete chemotactic activity for eosinophils. Blood, 1993; 81: 49-55.

6. Bruijnzeel PLB, Kuijper PHM, Rihs S, Betz S, Warringa RAJ, Koenderman L. Eosinophil migration in atopic dermatitis. In: Increased migratory responses to N-formyl-methionyl-leucyl-phenylalanine, neutrophil-activating factor, platelet activating factor, and platelet factor 4. J Invest Dermatol, 1993; 100: 137-42.

7. Watanabe O, Natori K, Tamari M, Shiomoto Y, Kubo S, Nakamura Y. Significantly elevated expression of PF4 (platelet factor 4) and eotaxin in the NOA mouse, a model for atopic dermatitis. J Hum Genet, 1999; 44: 173-6.

8. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh.), 1980; 92 (Suppl): 44-7.

9. Capron A, Joseph M, Ameisen JC, Capron M, Pancre V, Auriault C. Platelets as effectors in immune and hypersensitivity reactions. Int Arch Allergy Appl Immunol, 1987; 82: 307-312.

10. Rogala B, Gumprecht J, Glück J. Platelet aggregation in atopic dermatitis. Platelets, 1999; 10: 341-4.

11. Seguchi M, Nakajimas S. Induction of ICAM-1 expression on the eosinophilic leukemia cell line-3 (Eol-3) by platelet-activating factor and platelet factor 4. Ann Allergy Asthma Immunol, 1995; 74: 255-7.

12. Hayashi N, Chihara J, Kobayashi Y, Kakazu T, Kurachi D, Yamamoto T, Nakajima S. Effect of platelet-activating factor and platelet factor 4 on eosinophil adhesion. Int Arch Allergy Immunol, 1994; 104 (Suppl): 57-9.

13. De Sousa JR, Santos MC, Carlos ML, Carlos AG. Platelet reactivity to 'in vitro' allergen challenge in asthmatic patients. Allergol Immunopathol, 1992; 20: 13-16.

14. Wasserman SI, Ginsberg MH. Release of platelet factor 4 into the blood after cold challenge of patients with cold urticaria. J Allergy Clin Immunol, 1984; 74: 275-9.

15. Kasperska-Zając A, Rogala B. Platelet activity measured by plasma levels of beta-thromboglobulin and platelet factor 4 in seasonal allergic rhinitis during natural pollen exposure. Inflamm Res, 2003; (accepted).