Elevated Platelet Activation in Patients with Atopic Dermatitis and Psoriasis: Increased Plasma Levels of \( \beta \)-Thromboglobulin and Platelet Factor 4

Risa Tamagawa-Mineoka¹, Norito Katoh¹, Eiichiro Ueda¹, Koji Masuda¹ and Saburo Kishimoto¹

ABSTRACT

Background: Beyond their role in hemostasis and thrombosis, platelets are important for modulating inflammatory reactions. Activated platelets play a role in the pathomechanism of inflammatory diseases such as asthma, but little is known about platelet activation in chronic skin inflammation, including atopic dermatitis (AD) and psoriasis. Furthermore, the relationship between platelet activation and disease severity is not understood. This work was performed to investigate plasma levels of \( \beta \)-thromboglobulin (\( \beta \)-TG) and platelet factor 4 (PF4) as platelet activation markers in patients with AD or psoriasis, and to determine the relationships between these markers and disease severity.

Methods: Plasma levels of \( \beta \)-TG and PF4 were measured by enzyme-linked immunoassay in 22 healthy controls, 44 patients with AD, and 16 patients with psoriasis. The relationships between these markers and the scoring AD (SCORAD) index, blood eosinophilia, serum IgE and serum lactate dehydrogenase were investigated in AD patients, and relationships with the psoriasis area and severity index (PASI) score were examined in psoriatic patients.

Results: Plasma \( \beta \)-TG and PF4 levels were significantly higher in patients with AD or psoriasis compared with healthy controls. \( \beta \)-TG and PF4 levels correlated with the SCORAD index, and PF4 levels correlated with PASI scores. Elevated \( \beta \)-TG and PF4 levels were significantly reduced after treatments.

Conclusions: Our results show that blood platelets are activated in patients with AD or psoriasis, suggesting that activated platelets play a role in the pathomechanism of chronic skin inflammation. Furthermore, plasma \( \beta \)-TG and PF4 may be markers for the severity of AD and psoriasis.

KEY WORDS
atopic dermatitis, platelet factor 4, platelets, psoriasis, \( \beta \)-thromboglobulin

INTRODUCTION

The role of platelets in inflammatory processes is increasingly being recognized, in addition to their function in hemostasis and thrombosis.¹¹ Following activation, platelets rapidly release mediators stored in dense or \( \alpha \)-granules, including adenosine diphosphate, adenosine triphosphate, serotonin, cytokines and chemokines, and these molecules may affect induction and maintenance of allergic inflammatory reactions.¹ Indeed, involvement of platelets has been demonstrated in pathomechanisms of inflammatory disorders including asthma,²⁻⁶ arthritis⁷ and inflammatory bowel disease.⁸⁻¹⁰ The best known platelet chemokines are \( \beta \)-thromboglobulin (\( \beta \)-TG) and platelet factor 4 (PF4), which are released from \( \alpha \)-granules following activation of platelets¹²,¹³ and are therefore considered to be platelet activation markers.

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Atopic dermatitis (AD) and psoriasis are typical chronic inflammatory diseases of the skin. AD is frequently associated with high serum IgE levels, positive immediate-type hypersensitivity to environmental allergens, and eosinophilia.\(^\text{14}\) Th2-dominant immune responses to allergens in the skin based on undefined genetic predispositions are the central feature of AD,\(^\text{15}\) and scratching due to severe itch often results in excoriation and subsequent platelet aggregation at the inflamed lesion. The plasma levels of β-TG and PF4 are increased in patients with AD,\(^\text{16}\) but the relationship between platelet activation and disease severity has not been examined.

Psoriasis is a chronic relapsing inflammatory disease characterized by scaly, red cutaneous plaques. Epidemiological studies have shown that psoriasis represents a risk factor for thrombotic vascular diseases.\(^\text{17,18}\) In a large retrospective case-controlled study, the incidences of myocardial infarction, cerebrovascular accidents, thrombophlebitis and pulmonary embolism were significantly higher in psoriatic patients than in non-psoriatic dermatological patients.\(^\text{17}\) Several studies have suggested platelet activation in psoriatic patients,\(^\text{19,23}\) and Ludwig et al. showed increased platelet P-selectin expression in patients with psoriasis\(^\text{19}\) and a correlation between P-selectin expression levels in platelets and disease severity measured by the psoriasis area and severity index (PASI).\(^\text{19}\) These findings suggest that platelets play a role in psoriasis, but little is known about platelet chemokines in patients with psoriasis. In the current study, we evaluated platelet activity based on plasma β-TG and PF4 levels in patients with AD or psoriasis, and investigated the relationships between these markers and disease severity.

**METHODS**

**SUBJECTS**

Forty-four patients (21 men and 23 women) with AD were enrolled in the study. Diagnosis of AD was based on the criteria of Hanifin and Rajka.\(^\text{24}\) The average age of AD patients was 29.4 years (range: 17–52 years of age). The clinical severity of AD was determined using the scoring AD (SCORAD) index\(^\text{25}\) (median score: 43.2; range: 24.5–59.8). Peripheral eosinophil count (median: 637.3; range: 300–1600 /µl), serum total IgE (median: 16048.3; range: 839–87200 IU/ml), and serum lactate dehydrogenase (median: 275.3; range: 155–550 IU/l) were also examined. Each patient had a history of AD for more than three years, but none had received topical steroids or oral medications during the two weeks preceding the study.

Sixteen patients (11 men and 5 women) with psoriasis were also included in the study. Eligible patients had been diagnosed with plaque psoriasis for at least 6 months with at least 10% of total body surface area affected. The average age of the patients was 48.2 years (range: 28–78 years of age). Severity of psoriasis was assessed using the PASI score\(^\text{26}\) (median score: 15.8; range: 12.2–18.7). None of the patients had received topical or oral medication during the two weeks preceding the study.

The control group consisted of 22 healthy subjects (10 men and 12 women; average age: 30.2 years; range: 24–43 years). The control subjects did not take any medication for at least two weeks preceding the study.

The serum creatinine concentration was normal in all subjects, which is of significance since clearance of β-TG is impaired in renal insufficiency.\(^\text{27}\) Individuals were excluded from the study if their history indicated disorders affecting platelet activity, such as obesity, hypertension, dyslipidemia or diabetes. None of the patients or controls were smokers. All subjects gave written consent, and the study was approved by the Kyoto Prefectural University ethics committee and conducted in accordance with the Declaration of Helsinki.

**MEASUREMENT OF β-TG AND PF4 IN BLOOD SAMPLES**

To minimize platelet activation during sample collection, blood was drawn from antecubital veins through 20-gauge needles and mixed with 1/10 the volume of acid citrate dextrose. The blood was then centrifuged at 2500 × g for 20 minutes at 4°C, and the top third of the resultant plasma supernatant was collected and frozen at −80°C for evaluation. Plasma levels of β-TG and PF4 were measured by enzyme-linked immunosorbent assay kits for quantitative detection of β-TG (Diagnostica Stago, Asnieres, France) and PF4 (R&D Systems, Minneapolis, MN) according to the manufacturers’ instructions.

**STATISTICAL ANALYSIS**

Data are expressed as medians ± SD, and comparisons between groups were performed by the Mann-Whitney U-test. Correlation coefficients were obtained by Spearman tests. \(P\) values lower than 0.05 were considered to be significant.

**RESULTS**

**CONCENTRATIONS OF β-TG AND PF4**

Plasma β-TG and PF4 levels in patients with AD or psoriasis and in healthy controls are shown in Figure 1. Both β-TG and PF4 in patients with AD were significantly higher than in controls, and there was also a significant increase in β-TG and PF4 in patients with psoriasis compared with controls. Plasma levels of β-TG and PF4 did not differ significantly between patients with AD and those with psoriasis.

**RELATIONSHIPS OF β-TG AND PF4 WITH DISEASE SEVERITY IN AD PATIENTS**

Plasma levels of β-TG and PF4 were correlated with
F i g .  1  Plasma levels of \( \beta \)-TG and PF4 in patients with AD and psoriasis and in healthy controls. The plasma levels of \( \beta \)-TG and PF4 in patients with AD or psoriasis were significantly higher than those in the healthy controls. \(*P < 0.05.\)

(ng/ml)

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<tr>
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<th>Control</th>
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<th>Psoriasis</th>
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<tbody>
<tr>
<td>( \beta )-TG</td>
<td>10</td>
<td>30</td>
<td>50</td>
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<tr>
<td>PF4</td>
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Fig. 2 Relationships between plasma levels of \( \beta \)-TG or PF4 and the SCORAD index in patients with AD. Plasma \( \beta \)-TG and PF4 levels were correlated with the SCORAD index.

RELATIONSHIPS OF \( \beta \)-TG AND PF4 WITH DISEASE SEVERITY IN PSORIASIS PATIENTS
Plasma PF4 levels were correlated with severity of psoriasis measured by the PASI score, but plasma \( \beta \)-TG levels were not related to the PASI score (Fig. 3).

Elevated \( \beta \)-TG and PF4 levels were significantly reduced after treatments in patients with AD (Fig. 4A) and psoriasis (Fig. 4B).

PLATELET COUNTS
There was no significant difference in peripheral platelet count between healthy controls (median: 220.3 \( \times 10^3 \); range: 159–296 \( \times 10^3 \) /\( \mu l \)) and patients with AD (median: 269.2 \( \times 10^3 \); range: 194–365 \( \times 10^3 \) /\( \mu l \)) or psoriasis (median: 215.6 \( \times 10^3 \); range:138–315 \( \times 10^3 \) /\( \mu l \)).

DISCUSSION
The results of the study show that blood platelets are activated in patients with AD and psoriasis, and demonstrate for the first time that plasma levels of \( \beta \)-TG and PF4 are correlated with the SCORAD index in patients with AD, and plasma PF4 levels are also correlated with PASI scores in patients with psoriasis. In addition, elevated \( \beta \)-TG and PF4 levels were significantly reduced after treatments. These findings suggest that activated platelets may play a role in the
pathomechanism of chronic skin inflammation. We have recently shown that platelets have an important role in leukocyte recruitment of chronic skin inflammation in a murine model of chronic dermatitis, through formation of platelet-leukocyte aggregates via P-selectin in peripheral blood and secretion of chemokines at inflamed sites. Platelets have also been shown to be important in the development of cu-
taneous inflammation through direct activation of local vascular capillary endothelial cells and attraction of effector T cells into the tissue. Platelets contain various chemokines including β-TG and PF4, which attract and stimulate leukocytes and further activate other platelets. Interestingly, β-TG and PF4 are increased in inflammatory skin diseases and in other inflammatory diseases; for example, β-TG and PF4 are elevated in bronchoalveolar lavage fluid during the late inflammatory response after allergen challenge in asthmatics, and are increased in the peripheral blood in patients with inflammatory bowel disease.

In allergic skin inflammation such as AD, it has been shown that platelet-derived soluble factors including β-TG and PF4 may be important for leukocyte recruitment to skin. In AD, an elevated number of eosinophils in peripheral blood and inflamed sites is a characteristic feature. Eosinophils from the blood of AD patients show a potentiated migratory response to PF4 compared with eosinophils from normal controls. Moreover, PF4 not only modulates the chemotactic activity of eosinophils, but also increases eosinophil adhesion. It has been also reported that gene expression of PF4 is elevated in a mouse model of AD. Therefore, the platelet-derived chemokines such as PF4 may be related to recruitment and activation of leukocytes in AD. In psoriatic patients, the characteristic features of involved skin are epidermal proliferation and neutrophil infiltration. The specific factors contributing to keratinocyte proliferation have yet to be identified clearly, but activated platelets secrete mitogenic factors that stimulate proliferation in several cell lines, and therefore may be involved in epidermal proliferation in psoriasis. In addition, platelets affect neutrophil activation by release of many mediators, including PF4, thromboxane A2, and platelet-derived growth factor, which may lead to neutrophil infiltration in psoriasis.

The precise mechanism through which circulating platelets are activated in chronic skin inflammation remains to be elucidated. Platelet hyperaggregation in psoriatic patients has been shown and might be related to alterations in arachidonic acid metabolism in platelets; however, the function of platelet aggregation is not impaired in AD patients. The high and low affinity receptor for IgE is expressed on the surface of human platelets, suggesting that stimulation of platelets by IgE may induce platelet activation in AD. Endothelial cells activated by proinflammatory cytokines and chemokines such as platelet-activating factor released during skin inflammation may also be involved. Therefore, activation of the immune system may cause various proinflammatory mediators to induce concomitant activation of platelets.

In conclusion, our results indicate that blood platelets are activated in patients with AD and psoriasis. Plasma levels of β-TG and PF4 were also correlated with the SCORAD index in patients with AD, and plasma PF4 levels were also correlated with the PASI score in patients with psoriasis. In addition, elevated β-TG and PF4 levels were significantly reduced after treatments. These findings suggest that activated platelets may play an important role in the pathomechanism of chronic skin inflammation, and that β-TG or PF4 may be a marker for the severity of chronic skin inflammation such as AD or psoriasis. However, platelet activity as expressed by the β-TG or PF4 level was not correlated with other factors related to disease severity. This observation requires further investigation in a larger group of patients with AD or psoriasis.

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