

Thrombocytopoiesis in allergic asthma

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Summary: **Introduction:** Allergic asthma is characterized by bronchial inflammation and simultaneous repair processes that result in increased airway obstruction. **Objectives:** The aim of the present study was to evaluate platelet count, percentage of reticulated platelets, plasma concentration of thrombopoietin and interleukin 6 (IL-6) concentration in patients with bronchial asthma. **Patients and methods:** The study was performed in 12 allergic chronic asthma patients and 12 nonallergic chronic asthma patients. Patients were treated according to the GINA 2004 criteria for chronic moderate asthma. Seven healthy individuals served as negative controls. Blood was collected in the morning on CTAD. **Results:** The platelet-count was statistically higher in allergic asthma patients compared with healthy controls. In nonallergic asthma patients the platelet count was also higher but the difference was not statistically significant. The percentage of the reticulated platelets in allergic asthma patients was statistically higher as compared to nonallergic and healthy subjects. The concentration of IL-6 was elevated in allergic asthma patients as compared to healthy controls and the difference was statistically significant. In nonallergic asthma patients plasma thrombopoietin levels were slightly higher, but not statistically significant, in comparison to allergic asthma patients and control groups. **Conclusions:** Our findings suggest that platelets may be involved in allergic inflammation and may play a significant role in remodeling of the airways. Further studies on the role of platelets in the pathogenesis of asthma and their potential clinical implications are warranted.

Key words: reticulated platelets, allergic inflammation

INTRODUCTION

Asthma is a chronic inflammatory disease of the airways. Allergic inflammation is the main cause of shortness of breath with wheezing and tightness in the chest. It is connected with general but varying limitation of airflow in the airways. Allergic inflammation is characterized by accumulation of various cells, particularly mast cells, eosinophils, lymphocytes T and platelets in the bronchial wall [1]. Platelets initiate and maintain inflammation and take part in the regulation of airway remodeling by secreting platelet activating factor (PAF), transforming growth factors α and β (TGF α and β) and prostaglandins. Release of P-selectin by platelets enables their interaction with leukocytes [2]. Platelets can be activated by various pathogens: bacteria, fungi and allergens. They are capable of activating phagocytosis, increasing lysosome degranulation and intensifying the cytotoxicity of monocytes, neutrophils and natural killers (NK) [3].

The receptors with low and high affinity for IgE – Fc ϵ R1I, Fc ϵ R1 and for IgG – Fc γ R1I have been found on platelet surface [4,5]. In asthmatic patients the platelet count capable of binding IgE is fifty percent higher than in healthy controls [6]. Beasley et al. proved increased platelet accumulation in bronchial vessels after allergen provocation [7]. Taytard et al. showed significant decrease of platelet lifetime in asthmatic patients [8]. Jeffrey et al. suggest that this can be caused by platelet migration from the circulation to the airways [9]. Szczeklik et al. observed prolongation of bleeding time in allergic asthma patients [10]. Studies of Sullivan et al. on the platelet count dynamics after allergen provocation showed their decrease during late allergic reaction. Platelet count was still low 24 hours after provocation despite normalization of spirometry values [11]. Maccia et al. discovered a significant decrease of platelet aggregation after their induction with adenosinodiphosphorane (ADP) and adrenaline [12]. There were no significant changes in platelet aggregation following platelet activating factor (PAF) exposure. The latest findings show that platelets can participate in allergic inflammation.

Platelets are generated in bone marrow and also partly in lungs as a result of megakaryocyte fragmentation. Considering this it is interesting to evaluate thrombocytopoiesis in allergic asthma. The megakaryocyte development is stimulated

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by megakaryocyte – colony stimulating factor (Meg-CSF) and granulocyte – macrophage colony stimulating factor (GM-CSF). Thrombopoietin is the main hormone responsible for megakaryocytopoiesis. This glycoprotein with molecular weight 18 000–32 000 is produced in the kidneys and the liver [13]. Thrombopoietin is responsible for platelet proliferation, differentiation, maturation and migration to the circulation. The percentage of reticulated platelets with high RNA content can be very useful in the assessment of thrombopoietic activity [14,15]. Interleukin 6 (Il-6) and Il-3 are responsible for blood generation, stimulation of proliferation and differentiation of many stem cell pathways, particularly of megakaryocyte precursor cells, and generation of granulocyte and macrophage colonies [16]. The aim of our study was to evaluate the platelet count, percentage of reticulated platelets, concentration of Il-6 and thrombopoietin in the plasma of asthmatic patients.

PATIENTS AND METHODS

The study was performed on twelve patients (7 women, 5 men; mean age 42 ± 5.8) with documented moderate chronic allergic asthma and twelve patients (6 women, 6 men; mean age 48 ± 6.4) with documented moderate chronic nonallergic asthma that were hospitalized and ambulatory patients of the Department of Allergology and Internal Diseases at the Medical University of Białystok. Patients were treated according to GINA 2004 criteria for chronic moderate asthma. All patients presented in a stable disease phase. Seven healthy persons: 3 women and 4 men with a mean age of 24 ± 2.5 years served as negative controls. The research was approved by the Local Ethic Committee of the Medical University of Białystok (approval no. R-I-003/187/2005). The project was supported by a grant from the Medical University of Białystok (grant no. 3-35488 P).

The blood samples were taken in the morning from the ulnar vein on citrate-theophylline-adenosine-dipiridamole (CTAD).

The platelet count from the blood taken on ethylenediaminetetraacetic acid disodium salt p.a. (EDTA) was assessed on an Advial 650 (Bayer, Germany) analyzer. The number of reticulated platelets was assessed by flow cytometry (Epics XL, Coulter USA). The concentration of Il-6 and thrombopoietin was assessed on Quantikine Human HS ELISA Kits (R&D).

RESULTS

Platelet count

The platelet count was statistically higher in allergic asthma patients as compared to nonallergic asthma patients (281750 ± 58950 vs $268580 \pm 69740/\mu\text{l}$) and healthy controls (281750 ± 58950 vs $22050 \pm 5830/\mu\text{l}$; $p < 0.05$). In nonallergic asthma patients the platelet count was also higher as compared to the

control group but the difference was not significant (268580 ± 69740 vs 22050 ± 5830 tys/ μl) [fig. 1].

Reticulated platelets

The percentage of reticulated platelets in the allergic asthma patients was statistically higher as compared to nonallergic (2.58 ± 1.48 vs $1.5 \pm 0.91\%$; $p < 0.05$) and healthy subjects (2.58 ± 1.48 vs $1.32 \pm 0.55\%$; $p < 0.05$). There were no differences between nonallergic asthma patients and the control group (1.5 ± 0.91 vs $1.32 \pm 0.55\%$) [fig. 2].

Interleukin- 6

The concentration of Il-6 was elevated in allergic asthma patients as compared to healthy controls; the difference was statistically significant (0.57 ± 0.34 vs 0.25 ± 0.35 pg/ml, $p = 0.05$).

In nonallergic asthma patients the concentration of Il-6 was higher too, but was not statistically significant. There were no differences between allergic and nonallergic asthma patients (0.57 ± 0.34 vs 0.57 ± 0.38 pg/ml) [fig. 3].

Trombopoietin

In allergic asthma patients the concentration of thrombopoietin was higher as compared to nonallergic patients (41.55 ± 39.64 vs 25.48 ± 24.94 pg/ml) and the control group (41.55 ± 39.64 vs 22.19 ± 20.16 pg/ml) but was not statistically significant. There were no differences between allergic asthma patients and healthy controls [fig. 4].

DISCUSSION

The mechanisms responsible for airway remodeling in asthma still remain unclear. Recently published articles suggest that platelet activation may be crucial in that process [17]. In the study we have demonstrated a significantly higher reticulated platelet count in allergic asthma patients as compared to nonallergic asthma patients. The platelet count was also elevated in the blood of allergic asthma patients. Maybe that is because all patients presented stable disease phase and were properly treated. Recent publications about platelet action during bronchial provocation with *Dermatophagoides pteromyssinus* extract may bring some help in the explanation of the obtained results [18]. In the course of late allergic response substantial decrease of platelet count occurs in the blood, most probably associated with their migration to the site where the inflammatory process was triggered. Presence of platelet aggregates in pulmonary arterioles, bronchoalveolar lavage and airway epithelium in allergic asthma patients was documented which points out to their contribution in inflammatory processes [19,20]. Platelets participate in airway remodeling in-

directly through leukocyte and eosinophil chemotaction as well as directly through growth factors and matrix metalloproteinase expression [21]. Experimental mice with induced immunologic (platelet antibody) or non-immunologic (busulfan) thrombocytopenia showed decreased airway remodeling after ovalbumin nebulization in comparison to the control group that only had bronchial provokation. We conclude that in allergic asthma patients due to repeated allergen exposure, such as dust mite or animal fur, which cannot be completely eliminated from our environment, platelets migrate to the site where the inflammatory process arises. Taytard et al. demonstrated shortened platelet lifetime in allergic asthma patients which most likely was the result of the migration of platelets to the airways [8]. In our study we observed substantially increased reticulated platelet count in asthma patients which points to intensified megakariocytopoiesis as the result of these cells participation in inflammation development. Unfortunately we were not able to answer the question if Il-6 and thrombopoietin may significantly stimulate platelet formation in chronic allergic asthma. Il-6 has a very short half-life (about 5 h), and is generated as a result of cell activation. All

patients although chronically stimulated with small allergen doses presented in stable disease phase and were properly treated. Maybe that is why we were not able to observe significant differences in concentration of Il-6. Otherwise hypoxia, one of the most important factors that influence thrombopoietin generation does not play an important role in chronic, properly treated asthma. Difficulty in interpretation of our results is also due to the low count of asthma patients groups. We need further studies to draw final conclusions. Recent publication indicate that in the bronchoalveolar lavage fluid obtained from allergic asthma patients increased lymphocytes T count was detected. Activated platelets enable lymphocytes T adhesion to endothelium and migration to the inflammation site [22]. Lymphocytes T excrete among of others Il-3, GM-CSF which contribute to bronchial hyperreactivity and take part in megakariocytopoiesis [23]. Explanation of cytokine influence on platelet activation in asthma patients will be the subject of our further studies. The presented results indicate that in allergic asthma patients platelets migrate to bronchi and affect their remodeling. Are we able to hold back that process? Experiments revealed that dexamethasone administration nei-

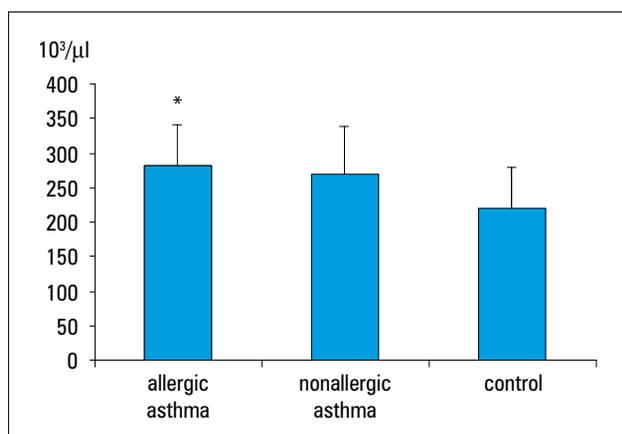


Fig. 1. Platelet count. * $p < 0.05$ (patients vs control)

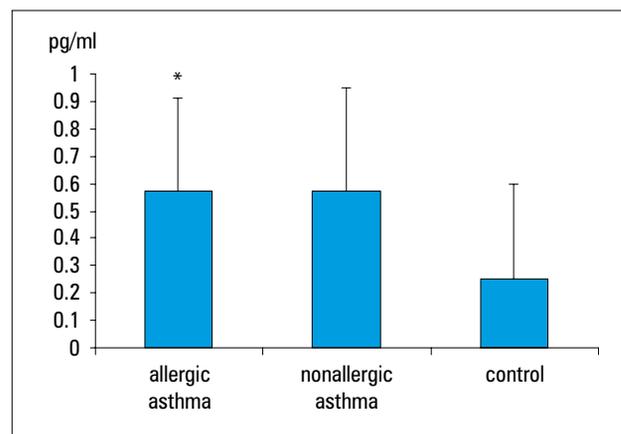


Fig. 3. Concentration of interleukin 6. * $p < 0.05$ (patients vs control)

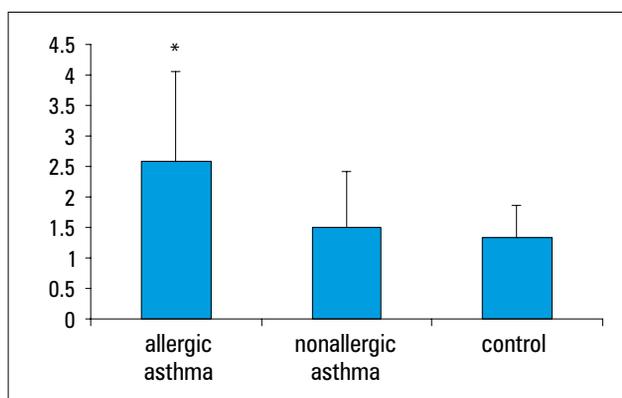


Fig. 2. Reticulated platelet count. * $p < 0.05$ (patients vs control)

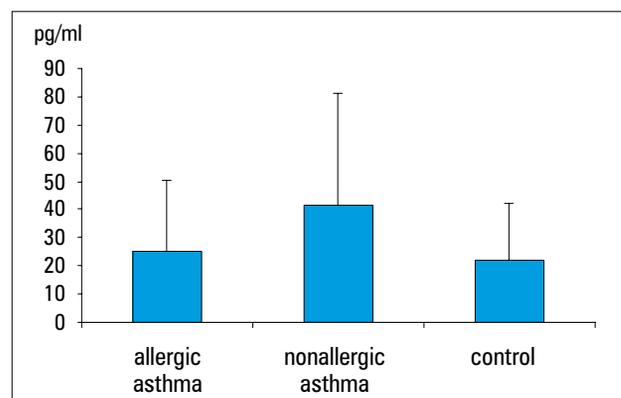


Fig. 4. Concentration of thrombopoietin. * $p < 0.05$ (patients vs control)

ther inhibits the thickening of bronchial epithelium nor inhibits subepithelial fibrosis even if migration was substantially inhibited. Possibly process leading to bronchial remodeling do not depend on leukocytes whereas platelets activation, as Pitchford et al. suggest, play the crucial role [21]. The results of our study are also consistent with previously presented data. Although both groups of patients were treated with inhaled steroids, we observed substantially increased platelet count only in the allergic asthma group.

Platelets may participate in allergy inflammation and remodeling of the airways. In our study we demonstrated a higher reticulated platelet count in allergic asthma patients which can indicate increased megakariocytopoiesis. Presently employed therapeutic methods do not influence this phenomenon. Further studies on the platelet role in asthma pathogenesis may contribute to the development of new, interesting therapeutic opportunities.

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