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Reprint

Features of intercellular interaction of platelets and neutrophils expressing adhesion molecules in psoriasis

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Abstract: Objective: to determine the features of intercellular interaction of platelets with neutrophils expressing adhesion molecules in psoriasis patients.
Material and Methods. The study included 82 patients 20-60 years of age with a plaque psoriasis or pustular psoriasis. As a control group, 50 virtually healthy individuals 20-60 years old were examined. The study of the adhesion molecules spectrum on neutrophils was carried out on a Beckman Coulter FC-500 flow cytometer (USA) using monoclonal antibodies. The platelet-neutrophil aggregates were determined sensu the method by Yu.A. Vitkovsky et al. (2006).
Results. In patients with psoriasis, compared with the control group, few intercellular contacts of neutrophils with platelets were revealed. Low values of platelet-neutrophil aggregates with 3, 4, and 5 lobes in the nucleus were observed. The formation of aggregates statistically significantly correlated with the expression of LFA-1 and PECAM-1 adhesion molecules by mature neutrophils.
Conclusion. Low rates of intercellular interaction of platelets with three-, four-, and five-lobed neutrophils in psoriasis were indicative of their augmented migration from the peripheral blood to the epidermis. The adhesion molecules LFA-1 and PECAM-1 play a key role in the migration of platelet-neutrophil aggregates.

Keywords: adhesion molecules, neutrophils, platelets, psoriasis

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Introduction

Studying the mechanisms of neutrophil migration into the skin in psoriasis, as well as establishing the features of this process regulation, are of practical interest, since high rates of infiltration activity of these cells with lobed nuclei in the epidermis are associated with a more severe manifestation of the disease [1, 2]. It is known that neutrophils move to the focus of inflammation due to the expression of adhesion molecules on them, and the latter are involved in all three stages of the migration process: rolling, strong adhesion, and transmigration [3–6].

The factual material available in the literature confirms that the physiological properties of platelets go beyond their conventional perception as hemocoagulation process participants [7]. Platelets, due to their adhesive properties and, entire complex of contractile actomyosin proteins, help neutrophils entering the inflammation zone, thereby dragging them into the area of the pathological process [8]. The absence of an actomyosin bridge in neutrophils limits their ability to migrate through the endothelial layer of capillaries, as a result of which, along with the expression of adhesion molecules, they require additional auxiliary interactions with platelet aggregates. The latter provide an enhanced migration of neutrophils into tissues [9].

It is known that the severity of psoriatic inflammation depends on the activity of neutrophil migration into the skin [10]. Based on this, it is of interest to study the features of intercellular contacts between platelets and neutrophils with lobed nuclei, assisted by adhesion molecules. Such intercellular contacts reflect the processes of neutrophil recruitment to the lesion and intensity of inflammatory process, and also act as a prerequisite for studying one of the links in the psoriasis pathogenesis [1, 2].

The objective of our study was to determine the features of intercellular interaction between platelets and neutrophils expressing adhesion molecules in psoriasis patients.

Materials and Methods

On the basis of the clinical diagnostic polyclinic of the Federal State Budgetary Institution of Higher Education, Northern State Medical University (Arkhangelsk), we organized the survey of 82 patients with either plaque psoriasis or pustular psoriasis (39 women and 43 men), 20–

60 years age (mean age of 36.5±16.5 years). The exclusion criteria encompassed age under 18 and over 60 years, other clinical forms of psoriasis: inverse, erythrodermic, palmoplantar psoriasis, and psoriatic arthritis. The control group included 50 virtually healthy individuals (28 women and 22 men) 20 to 60 years old (mean age of 35.0±13.5 years) without acute or chronic diseases in acute phase at the time of the study. The study was carried out with the written consent of the participants, in compliance with the basic rules of biomedical ethics, in accordance with the Ethical Principles for Medical Research Involving Human Subjects (World Medical Association Declaration of Helsinki, 1964, amended in 2008).

Venous blood for analysis was taken from the cubital vein in the morning on an empty stomach.

Determination of the spectrum of adhesion molecules on neutrophils was carried out by immunophenotyping on a flow cytometer FC-500, Beckman Coulter (USA), using monoclonal antibodies (Dako, Denmark): L-selectin - (CD62L, FITC); LFA-1 (CD11a, FITC); ICAM-1 (CD54, FITC); LFA-3 (CD58, FITC); PECAM-1 (CD31, FITC). The stages of migratory activity of neutrophils were assessed by the level of expression of adhesion molecules:

L-selectin (CD62L) is expressed on the neutrophil membrane, participates in the first migration phase (rolling) along the endothelium, promotes immobilization in the endothelium and transition to the second phase (strong adhesion) [3-5]; LFA-1 (lymphocyte function-associated antigen-1; CD11a) is associated with the function of lymphocytes, and belongs to the integrin family mediating the strong adhesion migration phase of neutrophils towards the endothelium [3-5];

ICAM-1 (intercellular adhesion molecule-1; CD54) are intercellular adhesion molecules-1, located on the surface of endothelial cells – neutrophils; they are integrin ligands, have structural homology with immunoglobulins, and are active during the strong adhesion phase [6];

LFA-3 (lymphocyte function-associated antigen-3; CD58) is associated with the function of lymphocytes, and participates in enhancing adhesion between leukocytes and antigen-presenting cells [3-5];

PECAM-1 (platelet endothelial cell adhesion molecule 1; CD31) are platelet/endothelial cell adhesion molecules that activate the final migration phase (transmigration) of leukocytes through the endothelium [3-5].

The value of platelet-neutrophil aggregates was determined sensu the method by Yu.A. Vitkovsky et al. (2006) [9]. To do so, smears of venous blood were dried, fixed in the mixture of Nikiforov for 20 minutes, then stained according to Romanowsky-Giemsa, after which the number of platelet-neutrophil aggregates per 100 cells was determined under magnification of ×1000, which represented the absolute number of platelet adhesion. A neutrophil that adhered three or more platelets on its surface was considered a platelet-neutrophil aggregate.

Statistical data processing was carried out using the SPSS 13.0 software for Windows. The distributions of the studied indicators were tested using the Shapiro-Wilk test. It was established that distributions differed from normal, and therefore the description of the samples was carried out via calculation of the median (Me) and interquartile range (Q25; Q75). The probability of differences was assessed by nonparametric Kolmogorov–Smirnov test (Z). When comparing two dependent groups by a quantitative trait, the Wilcoxon method was employed; to assess the relationship of qualitative traits, the $\chi 2$ test was used. Correlation analysis was carried out with the determination of the Spearman correlation coefficients (ρ). Differences were considered statistically significant at $p<0.05$.

Results

In psoriasis patients, there were 7.0 (3.0; 15.0) intercellular contacts with platelet aggregates per 100 neutrophils, which was three times as few as in the control group: 26.0 (23.0; 31.0); Z=3.18; p<0.001 (*Figure*).

It was established that contacts of band neutrophils with platelet aggregates were recorded only in half of the patients with psoriasis and half of the subjects from the control group: 52.1 and 55.1%; $\chi 2=0.1$; $p>0.05$. Contacts between neutrophils with two-lobed nuclei and platelet aggregates occurred more frequently: in 71.8% of patients with psoriasis vs 95.9% of control group subjects. The observed difference between patients with and without psoriasis was statistically significant ($\chi 2 =11.23$; $p<0.001$). The presence of contacts in platelets with three-, four- and five-lobed nuclei was observed in all subjects from the control group.

Such contacts in psoriasis group were much less common. Hence, neutrophils with three-lobed nuclei were in contact with platelet aggregates only in 80.3% of patients with psoriasis ($\chi 2=10.94$; $p<0.001$); those with four-lobed nuclei were in contact with platelet aggregates in 76.1% of subjects ($\chi 2=13.67$; $p<0.001$); finally, neutrophils with five or more nuclear lobes were part of platelet-neutrophil aggregates in 49.3% of examinees ($\chi 2=35.49$; $p<0.001$). Overall, in peripheral blood, neutrophils with three, four, five or more nuclear lobes were in contact with platelet aggregates, since there was just one contact with platelet aggregates per 100 neutrophils with one- and two-lobed nuclei. In psoriasis, the number of direct contacts of three-lobed forms of neutrophils was significantly less than in the control group: 4.0 (1.0; 11.0) vs 11.0 (9.0; 13.0); Z=3.07; p<0.001. Neutrophils containing four, five or more nuclear lobes were also significantly less likely to contact platelet aggregates: 2.0 (0.39; 8.0) vs 9.0 (6.0; 12.0); Z=3.08; p<0.001 and 1.0 (0.0;4.0) vs 4.0 (2.5;6.0); Z=2.57; p<0.001.

Thus, contacts of band neutrophils with platelet aggregates were recorded only in half of the patients with psoriasis and half of the subjects from the control group. In psoriasis, direct contacts of neutrophils with three, four, five or more lobes in their nuclei with platelet aggregates in peripheral blood were much less common than in the control group.

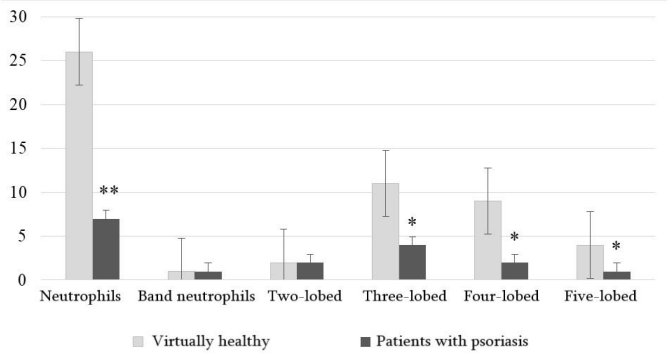

Figure. Number of platelet-neutrophil aggregates in virtually healthy people vs psoriasis patients

* $p<0.05$; ** $p\leq0.001$ (nonparametric Kolmogorov-Smirnov test)

Of particular interest is the issue of revealing what adhesion molecules are responsible for the contact of neutrophils with platelet aggregates at various stages of the migration process.

At the first stage of the migration process (rolling), the correlation analysis did not reveal statistically significant correlations between neutrophils carrying L-selectin adhesion molecules with the number of neutrophils that came into direct contact with platelet aggregates in patients with psoriasis. On the contrary, in the control group, a greater number of studied contacts was observed than in patients with psoriasis, statistically significant correlations were recorded between neutrophils carrying the L-selectin molecule and neutrophils that came into contact with platelet aggregates. These were mainly neutrophils containing three (ρ=0.35; p<0.01), four (ρ=0.42; p<0.001), five or more lobes in the nucleus (ρ=0.3; p< 0.01).

At the second stage of the migration process (strong adhesion), in patients suffering from psoriasis, a statistically significant correlation was detected only in the case when neutrophils expressed the LFA-1 molecule, and also had three (ρ=0.27; p<0, 01) and four lobes in the nucleus (ρ=0.31; p<0.001). In the control group, statistically significant correlations were recorded between neutrophils carrying the LFA-1 molecule and having three (ρ=0.61; p<0.001), four (ρ=0.48; p<0.001) and even two lobes in the nucleus (ρ=0.52; p<0.001).

No statistically significant correlations were found in psoriasis patients between the size of neutrophils expressing the ICAM-1 adhesion molecule and contacts with platelet aggregates. In contrast, in the group of virtually healthy individuals, statistically significant correlations were established between the number of four- (ρ=0.33; p<0.01) and five-lobed neutrophils (ρ=0.28; p<0.01) with expressed adhesion molecules of ICAM-1 and contacts with platelet aggregates.

In psoriasis patients, there were no statistically significant correlations with neutrophils having the LFA-3 molecule. In the control group, correlations were observed between neutrophils with the LFA-3 molecule and neutrophils with one (ρ=0.29; p<0.01), three (ρ=0.28; p<0.01) and four (ρ=0.29; p<0.01) lobes in their nucleus that were in contact with platelet aggregates in the peripheral blood.

At the third stage of the migration process (transmigration), in patients with psoriasis, contacts of neutrophils with platelet aggregates occurred only if neutrophils expressed PECAM-1 molecules, and also had three (ρ=0.31; p<0.01) and four (ρ=0.31; p<0.01) lobes in their nucleus. In the control group, contact of neutrophils with platelet aggregates was observed in case of neutrophils containing the PECAM-1 molecule, as well as three (ρ=0.47; p<0.01), five or more nuclear lobes (ρ=0.49; p<0 .01).

Discussion

Numerous studies have established that all types of leukocytes can interact with platelets, which can facilitate their migration to the focus of inflammation [7-9].

As a result of our study, in patients with psoriasis, a low content of platelet-neutrophil aggregates was revealed. We believe that this fact indicates an enhanced migration of neutrophils by means of intercellular contacts with platelets towards the areas of inflammation in the epidermis.

It was of interest to find out which forms of neutrophils with lobed nuclei migrate most actively and are in demand in the focus of inflammation.

In half of the patients with psoriasis and half of the control group, we revealed the platelet contacts with band neutrophils – immature cells, not adapted sufficiently to migration through the endothelial walls and suppression foreign antigens [11].

At the same time, in psoriasis, direct intercellular contacts between neutrophils with three-, four-, and five-lobed nuclei and platelet aggregates in peripheral blood were much less common than in the control group, which indicated their augmented migration from peripheral blood to the skin. Consequently, in psoriasis, there is an increased migration of neutrophils with three-, four-, and five-lobed nuclei into the epidermis; and such neutrophils are more mature and sufficiently active to suppress foreign antigens [11].

In the available literature, there is no information on the role of adhesion molecules in the migration of neutrophils with platelet aggregates from peripheral blood to the epidermis. It is not clear what adhesion molecules play a key role in the process of migration of neutrophils into the dermis.

When analyzing the obtained data, we established that in psoriasis, a registered decrease in the number of contacts between neutrophils and platelet aggregates in peripheral blood was associated with the presence of statistically significant correlations with neutrophils carrying LFA-1 and PECAM-1 molecules.

Therefore, the main role in the migration of platelet-neutrophil aggregates is played by LFA-1 molecules, initiating the second phase of migration (strong adhesion), and by PECAM-1, involved in the final (third) migration phase: transmigration.

Conclusion

The presented study results demonstrate the features of the intercellular interaction between platelets and neutrophils expressing adhesion molecules in psoriasis patients. Low levels of platelet aggregates with mature neutrophils with three, four and five lobes of their nuclei indicate increased migration of mature neutrophil forms from peripheral blood to the epidermis. Intercellular interactions of platelets and neutrophils with lobed nuclei are associated with high expression of LFA-1 and PECAM-1 adhesion molecules on their cell membranes.

Further research in the field of studying the intercellular interaction of platelets and neutrophils would draw the attention of clinicians to the modulation of inflammatory processes, and an emergence of new approaches to psoriasis treatment via influencing changes in the level of expression of adhesion molecules.

Conflict of interest: None declared.

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