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Spontaneous Neutrophil Extracellular Trap Formation During Pollinosis Exacerbation

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ABSTRACT

Aim. To study the morphological structure and functional activity of neutrophil extracellular traps in patients with seasonal allergic rhinitis during the exacerbation period.

Materials and methods. The study included 12 patients with seasonal allergic rhinitis during the exacerbation period. We studied the morphological structure and functional activity of neutrophil extracellular traps (NET) in pollinosis patients. Fluorescence microscopy with SYBR Green dye was used to visualize NETs.

Results. The study of neutrophils from allergic patients during the exacerbation period showed spontaneous NETs formation in the morphological form of neutrophil networks. The number of NETs during 1 hour of incubation is 10 [8.3–14] %, and their size was 28 [17.5–35] μm. Culturing neutrophils from allergic patients for 2 hours does not increase the number of NETs, but the size of NETs doubles, and 10% of NETs form as single threads and fibers. Neutrophil networks have a high functional activity.

Conclusion. The results specify that during the exacerbation of seasonal allergic rhinitis, there is an NET formation similar to that observed during infectious inflammation, which is combined with high functional activity of neutrophil networks.

Keywords: pollinosis, seasonal allergic rhinitis, exacerbation period, neutrophil extracellular traps, neutrophil networks, allergy

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Обострение поллиноза сопровождается спонтанным формированием нейтрофильных экстраклеточных ловушек в морфологической форме нейтрофильных сетей

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РЕЗЮМЕ

Цель. Исследование морфологической структуры и функциональной активности нейтрофильных экстраклеточных ловушек у больных сезонным аллергическим ринитом в период обострения заболевания.

Материалы и методы. В исследование включены 12 больных с поллинозом период обострения заболевания. Исследовали морфологическую структуру и функциональную активность нейтрофильных экстраклеточных ловушек (НЭЛ) у больных поллинозом. Для визуализации НЭЛ использовали флуоресцентную микроскопию с красителем SYBR Green.

Результаты. Исследование нейтрофилов больных в период обострения заболевания показало спонтанное формирование НЭЛ в морфологической форме нейтрофильных сетей. Количество НЭЛ в течение 1 ч инкубации составляет 10 [8,3–14]%, а их размеры – 28 [17,5–35] мкм. Культивирование нейтрофилов аллергических пациентов в течение 2 ч не увеличивает численность НЭЛ, размеры НЭЛ увеличиваются в 2 раза, при этом происходит формирование 10% НЭЛ в форме одиночных нитей и волокон. Нейтрофильные сети имеют высокую функциональную активность.

Заключение. Результаты свидетельствуют, что при обострении поллиноза наблюдается образование НЭЛ, подобное развивающемуся при инфекционном воспалении, сочетающееся с высокой функциональной активностью нейтрофильных сетей.

Ключевые слова: поллиноз, сезонный аллергический ринит, период обострения заболевания, нейтрофильные экстраклеточные ловушки, нейтрофильные сети, аллергия

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии источника финансирования при проведении исследования.

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INTRODUCTION

The rapid development of immunology has led to the creation of many new biological drugs for the treatment of allergic diseases, including anti-immunoglobulin E (anti-IgE), anti-interleukin-5 (anti-IL-5), and anti-thymic stromal lymphopoietin (anti-TSLP/IL-4), but the initiation of the allergic (atopic) process is still unclear [1]. The dysfunction of the immune system in allergic (atopic) diseases is associated with changes in the functioning of the body's defense.

One of the possible mechanisms is associated with the disruption of the protective functions of neutrophils as participants in the innate immunity. This particular pathogen link was identified during the examination of patients suffering from

hay fever (chronic allergic rhinitis) [2], where it was demonstrated that the neutrophils of these patients had impaired adhesion to plastic, reduced chemotaxis, and decreased bactericidal activity. An increased level of neutrophils in nasal mucosa biopsies was recorded in patients with allergies during the blooming season, compared to healthy individuals. Studies have shown that neutrophils in patients with seasonal allergic rhinitis are partially activated [3] and can, in turn, activate T-cells and enhance the migration of eosinophils.

Recently, researchers have gradually come to understand that in order to develop effective treatment strategies that take into account the complex interaction between immune system cells and pathogens, it is necessary to study in depth the mechanisms of neutrophil extracellular trap (NET) formation in allergic (atopic) diseases. It can be reasonably assumed that NETs can serve as potential biomarkers and therapeutic targets.

The aim was to study the morphological structure and functional activity of neutrophil extracellular traps in patients with seasonal allergic rhinitis during the exacerbation period.

MATERIALS AND METHODS

The study included 12 patients with hay fever during the exacerbation period. The inclusion criterion was the absence of other acute diseases at the time of the study and indications of other chronic diseases in history. The exclusion criterion was acute infectious diseases within a month before the day of the study.

The blood samples were analyzed at the Department of Pathophysiology and Clinical Pathophysiology, Institute of Biology and Human Pathology, Pirogov Russian National Research Medical University. All procedures were performed in accordance with the ethical standards of the WMA Helsinki Declaration (as revised in 2004) and the written informed consent of the patients. The study was approved by the local Ethics Committee of Pirogov University (Minutes No. 239 dated April 15, 2024).

Neutrophil isolation from EDTA-treated venous blood of patients was carried out as described by us before [4]. Immunofluorescence study of NETs to determine their morphological structure, as well as the study of the functional activity of neutrophil extracellular structures using a cell culture of *Klebsiella pneumoniae* (ATCC 700603) was carried out using the method [5].

Statistical data processing was carried out using STATISTICA 12.0 software packages (StatSoft, USA). Descriptive statistics are presented in the form of continuous quantitative data: for normal distributions, in the form of the mean value (M) ± standard error (m), and for distributions that differ from normal, as the median of the interquartile range Me [Q_1–Q_3]. Comparison of quantitative variables was performed using the Mann–Whitney test. The difference was considered to be statistically significant at a two-tailed p value of < 0.05.

RESULTS AND DISCUSSION

The study of neutrophils in patients with allergic rhinitis during the exacerbation period showed spontaneous formation of NETs in the morphological form of neutrophil networks.

The number of neutrophil networks during 1 hour of incubation was 10 [8.3–14] %, and their size was 28 [17.5–35] μm. Culturing neutrophils from allergic patients for 2 hours did not increase the number of NETs, but the size of neutrophil extracellular traps increased by almost 2 times (Table 1).

Table 1

Parameters of Neutrophil Extracellular Traps (NETs) in Patients with Seasonal Allergic Rhinitis (n = 12) during the Exacerbation Period, Me [Q_1–Q_3]

Number of NETs, %	NET size, microns	NET structure
Spontaneous formation of extracellular structures (1 hour of incubation)		
10 [8.3–14]	28 [17.5–35]	Networks
Spontaneous formation of extracellular structures (2 hours of incubation)		
11.1 [8.3–21]	52.5 [35–70]*	Networks, single strands, fibers

*$p < 0.05$ compared to cell incubation for 1 hour

The peculiarity of the NET formation in patients with seasonal allergic rhinitis during the exacerbation period is that, in addition to neutrophil networks, single strands, and fibers are detected during longer neutrophil cultivation (for 2 hours). The number of these abnormal structures is small and does not exceed 10% of the total number of neutrophil networks.

NET formation in the morphological form of neutrophil networks (Fig. 1), combined with single strands and fibers, confirms that the neutrophils of allergic patients are already pre-activated [3].

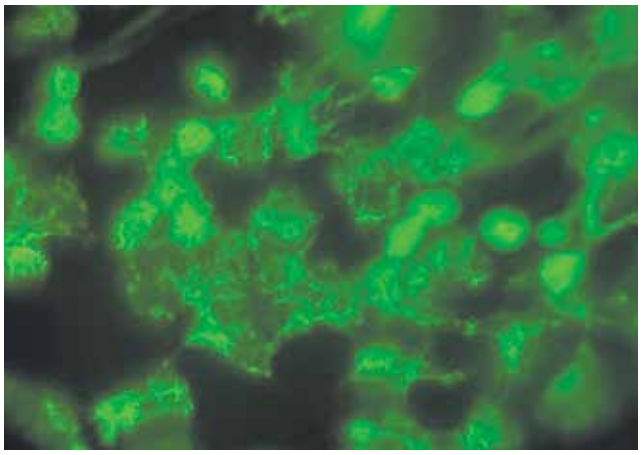

Fig. 1. Neutrophil networks in patients with seasonal allergic rhinitis during the exacerbation period. Incubation time 1 h. CYBR Green staining. Magnification × 1 000

These two morphological structures (neutrophil networks and single strands) may indicate two forms of neutrophil activation during the exacerbation period and may be caused by two different processes. Neutrophil network formation in patients with seasonal allergic rhinitis during the exacerbation period may be induced by both contact interactions with pathogens [5] and the action of IL-8, which can induce the production of free radicals and the formation of extracellular traps [6].

In severe infectious complications/sepsis, it was found that the IL-8 concentration in the patients' blood plasma was closely related to the NET formation, and the inhibition of IL-8 using monoclonal antibodies significantly weakened the formation of NETs [7]. Epithelial cells stimulated by pollen extracts release IL-8, as well as a number of pro-inflammatory cytokines, including IL-1, IL-6, and TNFα [8]. The secretion of pro-allergic alarmins (thymic stromal lymphopoietin TSLP, IL-33; and IL-25) contributes to a Th2-dominated immune response and the development of sensitization.

For that reason, the neutrophil network formation in the patients we examined is likely to be caused by the action of IL-8. However, as we have previously shown, the neutrophil network formation is a manifestation of the body's readiness to trigger protective anti-infective reactions [5]. The detection of NETs in the morphological form of single strands can be interpreted as a neutrophil response to contact interactions with damaged vascular endothelium [9].

The central neutrophil extracellular structure found in patients with seasonal allergic rhinitis during the exacerbation period is neutrophil networks. Functional activity study of these neutrophil structures showed that, despite the relatively small size of the neutrophil networks – 52.5 [35–70] microns, upon incubation for 2 hourthe pathogen *Klebsiella pneumoniae* in these extracellular structures is quite high (Table 2).

Table 2

Functional Activity of Neutrophil Networks, Single Strands, and Fibers in Patients with Seasonal Allergic Rhinitis (n = 12) during the Exacerbation Period, after Incubation for 2 hours, *Me* [Q_1-Q_3]

NET structure	NET size, microns	NET conversion after interaction with a pathogen	Capture of pathogen cells Klebsiella pneumoniae
Neutrophil networks	52.5 [35–70]	Neutrophil veils	50 [40–60]
Single strands, fibers	130 [112.5–157.5]	Single strands, fibers	0.00

Each neutrophil network binds 50 [40–60] pathogen cells. At the same time, the structure of the neutrophil network changes – it undergoes morphofunctional transformation, i.e., it transforms into a veil-like structure (Fig. 2).

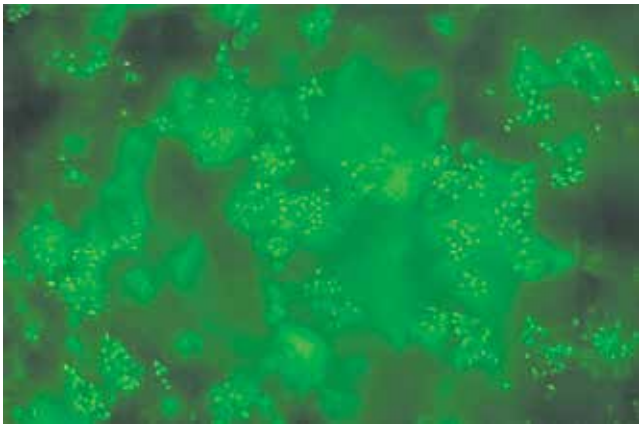

Fig. 2. Neutrophil networks of patients with seasonal allergic rhinitis during exacerbation capture and bind the cells of the pathogen *Klebsiella pneumoniae*.

We previously described such morphofunctional transformation of neutrophil networks during contact interactions with a pathogen in patients with infectious inflammation in the abdominal area [10].

It should be noted that during the neutrophil activation in healthy individuals, each neutrophil network is capable of capturing and retaining an average of 80 [60–100] *Klebsiella pneumoniae* cells. In patients with inflammatory diseases of the abdominal cavity (appendicitis, cholecystitis, and pancreatitis), the functional activity of NETs is reduced by several times compared to healthy donors in the postoperative period.

In these patients, neutrophil extracellular structures capture and bind an average of no more than 20 cells of the test microorganism, and some of the infectious pathogen cells remain unbound by the extracellular neutrophil structures, which increases the risk of postoperative infectious complications in these patients [9].

The functional activity of spontaneous neutrophil networks in patients with seasonal allergic rhinitis during the exacerbation period is 1.6 times lower than the activity of induced NETs in healthy donors, but 2.5 times higher than the same parameter in patients with inflammatory diseases of the abdominal cavity.

CONCLUSION

The results of a study of the morphological structure and functional activity of neutrophil extracellular traps in patients with hay fever (seasonal allergic rhinitis) during the exacerbation period indicate that during the exacerbation of hay fever, the NET formation is similar to that observed during infectious inflammation, accompanied by relatively high functional activity of neutrophil networks. The conducted research suggests that neutrophil extracellular trap formation in the morphological form of neutrophil networks is a universal response of neutrophils to pathogens.

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Author Contribution

Poryadin G.V., Salmasi J.M. – conception and design. Kazimirskii A.N., Kim A.E., Kim T.E. – experimental studies. Kazimirskii A.N., Turishcheva O.O. – preparation of illustrative material. Panina M.I. – statistical processing of the material. Kazimirskii A.N. – drafting of the manuscript. Panina M.I., Salmasi J.M. – editing of the manuscript. The contribution of the authors is equivalent.

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