УДК 616.233-002-092:612.59:616.2-021.5:616.248
https://doi.org/10.20538/1682-0363-2023-1-143-152

Clinical and pathogenetic aspects of neutrophilic bronchial inflammation in asthma patients with cold-induced airway hyperresponsiveness (literature review)

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ABSTRACT

The review presents data on the effect of neutrophilic bronchial inflammation on the clinical course, external respiration, and formation of the airway response to cold air in patients with asthma. According to the results of modern studies, activation of the structural and functional state of neutrophils in a mixed inflammatory pattern is associated with an increase in disease severity, more difficult achievement of asthma control, pronounced impairment of bronchial patency due to stimulation of epithelial destruction and remodeling, and development and maintenance of cold-induced airway hyperresponsiveness.

The mechanisms activating the Th1 cytokine profile and oxidative and halogenation stress and determining the activity of neutrophils and persistence of chronic inflammation lead to oxidative damage to lung parenchyma and epithelial dysfunction, which contributes to cold-induced bronchoconstriction. Cytolysis and NETosis, acting as alternative pathways of neutrophil death in the airways of asthma patients, are considered in terms of final stages of induced activity of neutrophil lysosomes in the mixed asthma phenotype.

Keywords: asthma, mixed inflammatory pattern, neutrophils, proinflammatory cytokines, oxidative stress, cold-induced airway hyperresponsiveness

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

Source of financing. The authors state that they received no funding for the study.

For citation: Pirogov A.B., Prikhodko A.G., Pirogova N.A., Perelman J.M. Clinical and pathogenetic aspects of neutrophilic bronchial inflammation in asthma patients with cold-induced airway hyperresponsiveness (literature review). *Bulletin of Siberian Medicine.* 2023;22(1):143–152. https://doi.org/10.20538/1682-0363-2023-1-143-152.

Клинические и патогенетические аспекты нейтрофильного воспаления бронхов у больных бронхиальной астмой с холодовой гиперреактивностью дыхательных путей (обзор литературы)

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РЕЗЮМЕ

Представлены данные о влиянии нейтрофилии бронхиального воспаления на клиническое течение, функцию внешнего дыхания и формирование реакции дыхательных путей к холодному воздуху у больных брон-

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хиальной астмой (БА). Согласно результатам современных исследований, активация структурно-функционального статуса нейтрофилов при смешанном паттерне воспаления связана с утяжелением течения и более сложным достижением контроля заболевания, выраженным нарушением проходимости бронхов вследствие стимуляции эпителиальной деструкции и ремоделирования, развитием и поддержанием холодовой гиперреактивности дыхательных путей.

Механизмы активации цитокинового профиля Th1, оксидативного и галогенирующего стресса, обусловливающие активность нейтрофилов и персистенцию хронического воспаления, приводят к оксидантному повреждению паренхимы бронхов и эпителиальной дисфункции, что способствует холодовому бронхоспазму. Цитолиз и нетоз, выступающие в качестве альтернативных апоптозу путей гибели нейтрофилов в дыхательных путях астматиков, рассматриваются с позиций финальных этапов индуцированной активности лизосом нейтрофилов при смешанном фенотипе БА.

Ключевые слова: бронхиальная астма, смешанный паттерн воспаления, нейтрофилы, провоспалительные цитокины, оксидативный стресс, холодовая гиперреактивность дыхательных путей, обзор

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

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

Для цитирования: Пирогов А.Б., Приходько А.Г., Пирогова Н.А., Перельман Ю.М. Клинические и патогенетические аспекты нейтрофильного воспаления бронхов у больных бронхиальной астмой с холодовой гиперреактивностью дыхательных путей (обзор литературы). *Бюллетень сибирской медицины.* 2023;22(1):143–152. https://doi.org/10.20538/1682-0363-2023-1-143-152.

BRONCHIAL NEUTROPHILS AND FEATURES OF THE CLINICAL COURSE OF THE DISEASE

Bronchial asthma (BA) has long been associated with eosinophilic inflammation. However, studies show that a number of patients have increased airway infiltration by neutrophils [1–3]. It indicates a potentially important role of neutrophils in BA development associated with phenotypic features of the disease. In a certain number of BA patients, a mixed pattern of bronchial inflammation is formed, which is characterized by a large number of eosinophils and neutrophils and accompanied by a more severe course of the disease [4, 5]. Clinically, this is manifested by persistence of symptoms, a decrease in asthma control, a decline in the lung function, and, to a greater extent, small airway patency [3].

It is estimated that an eosinophilic inflammatory phenotype is accompanied by a good response of asthma patients to treatment with inhaled corticosteroids (ICS), antileukotriene drugs (ALT), and interleukin (IL)-5 blockers [6]. Severe patients with a mixed BA phenotype have more frequent exacerbations of the disease, are difficult to treat with ICS, ALT, and require prolonged use of systemic corticosteroids (SCS) [4, 5], often responding poorly to the proposed therapy [7]. According to the literature data, neutrophilia accompanies resistance to corticosteroids and therapeutic resistance to high doses of SCS. It is associated not only with the severe course of BA, but also with the severity of its exacerbations [8].

In the period of extremely severe exacerbations and fatal attacks, the neutrophilic inflammation that dominates in the respiratory tract can lead to death [7, 8]. Thus, when studying bronchial biopsy specimens obtained from patients with severe exacerbations of BA and endotracheal intubation for respiratory failure, it was shown that during the exacerbation, the bronchial mucosa is heavily infiltrated by eosinophils and, to a greater extent, by neutrophils [9]. At the same time, a number of researchers do not exclude the presence of neutrophilia in bronchial secretion in such patients even before exacerbation due to the previous severe course of BA during treatment with high doses of ICS [10].

However, neutrophils are responsible not only for the severity of BA and resistance to ICS therapy, but are directly related to the nature of the pathological process in the respiratory tract, initiating or accompanying various exogenous reactions, for example, when exposed to cold air [3, 11]. The aggressive effect of cold leads to the formation of cold airway hyperresponsiveness (CAHR) in a significant number of BA patients (60–80%), which is accompanied by the aggravated course of BA in winter, difficulty in selecting adequate therapy due to

activation of neutrophilia, and emergence of a mixed (eosinophilic / neutrophilic) inflammatory pattern in the airways [3].

Difficulties in treating such patients are associated primarily with low efficiency of conventional anti-inflammatory therapy regimens due to the persistently high level of activated sputum neutrophils [3]. Patients with a mixed pattern of airway inflammation, even in mild BA, despite the controller therapy with ICS, more often experience respiratory discomfort and the need to use emergency drugs than patients with an eosinophilic pattern of inflammation. They also have lower values of FEV_1 and FEF_{25-75} and more pronounced bronchial lability in response to inhaled short-acting β_2-agonist and during bronchoprovocation with cold air [3]. In patients with a mixed pattern of airway inflammation, CAHR occurs 2 times more often than in patients with an eosinophilic pattern of inflammation [3]. The problem of endogenous regulation of inflammation, chemotaxis, proliferation, differentiation, apoptosis, and functioning of granulocytes in maintaining disturbed homeostasis in BA patients with CAHR remains little studied.

EXPRESSION OF PROINFLAMMATORY CYTOKINES AND THE NEUTROPHILIC LINK OF BRONCHIAL INFLAMMATION

Under the influence of a cold trigger, granulocyte neutrophils generate reactive oxygen species (ROS) and other mediators of cellular oxidation in the bronchi of BA patients [11]. The latter are represented as signaling molecules that regulate the activity of the nuclear factor NF-kB, which is one of the main transcription factors responsible for adaptive cell responses and is associated with the intensity of inflammation in BA and expression of proinflammatory cytokines [12, 13]. An elevated level of proinflammatory cytokines activates a cascade of inflammatory reactions that determine the severity of BA [13].

The impact of cold on the respiratory system of BA patients is accompanied by an increase in the concentration of IL-10, IL-5, and IL-1b in the sputum [14], which fits into the concept of antagonistic and synergistic relationships between Th2 and Th1 cytokines in BA. A pivotal role in the Th2 response of the asthma-specific transcription factor GATA-3, which is involved in the expression of Th2 cytokines, differentiation of CD4+T cells into Th2 cells, and inhibition of Th1development and mediates such components of the clinical course of the disease as CAHR and airway remodeling, has been proved [13, 15, 16].

If previously BA was classified as predominantly Th2 cell-mediated inflammation, in recent years there has been a paradigm shift, and it becomes increasingly clear that its severity is largely associated with neutrophils, which are present in large numbers in the sputum of asthma patients with CAHR [17]. The priority role in the development of neutrophilia with a mixed pattern of chronic bronchial inflammation in BA patients is attributed to the increased expression of non-Th2 cytokines, such as IL-17 and proinflammatory interferon (IFN) γ [1, 5]. They may participate in the immune response to the effects of physical and chemical environmental factors [17, 18].

The expression of IL-17 is associated with neutrophil NETosis, which often develops in BA patients along the non-lytic pathway with the formation of enucleated cytoplasts that induce differentiation of naive CD4+ helper T lymphocytes (CD4+Th0) into a subpopulation of T helper 17 (Th17) cells – producers of IL-17 [5, 19]. The enucleated cytoplasts found in the lungs in addition to neutrophil extracellular traps (NETs) result from nuclear desegmentation, disintegration of the nuclear envelope into multiple vesicles, and eruption of decondensed chromatin through ruptured neutrophil plasma membrane with cytolemma resealing. If the discarded double-stranded DNA can interact with dendritic cells (DC) localized among the epithelial cells of the airways through TLR2 receptors, causing the formation of CD4+Th2, then DC activation by cytoplasts, on the contrary, causes differentiation of Th0 cells into antigen-specific Th17 cells [5, 11].

Cytokines and activated enzymes interacting in Th17 and Th1 inflammatory responses modify the structure of the airways in BA patients, causing progression of bronchial obstruction with airway remodeling [20]. It has been known that IL-17 directly affects airway epithelial cells, fibroblasts, and smooth muscle cells [21], controlling both neutrophilic and T2 links of the inflammatory cascade [22]. Differentiation of airway Th2 cells into double positive Th2 / Th17 cells is of interest. According to *in vivo* studies, increased expression of Th2 / Th17 cells in bronchoalveolar lavage fluid is associated with the most severe airway obstruction and hyperresponsiveness, leading to an increase in corticosteroid resistance in BA [2, 18, 23].

Overproduction of Th17-related cytokines, including IL-17A and IL-17F, is thought to be a major

driver for neutrophil recruitment and activation by induction of cytokines and chemokines CXCL8, IL-6, G-CSF, GM-CSF, IL-8, CXCL1, and CXCL5, whose expression correlates with the severity of BA and neutrophilia of bronchial inflammation [24]. Thus, the neutrophilic inflammatory phenotype of BA (≥ 40% of neutrophils in the sputum) is associated with a decline in lung function, progression of disease symptoms, and an increase in the sputum concentration of IL-1b and MIP-3 alpha / CCL20 [4].

IL-8 plays an important role in neutrophilic and systemic inflammation, which primes the respiratory burst of neutrophils [25]. Among proinflammatory cytokines and chemokines, IL-8 preactivates respiratory reactions in cells [26]. As a chemokine, IL-8 plays a key role in neutrophil chemoattraction: it stimulates the migration of neutrophils from the bloodstream to the site of inflammation, increases the concentration of intracellular Ca^{2+} in them, which ensures the movement of leukocytes and activates the pentose phosphate pathway in these cells, causing the production of free radicals and degranulation and exocytosis of neutrophilic enzymes [27]. Integration of IL-8 with IL-1, GM-CSF, TNFα, and other proinflammatory cytokines constitutes a cytokine field that activates neutrophils [27], which, in turn, independently synthesize and produce neutrophilokines (GM-CSF, TNFα, IL-1, IL-6, IL-8), involved in the cooperation of phagocyte system cells and acting in a paracrine manner on macrophages and in an autocrine manner on neutrophils [28].

There is an opinion that neutrophils stimulated by IL-8 in BA lead to airway eosinophilia by modifying the migration of eosinophils through the basement membrane of the bronchial mucosa. Thus, due to increased migration of eosinophils induced by the neutrophil + IL-8 complex, the possibility of disease exacerbation is multiplied [29].

Expression of IL-8, TNFα, and other chemokines is controlled by NF-kB, which has a stimulatory effect on numerous genes involved in immune, acute phase, and inflammatory responses and airway smooth muscle responses [12]. In turn, NF-kB is activated by a direct synergistic effect of TNFα and the bifunctional enzyme CD38, which is expressed in cells of the immune system, as well as in vascular and bronchial leiomyocytes. The CD38 molecule, which combines the activity of adenosine diphosphate (ADP) ribosyl cyclase and cyclic ADP-ribose hydrolase (cADPRH), serves as a marker of immunopathological processes characteristic of BA [30]. TNFα-induced expression of CD38 potentiates the expression of multiple proinflammatory genes, increasing smooth muscle contractility, which leads to increased airway resistance to air flow and contributes to the development of bronchial obstruction [30, 31]. Under the influence of cold air in patients with CAHR, there is an increase in the concentration of IL-1b, IL-8, and TNFα [17]. There is a close relationship between the concentration of TNFα in the sputum and the severity of bronchoconstriction in response to inhalation of cold air [17].

In addition, it is likely that in patients with CAHR, there is a decrease in antiviral immunity due to the suppression of the antiviral and immunomodulatory activity of IFN through the activation of NF-kB and the expression of genes encoding proinflammatory cytokines [32]. This may cause persistence of infection in the airways and is considered as one of the potential independent mechanisms in the uncontrolled course and exacerbation of the disease [2, 13].

RESPIRATORY BURST AND AIRWAY MYELOPEROXIDASE ACTIVITY

The proinflammatory cytokines GM-CSF, TNFα, and IL-8 modulate the activity of NADPH oxidase in neutrophils via priming. NADPH oxidase is a multicomponent enzyme system catalyzing NADPH-dependent reduction of oxygen to the superoxide anion O_2^{2-}, i.e., capable of oxidizing reduced nicotinamide adenine dinucleotide phosphate (NADPH) and redeploying electrons from NADPH to molecular oxygen [26]. This process (respiratory burst) is regulated by many receptor and non-receptor reactions, which culminate in conformational changes in NADPH oxidase components and their readiness to interact with one other [26, 33].

The assembly of a single NADPH oxidase complex occurs at the center of the respiratory burst and is triggered by the activation of cell receptors, kinases, and guanosine triphosphatases, leading to phosphorylation and membrane relocation of oxidase components [26, 33]. Taking the first electron, an oxygen molecule turns into a superoxide radical anion $O_2^{\bullet-}$; with further reduction, either an H^+ ion is added to form a hydroperoxide radical $HO_2^{\bullet}$, or an electron is formed to shape a superoxide anion $O_2^{2-\bullet}$.

During the respiratory burst, the aggressive superoxide anion radical is removed spontaneously or under the effect of superoxide dismutase (SOD). Either an electron or an H^+ ion is attached to $O_2^{\bullet-}$ and a hydroperoxide anion HO_2^- is formed, which

is then reduced to a more stable hydrogen peroxide H_2O_2. At this stage, myeloperoxidase (MPO) released from neutrophil lysosomes is incorporated into the respiratory burst, converting oxidative stress into halogenation stress based on synthesis of highly reactive halogen-containing compounds [34, 35]. Hypochlorous acid (HOCl) is the main product of the interaction between MPO and H_2O_2 – the most active precursor of free radicals among reactive halogen species (RHS) [34, 35].

The cascade of free radical reactions caused by oxidative stress causes disruption of the structure and function of biomembranes and damage to all vital molecules in cells of the respiratory tract, leading to disorganization of the lung parenchyma and stroma [36]. MPO involved in the respiratory burst of neutrophils, when interacting with H_2O_2, catalyzes the oxidation of halide ions (Cl^-, Br^-, I^-), contributing to the production of hypohalogenites, hypohalogenite derivatives (HOCl, HOBr, HOI), and their ionized forms (hypochlorite, hypobromite and hypoiodite) [36]. As a result of the occurring reactions, a link between oxidative stress and halogenation stress is provided [34, 37].

MPO from azurophilic granules of neutrophils is secreted into the intercellular space as a result of degranulation during the respiratory burst. In asthma patients with CAHR, neutrophil degranulation in sputum occurs to the level of destruction [11]. Total degranulation, which, as a precursor of destruction, realizes the maximum oxidative capacity of neutrophils at the peak of the respiratory burst, is preceded by the activation of oxidases, which is proportional to the needs of bronchial inflammation in ROS and RHS. Enhanced synthesis and intragranular deposition of MPO, followed by extracellular exocytosis of the enzyme and products of its catalytic activity, prolong oxidative stress and provoke inflammatory damage to the epithelial parenchyma, accompanying the bronchial response to cold exposure.

Accumulation of peroxidase in neutrophils, stimulated by the accelerated utilization of HOCl, hypochlorite anion, and other ionized forms of hypohalogenites in the bronchial matrix, ends with functional depletion of cells, depletion of peroxidase-positive granules, disruption, and cytolysis with primary destruction of the cytoplasm, then – of the nucleus and cell lysosomes [11]. A differential sign of transformation of physiological degranulation (as a link in cellular adaptation to cold stress) into pathological destruction is vacuolization and fragmentation of the neutrophil cytoplasm in BA [38].

In contrast to degranulation, in which structure-preserving granules with enzymes deposited in them are secreted into the intercellular space, during neutrophil destruction, labilization of lysosome membranes occurs, and the lysosomal matrix penetrates into the environment. In sputum smears, the concentration of MPO in granulocytes is determined by the oxidation reaction of benzidine in the presence of H_2O_2 [38]. This reaction shows dense, diffuse, or granular location of a black-colored product in the cytoplasm of granulocytes, which marks peroxidase activity under conditions of intense synthesis and accumulation of the enzyme in granules. Due to the compact arrangement of granules larger than 0.3–0.4 μm, the cytoplasm of neutrophils is either filled with an intensely stained diffuse and granular material that masks or incompletely masks nuclear segments, or acquires a homogeneous black color, with which benzidine, oxidized by MPO, prevents detection of the cell nucleus [38].

Against the background of the clear and vacuolated cytoplasm in neutrophils during degranulation and destruction, peroxidase-positive inclusions marked with benzidine are present in the form of sparse or single, softly colored small granules, up to 0.2–0.3 μm in length [38]. The low concentration of MPO in neutrophils is regarded as a consequence of increased utilization of the enzyme during the generation of RHS, degranulation, destruction, and cytolysis of cells associated with respiratory burst and production of free radicals. All of the above stimulates the persistence of inflammation and the formation of an excessive airway response to cold exposure and leads to a decline in the lung function and a decrease in asthma control [3, 38].

NEUTROPHIL PROFILE AND DESTRUCTION OF BRONCHIAL EPITHELIUM

The most pronounced destruction of the epithelium is observed in patients with BA with a mixed inflammatory pattern due to ongoing intense destructive processes in the neutrophil pool [2, 39]. Destruction of the bronchial epithelium has a negative impact on airway patency and control of the disease, contributing to the formation of an increased response of the bronchi to cold exposure with the emergence of CAHR [39].

The generation of ROS and RHS exported by neutrophils is considered as a cause of free radical damage to mitochondrial cristae and destruction of the endoplasmic reticulum of the bronchial epithelium,

followed by cell death [40]. It is assumed that in this case, apoptotic signals are transmitted to the epithelium not via a direct pathway, from ligation of the death receptor to activation of the caspase cascade and cell death, but via a pathway mediated by de-energization of epitheliocytes [40]. Actively developing epithelial dysfunction in BA is associated with low expression of antioxidant defense factors, in particular, SOD, which contributes to an increase in the susceptibility of epithelial cells to the aggressive effect of oxidants [41]. Activation of the neutrophilic link of bronchial inflammation [42] is also associated with more pronounced mucociliary dysfunction in patients with BA who respond to cold air exposure [43]. Thus, in patients with CAHR, a close relationship was found between disorders in the structural organization of goblet cells, a large number of sputum neutrophils, and the severity of cold-induced bronchospasm, which served as a risk factor for escalation of mucociliary dysfunction [43].

According to a number of sources, oxidative damage to the airway epithelium leads to exocytosis from intraepithelial nerve endings of C-fibers (non-adrenergic / non-cholinergic) in substance P and neurokinins A and B – neurotransmitters with pronounced bronchoconstriction and vasodilator effects [41]. The produced substances activate mast cells, macrophages, and T and B lymphocytes and serve as chemoattractants for eosinophils and neutrophils. Inhibition of the vasoactive intestinal peptide (VIP), synthesized by neurosecretory cells of the bronchial epithelium, occurs, production of PgE_2 is impaired, surface cell receptors, proinflammatory cytokines, chemoattractants, and GM-CSF are expressed [41].

There is evidence that epithelial cells from bronchial biopsies of patients with BA are characterized by a significant increase in the production of GM-CSF and expression of proinflammatory IL-6 and IL-8, the concentration of which increases drastically under the influence of exogenous factors [2]. Airway exposure to cold air in patients with CAHR is accompanied by overproduction of IL-8 and TNFα, an increase in cytosis, and a rise in the proportion of neutrophils in the sputum, leading to a cytokine imbalance associated with bronchospasm [17]. In parallel, there is a decrease in the number of cells of the bronchial epithelium, which is associated with induction of Th1 cytokines and a shift in humoral inflammation to the Th1 cytokine profile. In addition, the decrease is associated with epithelial destruction, parenchymal modification of the airways, expression of the NF-kB factor, and release of proinflammatory cytokines during oxidative damage [2, 17]. The bidirectional behavior of NF-kB, which induces cytokines and is induced by cytokines, controlling the expression of genes encoding proinflammatory cytokines, is a fundamental point that brings together oxidative functions of neutrophils and proinflammatory activity of the airway epithelium, which makes an important contribution to the pathogenesis of airway hyperresponsiveness and remodeling [30].

In addition to oxidative damage, neutrophils produce proteases. The combined effect of both factors causes direct non-specific damage to lung tissues. The production of matrix metalloproteinases (MMPs) contributes to the degradation of the extracellular matrix in BA [2]. As it turned out, MMP-9 can degrade components of the extracellular matrix, including type IV basement membrane collagen, which makes a significant contribution to the integrity of the endothelium and (or) epithelium [2]. Studies show that remodeling is caused by the differentiation of mesenchymal cells of the bronchial subepithelial layer into myofibroblasts [44] with thickening of the reticular basement membrane, interstitial fibrosis, and atrophy of the surface epithelium [45]. Therefore, activation of the neutrophilic component of granulocytic inflammation in patients with BA is not only associated with the destruction of the airway epithelium and airway hyperresponsiveness, but also manifests escalation of airway remodeling, transferring BA into a more severe form.

CYTOLYSIS AND NETOSIS AS VARIANTS OF DEATH OF BRONCHIAL NEUTROPHILS ALTERNATIVE TO APOPTOSIS IN BA

If destruction of granulocytes is a process that reflects the general biological patterns in the dynamics of their functional activity [46], then intensification of destruction and cytolysis are interpreted as the result of stimulated functional activity associated with progressive inflammatory alteration, lysis of the cell membrane, cell isolation, and necrosis [47].

In BA, granulocytes show an IL-5-dependent increase in the expression of *bcl-2* genes, which causes suppression of apoptosis, prolongation of the damaging activity of granulocytes, and an increase in the proportion of dying cells [48]. Antiapoptotic factors regulating apoptosis are formed in the focus of inflammation, while the intermembrane space of neutrophil mitochondria contains proapoptotic

proteins that penetrate into the cytosol during apoptosis [49].

In respiratory diseases, mitochondrial membranes are the most vulnerable link in the pathogenesis of damage to subcellular structures. Changes in mitochondria cause disturbances in biological oxidation and cellular respiration, lead to a decrease in the intensity of energy exchange, ATP deficiency, and destruction of other organelles and the cell as a whole [5]. A decrease in the ability of neutrophils to undergo apoptosis correlates with destruction of mitochondrial cristae and indicates de-energization of cells [46, 49], while in patients with BA, there is a twofold (by 2.1 times compared with controls) increase in the pool of low-energy cells characterized by destruction of cristae and outer membrane mitochondria [46]. Activation of lysosomal acid phosphatases is also directly involved in the stimulation of neutrophil cytolysis, which causes an increase in the activity of neutrophil acid phosphatase by 22.0% in BA [49].

It is most likely that the mechanism responsible for the decrease in the number of neutrophils in the airways of asthma patients, in addition to cytolysis, includes such an alternative to apoptosis as "classical" NETosis, a process of programmed oxygen-dependent cell death aimed at producing highly active neutrophil extracellular traps (NETs) (an important tool for phagocytosis and elimination of pathogens and inflammation products) in response to the effects of stimuli [50–53]. Proinflammatory agents stimulating NETosis can include H_2O_2, bacterial lipopolysaccharides, phorbol myristate acetate, IL-8, and the cleavage product of the fifth complement component during its activation in the blood serum (C5a), but only after priming of neutrophils with interferons (IFNγ + C5a, IFNα+C5a) or GM-CSF (GM-CSF+C5a) [51]. It is believed that almost all neutrophils are capable of NETosis due to the presence in these cells of regulatory mechanisms for starting a program that ends with the formation of NETs [50].

The formation of NETs begins with neutrophil priming, triggering of the NADFH oxidase complex, respiratory burst, and generation of ROS that induce neutrophil elastase and PAD-4, converting arginine and methylarginine residues into citrulline in histones of the nucleus [51, 54]. As a result, chromatin decondensation occurs with a simultaneous violation of the structural integrity of the membranes in cytoplasmic granules [51, 53]. When decondensed chromatin (DNA strands, histones) is mixed with lysosomal granule enzymes, reticulated NETs are secreted into the extracellular space [50–53].

NETs include MPO, neutrophil elastase, cathepsin G, gelatinase, antibacterial peptides, histones, humoral pattern recognition receptor pentraxin 3, and peptidoglycan recognition proteins [51]. During the formation of NETs by mixing nuclear chromatin with the contents of lysosomal granules, the activated neutrophil still retains its viability and functional activity. Death of an activated ("damaged") cell occurs only after the release of reticulated structures into the extracellular space [49]. Therefore, we are talking about the existence of "vital" NETs that play a role in non-type 2 inflammation, correlate with the level of IL-17 and bronchoalveolar lavage neutrophilia [5, 19], and are found in the airways of patients following attacks of rhinovirus infections that provoke BA exacerbations [19]. Vital release of chromatin by neutrophils primed with proinflammatory cytokines GM-CSF and IL-5/IFN-γ and retaining their effector functions for some time after the formation of NETs develops much faster than the classical ("suicidal") NETosis. However, in any case, after the destruction of the pathogen and the completion of the infectious process, NETs should be eliminated via DNase I and lysis by macrophages. Overproduction of NETs, as well as disturbances of mechanisms for their elimination, for example, in the absence of DNase I, can cause the development of inflammation or autoimmune pathology [55].

Thus, even alternative pathways of neutrophil death in the airways – cytolysis as an outcome of enzymatic activity of cells induced by the respiratory burst and NETosis as a tool for the secretion of inflammatory mediators by ROS-stimulated NETs – can be considered from the standpoint of their role in the pathophysiological mechanisms of neutrophil activity in a mixed inflammatory BA phenotype.

Based on the above data, some potential areas for further research on the mixed inflammatory pattern in asthma patients with CAHR can be identified. It is advisable to study NETs as a marker of IL-17-mediated neutrophilic inflammation, which negatively affects clinical manifestations of BA, since the inducing effect of NETs on the differentiation of CD4+ T cells into Th17 cells and production of IL-17 was established [19]. As it is known, IL-17A and TNFα induce the production of the neutrophil chemokine CXCL-8 by the bronchial epithelium due to impaired inhibition of CXCL-8 expression by an internal repressor protein, which causes hyperreactivity and dysfunction of the

epithelium [56]. Normalization of the cytoplasmic translocation of the CXCL-8 repressor protein in the bronchial epithelium is a potential therapeutic target in neutrophilic BA. In addition to determining the level of IL-17 (IL-17A, IL-17F) in the sputum and bronchial epithelium of asthma patients with CAHR, which plays a key role in neutrophilic inflammatory phenotype, it is necessary to identify the components of the IL-17 signaling pathway that activates the transcription of IL-17A target genes encoding proinflammatory cytokines. The key component of the canonical IL-17 signaling pathway is the TRAF6 regulator (factor 6 associated with the tumor necrosis factor receptor) whose modification leads to the activation of the NF-kB and MAPK pathways.

Finally, taking into consideration that recruitment of neutrophils to the airways is associated with an increase in the level of proteolytic enzymes, including neutrophil elastase and MMP-9 [57], it is promising to study the activity of these enzymes in the sputum, since they cause destruction of collagen fibers and are involved in airway remodeling. High levels of neutrophil elastase and MMP-9 can be considered as markers of proteolysis activation, which intensifies airway remodeling and is controlled by the effects of IL-17A.

As potential targets in BA therapy in patients with CAHR with a mixed inflammatory pattern, development of biological preparations based on monoclonal antibodies should be considered. These include inhibitors and antagonists of Th17 cells, IL-17, TNFα, IL-8, IL-6, NF-kB-dependent proinflammatory cytokines, and Th1 immune responses.

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Received 14.04.2022;
approved after peer review 08.07.2022;
accepted 08.09.2022