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Interleukin-4 and interferon gamma in bronchial remodeling in asthma patients with cold airway hyperresponsiveness

Pirogov A.B., Prikhodko A.G., Pirogova N.A., Gassan D.A., Naumov D.E., Perelman J.M.

Far Eastern Scientific Center of Physiology and Pathology of Respiration
22, Kalinina Str., Blagoveshchensk, 675000, Russian Federation

ABSTRACT

Interleukin-4 (IL-4) and interferon gamma (IFNγ) are key participants in the polarization of the immune response toward Th1 or Th2 types in bronchial asthma. However, their role in bronchial remodeling in patients with asthma and cold airway hyperresponsiveness (CAHR) remains unclear.

Aim. To study the involvement of IL-4 and IFNγ in the disorganization of bronchial epithelium and the regulation of airway remodeling in asthma with CAHR.

Materials and methods. A total of 47 patients with mild persistent asthma were examined. Induced sputum collection, blood sampling for biochemical studies, spirometry, and the isocapnic hyperventilation test with cold (-20 °C) air (IHCA) were performed. The sputum was analyzed for cellular composition (in %), and the cytokine profile (IL-4 and IFNγ in pg / ml) was evaluated in peripheral blood.

Results. The patients were divided into groups with CAHR (group 1, 17 patients) and without cold-induced bronchoconstriction (group 2, 30 patients). Forced expiratory volume in 1 sec. (FEV_1) and maximal mid-expiratory flow (MMEF) in group 1 were lower compared to group 2: 84.0[83.0; 93.0]% and 99.0 [85.0; 105.0]% ($p = 0.012$); 55.0[51.0;67.0]% and 76.0[59.0;88.0]% ($p = 0.021$), respectively. The blood content of IL-4 and IFNγ in group 1 was 11.48[10.82;22.48] pg / ml and 26.98[17.24; 73.5] pg / ml, while in group 2, it was 1.88 [0.66; 5.96] ($p = 0.003$) and 7.24[1.5; 26.98] pg / ml ($p = 0.047$), respectively. In group 1, an association was found between blood IL-4 and IFNγ levels ($Rs = 0.65$; $p = 0.016$), between FEV_1 and the number of epithelial cells in sputum ($Rs = –0.74$; $p = 0.0003$), and between IL-4 and airway response (ΔFEV_1/Vital Capacity) after the IHCA ($Rs = –0.70$; $p = 0.007$).

Conclusion. The escalation of the proinflammatory and pro-oxidant function of IFNγ indicates a shift from Th2 immune response activation, regulated by IL-4, toward a Th1 response, which stimulates bronchial remodeling in patients with asthma and CAHR.

Keywords: bronchial asthma, cold airway hyperresponsiveness, IL-4, IFNγ, bronchial epithelium, airway remodeling

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Conformity with the principles of ethics. All patients signed an informed consent to participate in the study. The study was approved by the local Bioethics Committee at the Far Eastern Scientific Center of Physiology and Pathology of Respiration (Protocol No. 148 of 24.05.2023).

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✉ *Perelman Juliy M.,* jperelman@mail.ru

Интерлейкин-4 и интерферон-гамма в ремоделировании бронхов у больных бронхиальной астмой с холодовой гиперреактивностью дыхательных путей

Пирогов А.Б., Приходько А.Г., Пирогова Н.А., Гассан Д.А., Наумов Д.Е., Перельман Ю.М.

Дальневосточный научный центр физиологии и патологии дыхания (ДНЦ ФПД)
Россия, 675000, г. Благовещенск, ул. Калинина, 22

РЕЗЮМЕ

Интерлейкин-4 (IL-4) и интерферон-гамма (IFNγ) – одни из основных участников поляризации иммунного ответа по Th1 или Th2 типу при бронхиальной астме (БА). Неизвестна их роль в ремоделировании бронхов у больных БА с холодовой гиперреактивностью дыхательных путей (ХГДП).

Цель. Изучение путей участия IL-4 и IFNγ в дезорганизации бронхиального эпителия и регуляции ремоделирования дыхательных путей при БА с ХГДП.

Материалы и методы. Обследованы 47 пациентов с легкой персистирующей БА. Проводился сбор индуцированной мокроты, забор крови для биохимических исследований, выполнялись спирометрия и бронхопровокационная проба изокапнической гипервентиляции холодным (–20 °С) воздухом (ИГХВ). В мокроте исследовали клеточный состав (в %), в периферической крови – цитокиновый профиль (IL-4, IFNγ, в пг/мл).

Результаты. Пациенты разделены на группы с холодовой гиперреактивностью дыхательных путей (1-я группа, 17 человек) и с отсутствием холодовой бронхоконстрикции (2-я группа, 30 человек). Объем форсированного выдоха за 1 с ($ОФВ_1$) и средняя объемная скорость выдоха ($СОС_{25–75}$) на уровне 25–75% жизненной емкости легких (ЖЕЛ) в 1-й группе были ниже по сравнению со 2-й группой: 84,0 [83,0;93,0] и 99,0 [85,0;105,0]% (p = 0,012); 55,0 [51,0;67,0] и 76,0 [59,0;88,0]% (p = 0,021) соответственно. Содержание в крови IL-4 и IFNγ в 1-й группе составляло 11,48 [10,82;22,48] и 26,98 [17,24;73,5] пг/мл, во 2-й группе 1,88 [0,66;5,96] (p = 0,003) и 7,24 [1,5;26,98] пг/мл (p = 0,047) соответственно. В 1-й группе найдена связь между содержанием в крови IL-4 и IFNγ (Rs = 0,65; p = 0,016), между $ОФВ_1$ и количеством эпителиоцитов в мокроте (Rs = –0,74; p = 0,0003), а также между IL-4 и реакцией дыхательных путей ($\Delta ОФВ_1$/ЖЕЛ) в ответ на пробу ИГХВ (Rs = –0,70; p = 0,007).

Заключение. Эскалация провоспалительной и прооксидантной функции IFNγ свидетельствует о смещении баланса активации Th2 иммунного ответа, регулируемого сигналами IL-4, в сторону Th1 иммунного ответа, стимулирующего ремоделирование бронхов у больных БА с ХГДП.

Ключевые слова: бронхиальная астма, холодовая гиперреактивность дыхательных путей, IL-4, IFNγ, бронхиальный эпителий, ремоделирование дыхательных путей

Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов, связанных с публикацией настоящей статьи.

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

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным комитетом по биомедицинской этике ДНЦ ФПД (протокол № 148 от 24.05.2023).

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INTRODUCTION

Airway remodeling in patients with bronchial asthma (BA) appears as a change in the structural and functional organization of the parenchymal and stromal elements of the bronchi, induced by damage and impaired restoration of the epithelial barrier. Under the influence of various triggers, such as allergens, viruses, alarmins, and low temperatures, signaling pathways of inflammation are initiated in the disrupted epithelium, involving immunocompetent cells. Epithelial – mesenchymal units of the bronchi

are activated and proinflammatory cytokines are secreted, leading to persistent chronic inflammation, airway hyperresponsiveness, and obstruction [1–4].

The sources of inflammatory mediators in the bronchi of patients with BA are granulocytes, lymphocytes, macrophages, mast cells, interstitial cells, and smooth muscle cells. However, primary producers of cytokines and growth factors are damaged parenchymal cells. Activated epithelium generates alarmins, such as TSLP, IL-25, and IL-33, which stimulate the polarization of naive T-helper cells into Th2, the expression of IL-4, IL-5, and IL-13, and eosinophilic inflammation. IL-5 and GM-CSF together with eotaxins, CCL5/RANTES and MCP, regulate the production, maturation, recruitment, and activation of eosinophils. IL-9 and IL-13 induce the metaplasia of ciliated epithelial cells into secretory mucocytes. CCL17/TARC and CXCL8/IL-8 recruit Th17 cells and neutrophils, respectively. Proinflammatory cytokines and chemokines, such as IL-1β, IL-2, IL-6, IL-12, IL-18, IL-36, TNFα, CXCL5, CCL20, CCL22, CCL5/RANTES, CXCL10, interferons I (IFNα/β), III (IFNλ1, 2, and 3), and IFNγ, are secreted [1, 3, 5, 6].

The expression of CXCL2, CXCL8, IL-12, CCL20, IFNγ, IL-6, IL-18, IL-36, and TNFα is associated with activation of epithelium by viruses and other infectious agents, leading to the mobilization of neutrophils, neutrophil-macrophage infiltration of the bronchi, and neutrophil response to IL-12 and IFNγ signals in the form of proinflammatory cytokine release [6]. This Th1 variant of the immune response is characteristic of cold airway hyperresponsiveness (CAHR), which is associated with a mixed pattern of bronchial inflammation, neutrophil destruction, and cytolysis, accompanied by the escalation of proinflammatory cytokine synthesis and structural signs of epithelial dysfunction [7]. Clinically, this manifests as uncontrolled asthma with increased symptoms during the cold season, requiring higher doses of medication and/or the inclusion of systemic glucocorticoids in therapy [7].

Given that the central cytokine responsible for the differentiation, growth, and effector functions of Th1 cells, polarizing the immune response toward the Th1 type, is IFNγ [8–10] and IL-4 is one of the main activators of the Th2 immune response and allergic inflammation in the bronchi [5, 11], a study was planned to investigate the involvement of IL-4 and IFNγ in the disorganization of bronchial epithelium and the regulation of airway remodeling in patients with BA and CAHR.

MATERIALS AND METHODS

The study included 47 patients who sought outpatient care at the clinic of the Far Eastern Scientific Center of Physiology and Pathology of Respiration (FSCPPR) with a diagnosis of mild persistent BA [12] and who had not previously received inhaled glucocorticoid therapy on a regular basis.

This clinical study was conducted with the approval of the local Bioethics Committee of FSCPPR (Protocol No. 148 of 24.05.2023). All patients were familiarized with the clinical study protocol, the procedure for functional testing was explained, and they signed an informed consent to participate in the study.

The study design included a period to assess the patient's clinical condition and asthma severity, and a visit for induced sputum collection (day 1), blood sampling for biochemical studies and the isocapnic hyperventilation test with cold air (day 2). Patients were then divided into groups based on the presence or absence of CAHR (group 1 and group 2, respectively).

Inclusion criteria for the study were: forced expiratory volume in one second (FEV_1) > 75% of the predicted value according to spirometry; absence of a documented cold allergic reaction as confirmed by an allergist (Douglas method).

Patients with obstructive ventilatory disorders (FEV_1 < 75% of the predicted value), concomitant respiratory diseases (acute bacterial or viral infections at the time of testing, COPD, etc.), clinically significant comorbidities in other organs and systems, pregnant women, as well as those taking medications that could affect the interpretation of study results were excluded from the study.

Instrumental testing was performed by qualified medical staff in the Laboratory of Functional Research of the Respiratory System at FSCPPR.

Induced sputum collection was performed using a standard method under the control of FEV_1, which was evaluated by spirometry at the beginning of the collection and after each inhalation of 3, 4, and 5% sodium chloride (NaCl) solution. Before each sputum collection procedure, the patient rinsed their mouth with distilled water. The sputum samples were analyzed no later than two hours after the collection. Sputum smears were dried (5–10 minutes at 37 °C)

in a TM-2 thermostat, fixed in formaldehyde vapors (40% solution, 10 minutes), and stained with aqueous Romanowsky – Giemsa stain (4–5%, pH 6.8). A light optical immersion microscope was used to analyze the cellular composition by counting at least 400 cells in the fields of view (central and peripheral regions); the number of cellular elements was expressed as a percentage of the total content. In order to differentiate goblet epithelial cells, a cytochemical reaction was performed by staining formalin-fixed preparations with Alcian blue, which selectively binds mucins (acidic glycosaminoglycans) present in the cytoplasm of goblet cells [13].

Blood samples were collected from the median cubital vein in the morning hours (9:00 AM) into a vacutainer (5 ml) and stored frozen at –80 °C until the biological sample analysis was performed. The cytokine profile (IL-4, IFNγ, in pg / ml) was studied using a flow cytometer (BD FACSCanto II, BD, USA) with LEGENDplex HU Essential Immune Response Panel kits (BioLegend, USA), following the manufacturer's protocols precisely.

All tests involving spirometry were performed using the Easy on-PC device (NDD Medizintechnik AG, Switzerland). The following lung function parameters were measured: vital capacity (VC), forced expiratory volume in one second (FEV_1, in liters), mean mid-expiratory flow at 25–75% of forced VC (MMEF, % of predicted), and maximal expiratory flow rates at 50% (MEF_{50}, % of predicted) and 75% (MEF_{75}, % of predicted) of forced VC. Predicted values according to ECSC standards were used for individuals of European descent older than 18 years.

Isocapnic hyperventilation cold air test (IHCA) was conducted in a mode of submaximal hyperventilation (60% of the predicted maximum ventilation) with an air mixture containing 5% CO_2 for three minutes with individual selection of breathing depth and frequency during the load. Before and after IHCA (at 1 and 5 minutes), FEV_1 was recorded (in liters). The maximum changes in this parameter after IHCA relative to baseline were analyzed. The difference between the obtained values was expressed as a percentage of the baseline (ΔFEV_1, %). A decrease in FEV_1 by 10% or more indicated the presence of CAHR in the patient [14].

Statistical analysis of the obtained results included testing for normality of distribution using the Kolmogorov – Smirnov and Pearson – von Mises criteria. Variables with normal (Gaussian) distribution were compared using the Student's *t*-test (when homogeneity of group variances was confirmed by the Fisher's test). Variables with non-Gaussian distribution were compared by the Mann – Whitney test. Quantitative variables were presented as $M \pm m$ (M – arithmetic mean, m – standard error of the mean) or as $Me\ [Q_1; Q_3]$ (median and interquartile range). The nonparametric Spearman's rank correlation coefficient (*Rs*) was used to determine the degree of correlation between two variables. The differences were statistically significant at $p < 0.05$.

RESULTS

Of the 47 patients included in the study, 17 were included in group 1 with cold airway hyperresponsiveness, while 30 were in group 2 without a cold trigger response. The patients in both groups were comparable in terms of gender and key physiological parameters: age 37.1 ± 3.5 and 43.2 ± 2.9 years ($p = 0.188$), respectively; height 174.3 ± 2.6 and 170.1 ± 1.5 cm ($p = 0.151$); body mass index 26.0 ± 1.5 and 27.6 ± 1.2 kg / m² ($p = 0.419$), respectively. Smokers comprised 35% of group 1 and 23% of group 2 ($\chi^2 = 0.29$, $p > 0.05$).

The groups differed significantly in several flow parameters recorded during the initial evaluation (Table 1). Median FEV_1 and MMEF values in patients with CAHR were significantly lower, indicating bronchial obstruction. These patients also had lower MEF_{50} (60 [56;87]%) and MEF_{75} (46 [42;54]%) compared to group 2 patients (76 [66;94]%, $p = 0.021$, and 61 [49;83]%, $p = 0.012$, respectively), suggesting the persistence of chronic inflammation in the small airways.

Table 1

Initial lung ventilation parameters and bronchial response to IHCA, $Me\ [Q_1; Q_3]$

Group	FEV_1 % predicted	FEV_1/ VC %	MMEF % predicted	ΔFEV_1 %
Group 1	84.0 [83.0; 93.0]	73.0 [70.0; 76.8]	55.0 [51.0; 67.0]	–16.0 [–19.0; –12.0]
Group 2	99.0 [85.0; 105.0]	78.1 [72.8; 82.4]	76.0 [59.0; 88.0]	–2.2 [–3.5; 0.2]
p between group 1 and group 2	0.012	0.165	0.021	0.0001

When assessing the cytokine content in the blood serum of patients in group 1, higher median values of IL-4 and IFNγ were registered compared to group 2

(Table 2). In the group of patients with CAHR, there was a positive correlation between IL-4 and IFNγ in the blood (Rs = 0.65; p = 0.016). Additionally, IL-4 was inversely correlated with the airway response to the bronchoprovocation test ($\Delta FEV_1/VC$) (Rs = –0.70; p = 0.007).

In the sputum of group 1 patients, a greater number of neutrophils and desquamated epithelial cells was observed (Table 3), and the levels of neutrophils, eosinophils and macrophages directly influenced the severity of the bronchoconstrictor response (ΔFEV_1) during the IHCA test (Rs = –0.50; p = 0.029; Rs = –0.51; p = 0.027; Rs = 0.56; p = 0.013, respectively). It is important to note that in this group there was an inverse correlation between the baseline FEV_1 value, reflecting bronchial patency, and the number of epithelial cells found in sputum (Rs = –0.74; p = 0.00003). Figures 1 and 2 illustrate the various degrees of destructive changes in epithelial cells.

Table 2

IL-4 and IFNγ content in the blood serum of asthma patients, *Me* [Q_1; Q_3], pg / ml

Group	IL-4	IFNγ	IL-4/IFNγ
Group 1	11.48 [10.82; 22.48]	26.98 [17.24;73.51]	0.43[0.31;0.70]
Group 2	1.88 [0.66; 5.96]	7.24 [1.54;26.98]	0.16[0.22;0.40]
p between group 1 and group 2	0.003	0.047	0.049

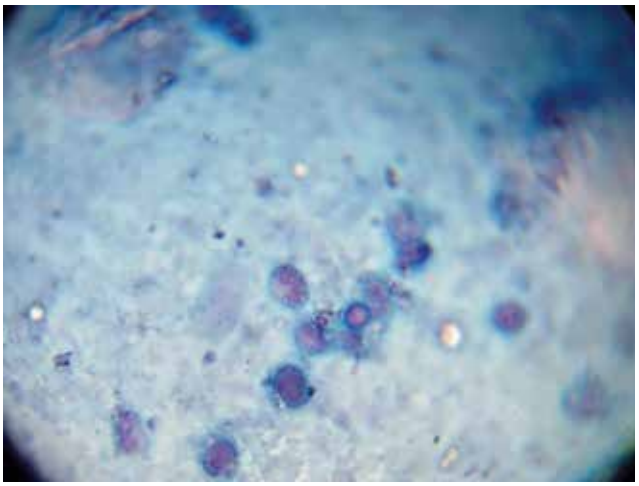

Fig. 1. In the center of the preparation, goblet cells show varying degrees of cytoplasmic and nuclear destruction. Toward the peripheral areas, there are fully destroyed epithelial cells with disintegration of the nucleus and cytoplasm, containing mucins. Here and in Fig.2, induced sputum smear from a patient with BA and CAHR. Stained with Alcian blue. Magnification x 1,250.

Table 3

Cellular composition of induced sputum in asthma patients, *Me* [Q_1; Q_3], %

Group	Neutrophils	Macrophages	Eosinophils	Epithelial cells
Group 1	22.5 [19.5; 26.3]	54.7 [45.6; 66.8]	19.8 [12.6; 21.2]	1.6 [1.2; 2.7]
Group 2	16.9 [15.4; 20.0]	60.4 [56.9; 67.6]	17.0 [3.0; 21.3]	0.2 [0.1; 1.0]
p between group 1 and group 2	0.049	0.119	0.112	0.0013

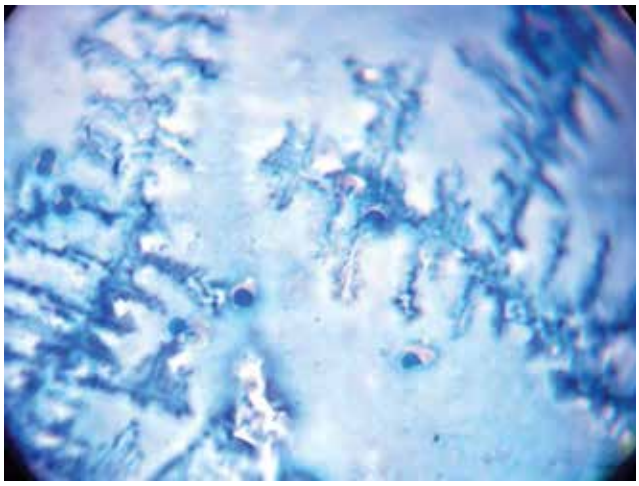

Fig. 2. Goblet cells containing large amounts of mucoproteins are located within an abundance of mucus exhibiting signs of biocrystallization

DISCUSSION

Airway remodeling in BA, affecting all parts of the walls of small bronchi, involves changes in the connective tissue due to fragmentation and homogenization of its fibrous framework by metalloproteinases, hyperproduction and accumulation of proteoglycans, increased fibroblastic synthesis, decreased extracellular matrix protein degradation, fibrillogenesis, and the development of subepithelial fibrosis and diffuse sclerosis. This process affects smooth muscle cells, transforming them from a contractile to a secretory and proliferative phenotype due to hypertrophy and hyperplasia occurring against the background of myofibroblast differentiation and enhanced angiogenesis mediated by vascular endothelial growth factor (VEGF) release. Additionally, epithelial lining disruptions occur in the form of desquamation and destruction of cells, exposing the hyalinized basement membrane, destroying ciliated epithelium, goblet cell hyperplasia, and metaplasia [2, 4].

IL-4 and IFNγ, whose main functions include mutual inhibition, belong to a wide range of cytokines involved in the disruption of the structural integrity of the epithelial barrier and causing the development of epithelial dysfunction of the bronchi [3]. In this study, we observed an increase in IL-4 and IFNγ levels in patients with CAHR compared to those who did not respond to cold air provocation (Table 2). IFNγ activity is linked to the weakening of the atopic phenotype of BA [6, 9] due to significant antagonistic role of the IFNγ/STAT1 signaling pathway (T-bet pathway) against GATA-3 expression, which suppresses Th1 development and activates Th2 proliferation [8–10]. IL-4 induces GATA-3 expression via the STAT6-dependent pathway, which suppresses Th1-specific transcription factors and stimulates Th2 cytokine synthesis, resulting in Th2-associated eosinophilic inflammation, epithelial destruction, secretory hyperplasia, and ciliary dysfunction, accompanying hyperresponsiveness and airway remodeling in BA [2,11,15].

A well-studied effect of Th2 cytokines, induced by IL-4, on the bronchial epithelium in BA is mucus hypersecretion [3, 15]. Increased expression and secretion of mucins MUC5AC, produced by goblet cells, and MUC5B, synthesized by glandular epithelium, intensifies as the disease progresses. This is accompanied by impaired tissue fluid circulation in the bronchi, dehydration of the mucin gel, increased viscosity due to elevated chondroitin sulfate levels, and decreased hyaluronic acid and heparin content in mucins, resulting in firmer adhesion of the gel to the epithelial surface. It has been shown that cold air induces MUC5AC hypersecretion by bronchial epithelium through TRPM8 ion channels [16].

In our previous studies, we demonstrated that BA patients with CAHR have an elevated baseline concentration of glycoproteins and glycosaminoglycans (GAGs) in the bronchial lining. After the IHCA test, simultaneously with the increase in the number of goblet cells and the generation of mucopolysaccharides, disorganization and desquamation of the epithelium, destruction and cytolysis of mucocytes intensify. [17]. During prolonged exposure to cold air *in vitro*, pronounced destructive changes were observed in ciliated epithelial cells, with positive staining for mucins and an abundance of mucus secretions containing high amounts of GAGs and microorganisms on the surface of the epithelial layer [17, 18].

It is suggested that mucous ciliated cells represent a molecular phenotype unique to the respiratory tracts of BA patients, wherein ciliated epithelial cells can express MUC5AC and other goblet cell-specific genes. These metaplastic cells, whose formation is induced by IL-4/IL-13 signaling, express IL-4/IL-13-induced genes and are considered transitional from the ciliated epithelium phenotype to the secretory cell phenotype [2]. In BA, IL-4/IL-13 signaling is linked to the stimulation of the Notch signaling pathway and high levels of Notch signaling, which lead to the activation of differentiation and an increase in the number of goblet cells that produce mucus [15].

Among our patients with CAHR, varying degrees of destructive changes were observed in epithelial cells synthesizing and secreting glycoproteins: from mild, with partial (no more than 1/2) cytoplasmic destruction and preservation of normal nuclear structure, to complete destruction with disintegration of the cytoplasm and nucleus (Fig. 1). In cases where fully destroyed cells containing mucins were found in the induced sputum smears (Fig. 1), it was difficult or impossible to differentiate them as goblet cells or ciliated epithelial cells that had undergone secretory metaplasia. The presence of goblet cell clusters containing large amounts of mucoproteins in abundant, viscous mucus (Fig. 2) in patients from group 1 indicated the development of pronounced mucociliary dysfunction, which exacerbated airway remodeling and obstruction and was associated with increased IL-4 levels in the cytokine profiles of these patients.

Epithelial desquamation in BA patients with CAHR was more intense compared to patients without cold-induced bronchoconstriction: a greater number of desquamated epithelial cells were found in the induced sputum of group 1 patients (Table 3), indicating increased damage to intercellular junctions and heightened epithelial barrier permeability in the bronchi in CAHR. Intercellular junctions in the bronchial epithelium include tight junctions (TJs), located at the apical surface, which contain proteins, such as claudins, occludins, and junctional adhesion molecules (JAMs), forming a multi-protein complex known as the zonula occludens (ZO); adherens junctions (AJs), which contain cadherins and catenins; desmosomes, connecting intermediate filaments of adjacent cells; and hemidesmosomes, anchoring basal cells and other epithelial cells to the basement membrane [6, 15].

The loss of several proteins from intercellular TJs and AJs is considered a key feature of airway hyperreactivity and remodeling in BA [2, 6]. Deficiency of E-cadherin, as the main membrane protein of AJs, is associated with desquamation of ciliated cells, exposure of the basement membrane, induction of proliferation of club cells, and suppression of their differentiation, leading to impaired epithelial repair and the development of proinflammatory and non-regenerative reactions in the airways [2].

It has been shown that IL-4 and IL-13 play a central role in inhibiting the surface expression of ZO-1, occludin, α-catenin, β-catenin, and E-cadherin in bronchial epithelial cells, with decreased levels of E-cadherin in sputum correlating with BA severity [6]. Clinical findings regarding role of IL-4 in disruption of the epithelial barrier in the airways align with *in vitro* studies, which show that the cytokine inhibits the expression of membrane components of AJs: when acting on the apical and basolateral monolayers of cultured epithelial cells, IL-4 increases paracellular permeability and decreases transepithelial resistance [19].

Higher IL-4 concentrations detected in group 1 patients compared to group 2 suggest that IL-4 is a triggering factor for barrier dysfunction and bronchial remodeling in patients with CAHR, associated with Th2-type allergic inflammation. In addition, the bronchial response to cold stimuli is linked to Th1 immune response, and the role of IFNγ in the development and exacerbation of bronchial remodeling and its connection to the neutrophil count, which was higher in the induced sputum of CAHR patients, should not be overlooked (Table 3).

IFNγ marks the Th1 immune response in non-allergic BA phenotypes, which is associated with chronic inflammation persistence, increased neutrophil survival, and activation of the neutrophil inflammatory component, while reducing atopic activity, a factor that contributes to the development of glucocorticoid resistance [20]. Neutrophil pool mobilization in CAHR patients was associated with induction of proinflammatory cytokines and chemokines that recruit neutrophils to the bronchial infiltrate. Neutrophil infiltration stimulated the persistence of chronic inflammation, culminating in diffuse interstitial sclerosis, leading to structural modification of the bronchi and progression of airway obstruction and remodeling.

IFNγ involvement in bronchial epithelial destruction was also linked to the impact of proinflammatory cytokines expressed under its influence, along with oxidative damage caused by reactive oxygen species (ROS) and other toxic metabolites. A critical factor in free-radical epithelial damage is the activation of the respiratory burst in macrophages, stimulated by IFNγ through the induction of cytosolic components of NADPH oxidase [8, 10, 21, 22], associated with IFNγ-regulated phagocyte differentiation. When IFNγ interacts with its receptor on macrophages, the T-bet signaling pathway is activated, which induces STAT1 target genes [22, 23] and polarizes lung interstitial macrophages, which interact with neutrophils in the Th1/Th17 cytokine cascade, into the classic M1 inflammatory phenotype [23, 24].

A possible reason for the lower median macrophage values in the sputum of CAHR patients (Table 3) may have been cytolysis resulting from the intensification of the respiratory burst induced by IFNγ. The escalation of proinflammatory and pro-oxidant functions of IFNγ in these patients indicates a shift in the balance of Th2 cytokine activation, regulated by IL-4, towards the Th1 immune response, which, alongside Th2 immune responses, contributes to airway remodeling in BA patients with CAHR.

CONCLUSION

Patients with BA and CAHR exhibit higher levels of IL-4, associated with increased desquamation, destruction, and marked secretory activity of bronchial epithelial cells, and IFNγ, linked to neutrophil pool mobilization and an increase in neutrophil counts in the inflammatory pattern of the bronchi. Desquamation, destruction, goblet cell hyperplasia and metaplasia, and mucus hypersecretion in the bronchial epithelium, stimulated by IL-4 activation and exacerbating mucociliary and barrier dysfunction, contribute to more pronounced airway obstruction in BA patients with CAHR.

The escalation of the proinflammatory and pro-oxidant functions of IFNγ in BA patients with CAHR indicates a shift from IL-4-regulated Th2 cytokine activation, traditionally responsible for structural reorganization of the bronchial walls in BA, toward a Th1 immune response, which stimulates bronchial remodeling in CAHR.

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Authors' contribution

Pirogov A.B. – conception, analysis of the data, drafting of the manuscript. Prikhodko A.G. – analysis of the manuscript, critical revision of the manuscript for important intellectual content. Pirogova N.A. – analysis of literature data, drafting of the manuscript. Naumov D.E. – analysis of the data, carrying out of biochemical studies. Gassan D.A. – carrying out of biochemical studies. Perelman J.M. – critical revision of the manuscript for important intellectual content, final approval of the manuscript for publication, responsibility for the integrity of all parts of the article.

Authors' information

Pirogov Aleksey B. – Cand. Sci. (Med.), Associate Professor, Senior Researcher, Laboratory of Functional Research of the Respiratory System, Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, dncfpd@dncfpd.ru, https://orcid.org/0000-0001-5846-3276

Prikhodko Anna G. – Dr. Sci. (Med.), Chief Researcher, Laboratory of Functional Research of the Respiratory System, Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, prih-anya@ya.ru, https://orcid.org/0000-0003-2847-7380

Pirogova Natalia A. – Cand. Sci. (Med.), Researcher, Laboratory of Functional Research of the Respiratory System, Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, dncfpd@dncfpd.ru, https://orcid.org/0000-0001-6350-7392

Gassan Dina A. – Cand. Sci. (Med.), Head of the Laboratory for Mechanisms of Virus-Associated Pathologies, Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, dani-shi@mail.ru, https://orcid.org/0000-0003-3718-9962

Naumov Denis E. – Cand. Sci. (Med.), Head of the Laboratory for Molecular and Translational Research, Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, denn1985@bk.ru, https://orcid.org/0000-0003-3921-8755

Perelman Juliy M. – Corresponding Member of the Russian Academy of Sciences, Dr. Sci. (Med.), Professor, Head of the Laboratory of Functional Research of the Respiratory System, Far Eastern Scientific Center of Physiology and Pathology of Respiration, Blagoveshchensk, jperelman@mail.ru, https://orcid.org/0000-0002-9411-7474

(✉) **Perelman Juliy M.,** jperelman@mail.ru

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