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РОЛЬ СУПРЕССОРА ЦИТОКИНОВОЙ СИГНАЛИЗАЦИИ SOCS2 В РЕГУЛЯЦИИ ПРОВОСПАЛИТЕЛЬНОЙ АКТИВНОСТИ КЛЕТОК ЦЕЛЬНОЙ КРОВИ ПОСЛЕ ПЕРЕНЕСЕННОЙ ИНФЕКЦИИ НИЖНИХ ОТДЕЛОВ РЕСПИРАТОРНОГО ТРАКТА

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The Role of SOCS2 Cytokine Signaling Suppressor in the Regulation of Pro-Inflammatory Activity of Whole Blood Cells after Lower Respiratory Tract Infection

Резюме

Цель исследования — изучение взаимосвязи содержания в мононуклеарных лейкоцитах цельной крови при пневмонии и у практически здоровых лиц супрессора цитокиновой сигнализации 2 (SOCS2) с продукцией цитокинов (ФНОα, TGFb, ИФНα, ИФНβ, ИФНγ, ИЛ-1β, ИЛ-2, ИЛ-4, ИЛ-5, ИЛ-10, ИЛ-12, ИЛ-17А, РАИЛ-1, RANTES) и отдельными факторами NF-kB и JAK/STAT-сигнальных путей (NF-kB2, p65, p50, STAT1, STAT3, STAT5B, STAT6). **Материалы и методы исследования.** Материалом исследования служили мононуклеарные клетки, выделяемые из образцов венозной крови, а также плазма крови практически здоровых лиц и больных пневмонией. В ядерно-цитоплазма-

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тических лизатах мононуклеарных клеток крови методом иммуноферментного анализа оценивали концентрацию компонентов ядерного фактора транскрипции NF-κB: p65, p50, NF-κB2, факторов STAT1, STAT3, STAT5B, STAT6, протеина SOCS2. Также определяли концентрацию ФНОα, ИЛ-1β, TGFb, ИФНα, ИФНβ, ИФНγ, ИЛ-1β, ИЛ-2, ИЛ-4, ИЛ-5, ИЛ-10, ИЛ-17А, РАИЛ-1, RANTES. **Результаты** проведенного исследования свидетельствует о том, что стадия реконвалесценции пневмонии сопровождается дисрегуляцией продукции основных провоспалительных цитокинов, проявляющейся снижением уровня ФНОα, TGFb, RANTES, ИЛ-4, ИЛ-17А, ИФНβ, ИФНγ и повышением продукции ИЛ-2 и ИФНα. На этом фоне отмечено снижение фосфорилирования факторов STAT3 и STAT4, а также снижение содержания в МНК протеинов p50 и p65. Указанные изменения ассоциировались с повышенным содержанием в МНК фактора SOCS2. Проведенный анализ показал, что повышение содержания в МНК SOCS2 от минимального уровня, определяющегося концентрацией, соответствующей 1 квартилю выборки (1,3 нг/мл) до максимального, определяющегося 4-м квартилем выборки (1,7 нг/мл) ассоциировано со снижением продукции ИЛ-1β, ИЛ-4, ИЛ-4, ИЛ-5, ИЛ-10, ИЛ-17А, TGFb, RANTES и ИФНβ на фоне повышения уровня ИНФα, ИНФγ и ИЛ-2. Изменения продукции цитокинов сопровождались повышением содержания STAT5B, STAT4 и NF-kB2 и снижением фосфорилирования STAT3. уменьшением содержания в клетке компонентов ядерного фактора транскрипции NF-κB, в частности, p50, p65. **Заключение.** Особенности взаимосвязей SOCS2 с исследуемыми факторами позволяет говорить о том, что его высокий уровень способствует ограничению продукции провоспалительных цитокинов, в особенности, продуцирующихся Т-хелперами 2 типа и Th17, стимулирует усиление чувствительности ИКК к ИЛ-2 и стимуляции Т-хелперов 1 типа. Указанные эффекты реализуются за счет повышения фосфорилирования факторов STAT5 и STAT4, снижения активности STAT3, изменения соотношения в клетке компонентов p50, p65 и NF-κB2 ядерного фактора транскрипции NF-κB.

Ключевые слова: *SOCS2, NF-κB, STAT3, STAT5, ИФНα, ИЛ-2, пневмония*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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Abstract

The aim of the investigation was to study the relationship between the content of whole blood in mononuclear leukocytes in pneumonia and in apparently healthy individuals of cytokine signaling suppressor 2 (SOCS2) with the production of cytokines (TNFα, TGFb, IFNα, IFNβ, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-10, IL-12, IL-17A, RAIL-1, RANTES) and individual factors of the NF-kB and JAK / STAT signaling pathways (NF-kB2, p65, p50, STAT1, STAT3, STAT5B, STAT6). **Materials and research methods.** The research material was mononuclear cells isolated from venous blood samples, as well as blood plasma of practically healthy individuals and patients with pneumonia. In nuclear-cytoplasmic lysates of mononuclear blood cells, the concentration of the components of the nuclear transcription factor NF-κB, p65, p50, NF-κB2, factors STAT1, STAT3, STAT5B, STAT6, and protein SOCS2, was assessed by enzyme immunoassay. We also determined the concentration of TNFα, IL-1β, TGFb, IFNα, IFNβ, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-10, IL-17A, RAIL-1, RANTES. **The results** of this study indicate that the stage of pneumonia convalescence is accompanied by dysregulation of the production of the main proinflammatory cytokines, manifested by a decrease in the level of TNFα, TGFb, RANTES, IL-4, IL-17A, IFNβ, IFNγ and an increase in the production of IL-2 and IFNα. Against this background, a decrease in the phosphorylation of the STAT3 and STAT4 factors was noted, as well as a decrease in the content of p50 and p65 proteins in MNCs. These changes were associated with an increased content of the SOCS2 factor in MNCs. The analysis showed that an increase in the content of SOCS2 in MNCs from the minimum level determined by the concentration corresponding to the 1st quartile of the sample (1.3 ng / ml) to the maximum, determined by the 4th quartile of the sample (1.7 ng / ml) is associated with a decrease in production IL-1β, IL-4, IL-4, IL-5, IL-10, IL-17A, TGFb, RANTES and IFNβ against the background of an increase in the level of INFα, INFγ and IL-2. Changes in cytokine production were accompanied by an increase in STAT5B, STAT4, and NF-kB2 levels and a decrease in STAT3 phosphorylation. a decrease in the content in the cell of the components of the nuclear transcription factor NF-κB, in particular, p50, p65. **Conclusion.** The peculiarities of the relationship of SOCS2 with the studied factors suggests that its high level helps to limit the production of proinflammatory cytokines, in particular those produced by type 2 T-helpers and Th17, stimulates an increase in ICC sensitivity to IL-2 and stimulation of type 1 T-helpers. These effects are realized due to an increase in the phosphorylation of the STAT5 and STAT4 factors, a decrease in the STAT3 activity, and a change in the ratio of the components p50, p65 and NF-κB2 of the nuclear transcription factor NF-κB in the cell.

Key words: *NF-kB, STAT3, STAT5, SOCS2, IFNa, IL-2, pneumonia*

Conflict of interests

The authors declare no conflict of interests

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BMI — body mass index; CAP — community-acquired pneumonia; CCL5 — chemokine (c-c motif) ligand 5; CRP — C-reactive protein; ICC — immunocompetent cells; GM-CSF — granulocyte-macrophage colony-stimulating factor; IFN — interferon; IL — interleukin; JAK — Janus kinase; LDH — lactate dehydrogenase; Me — sample median value; MNC — mononuclear cells; NF-κB — transcription factor nuclear factor-kappa B; p50, p52, p65 — subunits of nuclear factor-kappa B; PIAS — protein inhibitor of activated STAT; SOCS — suppressor of cytokine signaling proteins; STAT — signal transducer and activator of transcription; TGF — transforming growth factor; 25 %, 75 % — 25th and 75th percentiles of the sample; TNF — tumor necrosis factor

The reactivity of immunocompetent cells (ICC) with respect to cytokines is largely determined by the state of the JAK/STAT/SOCS signaling pathway [1]. By participating in the regulation of inflammatory response, this signaling pathway plays a critical role in the development and maintenance of the activity of adaptive immune response in cases of various infectious, autoimmune or allergic pathologies, ensuring the perception of signals by a cell and their transmission by cytokines to the executive apparatus [2–4]. The development of an infectious process that initiates an acute phase response is accompanied by the production of cytokines, such as tumor necrosis factor-alpha and interleukin-1, which ensure the activation of non-specific defense mechanisms, as well as innate and acquired immunity. The activation of NF-κB transcription factor, a dimer that may include subunits p65, p52, p50, etc., plays a key role in the production of these cytokines [1].

Exposure to these cytokines increases the production of cytokines that stimulate an adaptive immune response, particularly IL-2, IL-4, IL-5, interferon-gamma (IFNγ), IL-10, IL-17A, TGFβ1; the balance of their production determines the ratio between humoral and cell-mediated immune responses. In response to chemokines, particularly CCL5, activated cells of adaptive immune response are attracted to the inflammatory focus. Janus kinase 1 (JAK1) provides intracellular signal transmission from interferon receptors, cytokines of the IL-10 family, IL-6, JAK2 — IL-3, -5 receptors, erythropoietin, granulocyte-macrophage colony-stimulating factor (GM-CSF), etc., JAK3 — IL-2, IL-4, IL-7, etc. Therefore, phosphorylation in response to cytokine stimulation of Janus kinases, followed by phosphorylation and dimerization of transducers and activators of transcription (STAT), leads to the activation of this signaling pathway and differentiation of ICC, particularly T- and B-lymphocytes and NK cells, which largely determines the nature of the developing pathological process and its outcome [2, 4]. In addition, the activation of STAT1 factors in cells under the influence of interferons alpha and beta (IFNα, β) contributes to an increase in their antiviral protection due to the expression of specific proteins [3]. In turn, the activation of STAT3, -4, -6 determines the differentiation of different subpopulations of T helper cells (T helper cells of types 1 and 2, T helper cells 17, etc.), phosphorylation of STAT5 is required for the normal course of hematopoietic processes [2, 4].

It should also be noted that the activation of the signaling pathway under consideration plays an important role in repair processes, promoting the proliferation and differentiation of mesenchymal fibroblast progenitor cells, which is most important in the reparative phase of pathological processes [3].

Negative regulation of the immune response mediated by the JAK/STAT/SOCS signaling pathway is provided by a family of cytokine signaling suppressors, represented by SOCS1-7 and PIAS proteins capable of dephosphorylating Janus kinases and STAT factors, as well as other intracellular proteins, particularly components of NF-κB transcription factor [2, 4]. It should be noted that the production of SOCS proteins is stimulated by STAT factors [5–8]. Therefore, suppressors of cytokine signaling reduce the sensitivity of the corresponding cells to cytokines by regulating the activity of Janus kinases and STAT factors.

One of the regulators of the JAK/STAT signaling pathway is SOCS2 protein, which plays an essential role in the regulation of infectious inflammatory and autoimmune processes. Its role in the differentiation of T helper cells of types 1 and 2, T helper cells 17, and T regulatory cells was demonstrated. A low level of SOCS2 contributes to the differentiation of type 2 T helper cells and the development of an allergic response [9–12]. The important role of SOCS2 in the processes of sanogenesis during autoimmune inflammation in its recovery phase was also established [13]. Controlling the activity of SOCS proteins can be considered a promising therapeutic approach to the management of pathological conditions associated with increased activation of the JAK/STAT signaling pathway, as well as in patients with certain types of immunodeficiencies [14, 15].

Considering the important role of SOCS2 in the processes of sanogenesis and regulation of JAK/STAT activity, as well as of NF-κB signaling pathways, the aim of this study was to analyse the relationship between cytokine signaling suppressor SOCS2 in the MNC of patients with past community-acquired pneumonia and the blood levels of cytokines that regulate adaptive immune response, as well as JAK/STAT and NF-κB signaling pathway components in cells.

Material and methods

In accordance with the objective of this work, 45 male patients with non-severe bacterial community-acquired pneumonia were examined on days 13–15 of the disease before their discharge from the hospital; they were the test group. The diagnosis was confirmed in accordance with national guidelines for the diagnosis of pneumonia [16]. The control group included 15 apparently healthy young individuals who were blood donors.

Clinical features and laboratory test results of the examined individuals are presented in Table 1.

S. pneumoniae was found during the microbiological test of sputum samples in 34 (76 %) patients of the test group; S. aureus was found in three cases (6.7 %); E. coli — in one case (2.2 %). The causative agent of the disease in other patients was not identified. All patients of the test group received antibiotic therapy. Empiric antibiotic therapy in cases of non-severe pneumonia included protected aminopenicillins (amoxicillin / clavulanic acid, or amoxicillin/sulbactam; in case of intolerance, respiratory fluoroquinolones — levofloxacin). In severe cases, combined antibiotic therapy was prescribed, which included third-generation cephalosporins (ceftriaxone or cefotaxime) and respiratory fluoroquinolones (levofloxacin or moxifloxacin) at medium therapeutic doses. Antibacterial therapy was adjusted according to the results of bacteriological tests. According to indications, patients also received symptomatic, respiratory and infusion therapy [16].

The material for the study of cytokines and immunoregulatory factors was venous blood taken in the morning from the cubital vein of examined individuals. For the detection of intracellular markers, 1 mL of whole blood was put into a vial with 4 mL of DMEM medium, heparin (2.5 U/mL), gentamicin (100 μg/mL) and L-glutamine (0.6 mg/mL), followed by isolation of MNC using ficoll-verographin density-gradient separation (ρ = 1.077) and preparation of cell lysates [5, 8]. Nuclear cytoplasmic lysates of MNC were assessed for the concentration of the components of NF-κB transcription factor — p65, p50, p52, signal transducers and activators of transcription (STAT) — STAT1, STAT3, STAT5B, STAT6, as well as suppressor of cytokine signaling proteins

Table 1. *Clinical and laboratory characteristics of the examined persons*

Characteristic	Main group (n = 45)	Control group (n = 15)
Age (years), mean (minimum, maximum)	25 (18 — 42)	26 (18 — 44)
Gender, n (%)		
Male	24 (53,3)	9 (60,0)
Female	21 (46,7)	6 (40,0)
Concomitant pathology, n (%)		
Obesity (BMI >35 kg/m^2)	8 (17,8)	3 (20,0)
Chronical bronchitis	7 (15,6)	2 (13,3)
Arterial hypertension	4 (8,8)	1 (6,7)
Diabetes	5 (11,1)	2 (13,3)
Clinical symptoms, n (%)		
Fever	38 (84,4)	-
Cough	30 (66,7)	-
Chest pain	5 (11,1)	-
Dyspnea	22 (48,8)	-
Laboratory indicators, n (%)		
Leukocytosis >12.0×10^9 /l	40 (88,9)	-
CRP >10 mg/l	45 (100,0)	-
Urea >7 mmol/l	14 (31,1)	-
LDH >300 mg/l	16 (35,6)	-
Saturation less than 90 %	11 (24,4)	-
Severity of condition on admission, n (%)		
Non-Severe Condition	32 (71,1)	-
Grave condition	13 (28,9)	-
X-ray symptoms, n (%)		
Alveolar type of infiltration	38 (84,4)	-
Focal type of infiltration	7 (15,6)	-
Unilateral lesion within 1-2 segments of the lung	36 (80,0)	-
Polysegmental lesion	5 (11,1)	-
Bilateral localization	8 (17,8)	-
Exudative pleurisy	6 (13,3)	-
The presence of residual (small) forms of infiltration at discharge (low-intensity focal, peribronchial infiltration, increased vascular pattern)	5 (11,1)	-

Note: BMI — body mass index, CRP — C-reactive protein, LDH — lactate dehydrogenase

(SOCS) SOCS2 by enzyme immunoassay (ELISA). The concentration of tumor necrosis factor-alpha (TNFα), interleukins (IL) — IL-1β, IL-4, IL-5, IL-10, IL-17A, interferons (IFN) — IFNα, IFNβ, IFNγ, and CCL5 chemokine was defined in cell supernatants. ELISA was performed using Cusabio Biotech (PRC) reagent kits. Statistical analysis was performed using Statistica 7.0 software. The study results are presented as follows: median (Me); 25th and 75th percentiles (25 %, 75 %). The statistical significance (p) for intergroup differences was assessed using the Mann-Whitney U-test.

Results

The analysis revealed that the recovery phase of CAP was characterized by a statistically significant decrease in the production of TNFα, CCL5, IL-4, IL-17A, IFNβ, and increased production of IFNα. In this context, in MNC, there was decreased phosphorylation of STAT3 factors with increased levels of STAT5B and STAT1, as well as decreased levels of proteins p50, p65, JAK1 protein kinase in cells and increased levels of JAK3 and SOCS2. It should be noted that there was no statistically significant difference in the production of IL-1β, IL-5 and IL-10, as well as the level of JAK2, STAT4, STAT6 and p52 factors in MNC in convalescents and apparently healthy individuals.

The results of the study are presented in Table 2.

Therefore, the production of cytokines that regulate adaptive immune response is inhibited in CAP convalescents, which is associated with decreased intracellular levels of certain components of NF-κB and the JAK/STAT signaling pathway. In this context, there is a statistically significant increase in the production of IL-2 and phosphorylation of STAT5B factor, as well as the level of Janus kinase 3 in MNC. The data obtained indicate a decrease in the activity of type 2 T helper cells and T helper cells 17 (Th17), as well as in the sensitivity of MNC to IL-2, IL-4, IL-7, IL-15, and IL-21 in convalescents. It should be noted that the changes found are associated with an increased SOCS2 / STAT3 ratio.

In accordance with the objective of this study, depending on the level of SOCS2 in the MNC of CAP

Table 2. *The level of the studied factors in the groups*

Исследуемый фактор/ Researched factor	Группа контроля/ Control group	Основная группа/ Main group	Δ, %	p
	Me (25; 75 %)	*Me* (25; 75 %)		
TNFa, pg/ml	15,3 (14,7; 16,3)	14,0 (13,6; 14,9)	-8,5	0,047
CCL5, pg/ml	7,36 (6,3; 8,7)	6,5 (5,8; 7,1)	-11,7	0,0001
IL-1b, pg/ml	16,1 (12,7; 18,1)	14,7 (12,5; 17,4)	-8,7	0,58
IL-4, pg/ml	3,15 (2,7; 3,4)	2,50 (2,0; 2,6)	-20,6	0,0001
IL-5, pg/ml	2,47 (2,0; 3,1)	2,42 (2,2; 2,8)	-2,0	0,71
IL-10, pg/ml	13,4 (12,9; 15,7)	14,1 (12,8; 16,0)	5,2	0,56
IL-17A, pg/ml	2,59 (2,3; 2,8)	2,24 (1,9; 2,4)	-13,5	0,0023
IFNa, pg/ml	11,4 (10,1; 13,1)	17,2 (15,5; 19,6)	50,9	0,0001
IFNb, pg/ml	2,46 (2,0; 2,8)	1,84 (1,7; 1,9)	-25,2	0,005
IFNg, pg/ml	3,1 (2,9; 3,9)	3,06 (2,8; 3,3)	-1,3	0,31
STAT5B, ui/ng	0,73 (0,6; 0,8)	1,45 (0,9; 1,7)	98,6	0,0001
STAT6, ui/ng	2,35 (2,2; 2,5)	2,21 (2,0; 2,9)	-6,0	0,26
STAT1, ui/ng	1,1 (0,8; 1,6)	1,37 (1,1; 1,5)	24,5	0,06
STAT3, ui/ng	1,42 (1,0; 2,1)	1,13 (1,0; 1,5)	-20,4	0,051
STAT4, ui/ng	0,8 (0,7; 1,4)	0,86 (0,7; 1,1)	7,5	0,96
p50, ng/ml	0,73 (0,7; 0,8)	0,68 (0,4; 0,7)	-6,8	0,002
p65, ng/ml	0,58 (0,5; 0,7)	0,56 (0,3; 0,6)	-3,4	0,03
p52, ng/ml	0,75 (0,68; 0,82)	0,71 (0,67; 0,87)	-5,3	0,88
JAK1, ng/ml	52,0 (51,3; 52,5)	51,2 (50,7; 52,7)	-1,5	0,05
JAK2, ng/ml	5,28 (4,9; 5,4)	5,3 (5,1; 5,4)	0,4	0,18
JAK3, ng/ml	24,8 (22,5; 25,0)	26,27 (24,2; 27,0)	5,9	0,007
SOCS2, ng/ml	1,38 (1,31; 1,4)	1,59 (1,5; 1,7)	15,2	0,0001
SOCS2 / STAT3, ui.	1,07 (0,66; 1,41)	1,33 (1,09; 1,77)	24,3	0,0001

Note: Δ is the difference in the concentration of the studied factors in the first and third subgroups against the background of low and high levels of SOCS2, respectively (%); Me, 25 %, 75 % — median, percentile values of the sample, IL-1β — interleukin 1 beta, IL-4 — interleukin-4, IL-5 — interleukin-5, IL-10 — interleukin-10, IL-17A — interleukin 17A, IFNα — interferon alpha, IFNβ — interferon beta, IFNγ — gamma interferon, STAT1 — signal transducer and transcription activator 1, STAT3 — signal transducer and transcription activator 3, STAT4 — signal transducer and transcription activator 4, STAT5B — signal transducer and transcription activator 5B, STAT6 — signal transducer and transcription activator 6, p50 — p50 subunit of nuclear transcription factor NF-kB, p52 — p52 subunit of nuclear transcription factor NF-kB, p65 — p65 subunit of nuclear transcription factor NF-kB, JAK1 — Janus kinase 1, JAK2 — Janus kinase 2, JAK3 — Janus kinase 3, SOCS2 — cytokine signaling suppressor 2

convalescents (Table 1), the test group was divided into three subgroups. Subgroup 1 included MNC samples with SOCS2 levels less than 1.48 pg/mL (corresponding to the 1st quartile of the sample); subgroup 2 included samples with levels from 1.48 pg/mL to 1.66 pg/mL, which corresponds to the 2nd quartile of the sample; subgroup 3 included samples with SOCS2 levels of more than 1.66 pg/mL, which corresponds to the 3rd quartile. Therefore, subgroup 1 includes samples with a minimum level of SOCS2, subgroup 2 corresponds to the average values, and subgroup 3 represents samples with the maximum level of the studied factor in the sample population.

The concentration of the studied factors depending on the SOCS2 level in MNC is presented in Table 3.

The analysis showed that the increased level of the suppressor of cytokine signaling proteins SOCS2 in MNC contributed to a decrease in the production of IL-1β, IL-4, IL-5, IL-10, IL-17A, CCL5, and IFNβ. At the same time, the increased level of SOCS2 was associated with a statistically significant increase in the production of IFNα and IL-2. It should be noted that the production of IL-4, IL-5, IL-17A, and CCL5 decreased most significantly in connection with the increased level of SOCS2.

These changes were accompanied by the increased phosphorylation of factors STAT5B and STAT4, increased level of the component of NF-κB transcription factor — p52 and Janus kinase 3 in MNC, a decrease in STAT3 phosphorylation and the levels of factors p50 and p65, as well as Janus kinase 1. It should be noted that the suppression of the production of key cytokines that determine the activity of adaptive response in CAP convalescents compared with practically healthy individuals is a potentially unfavorable factor in terms of the development of recurrent pneumonia and other infectious and inflammatory pathologies [16, 17].

Results of this study suggest that SOCS2 is one of the regulators of the activity of intracellular signaling pathways, which affects not only the production of cytokines (primarily IL-5, IL-12, IL-17A, CCL5), but also ICC reactivity to them, which is determined by changes in the phosphorylation of STAT5B, STAT3, and STAT4 factors, as well as the level of NF-κB nuclear factor components in cells, particularly p50, p65, and NF-κB2 [15].

Table 3. *The level of the studied factors depending on the content of the SOCS2 protein in the MNC*

Factor	Subgroup № 1 n = 16	Subgroup № 2 n = 15	Subgroup № 3 n = 14	Δ, %	p
	Me (25 % 75 %)	*Me* (25 % 75 %)	*Me* (25 % 75 %)		
IL-1b, pg/ml	16,1 (15,8; 16,3)	15,9 (11,9; 18,3)	13,6 (12,4; 17,0)	-15,5	0,028
IL-4, pg/ml	2,72 (2,3; 3,2)	2,62 (2,4; 3,1)	2,24 (1,9; 2,5)	-17,6	0,0028
IL-5, pg/ml	2,9 (2,3; 3,5)	2,38 (2,1; 2,8)	2,37 (2,0; 2,5)	-18,3	0,0028
IL-10, pg/ml	15,2 (12,8; 17,5)	14,1 (12,9; 14,9)	13,5 (12,8; 15,4)	-11,2	0,29
IL-17A, pg/ml	2,57 (2,3; 2,8)	2,36 (2,2; 2,6)	1,87 (1,6; 2,3)	-27,2	0,0005
IFNg, pg/ml	2,89 (2,7; 3,1)	3,02 (2,8; 3,3)	3,19 (2,8; 3,4)	10,4	0,053
TNFa, pg/ml	14,7 (14,2; 15,2)	15,0 (14,3; 17,2)	13,7 (13,6; 14,4)	-6,8	0,005
CCL5, pg/ml	8,65 (8,1; 9,2)	6,84 (5,9; 7,1)	5,97 (5,7; 6,5)	-31,0	0,0001
IFNa, pg/ml	11,0 (9,6; 12,4)	15,5 (13,8; 17,1)	17,4 (15,6; 19,6)	58,2	0,0002
IFNb, pg/ml	2,04 (1,5; 2,5)	1,93 (1,8; 2,3)	1,72 (1,7; 1,8)	-15,7	0,87
STAT5B, ui/ng	0,81 (0,8; 0,8)	0,86 (0,7; 1,5)	1,45 (1,0; 1,7)	79,0	0,0028
STAT6, ui/ng	2,34 (2,1; 2,5)	2,24 (2,0; 2,4)	2,47 (2,0; 2,9)	5,6	0,26
STAT1, ui/ng	1,31 (0,8; 1,8)	1,44 (0,9; 1,5)	1,33 (1,1; 1,4)	1,5	0,88
STAT3, ui/ng	1,68 (1,0; 2,4)	1,37 (1,1; 1,9)	1,01 (0,9; 1,3)	-39,9	0,08
STAT4, ui/ng	0,72 (0,7; 0,8)	0,89 (0,7; 1,6)	1,00 (0,8; 1,1)	38,9	0,0005
p50, ng/ml	0,74 (0,6; 0,8)	0,68 (0,6; 0,7)	0,68 (0,4; 0,7)	-8,1	0,06
p65, ng/ml	0,62 (0,5; 0,7)	0,49 (0,3; 0,6)	0,56 (0,5; 0,6)	-9,7	0,051
p52, ng/ml	0,68 (0,6; 0,7)	0,74 (0,7; 0,9)	0,78 (0,6; 0,9)	14,7	0,038
JAK1, ng/ml	51,3 (51,3; 51,4)	52,0 (51,2; 52,5)	50,9 (50,4; 52,5)	-0,8	0,004
JAK2, ng/ml	5,05 (4,6; 5,5)	5,20 (5,1; 5,3)	5,36 (5,2; 5,4)	6,1	0,53
JAK3, ng/ml	25,0 (24,8; 25,2)	24,7 (23,7; 26,3)	26,3 (23,4; 27,0)	5,2	0,02
SOCS2, ng/ml	1,3 (1,26; 1,33)	1,47 (1,4; 1,5)	1,66 (1,6; 1,7)	27,7	0,001
SOCS2 / STAT3, ui.	0,97 (0,53; 1,42)	1,18 (0,78; 1,38)	1,68 (1,20; 1,91)	73,2	0,0001

Note: Δ is the difference in the concentration of the studied factors in the first and third subgroups against the background of low and high levels of SOCS2, respectively (%); Me, 25 %, 75 % — median, percentile values of the sample, IL-1β — interleukin 1 beta, IL-4 — interleukin-4, IL-5 — interleukin-5, IL-10 — interleukin-10, IL-17A — interleukin 17A, IFNα — interferon alpha, IFNβ — interferon beta, IFNγ — gamma interferon, STAT1 — signal transducer and transcription activator 1, STAT3 — signal transducer and transcription activator 3, STAT4 — signal transducer and transcription activator 4, STAT5B — signal transducer and transcription activator 5B, STAT6 — signal transducer and transcription activator 6, p50 — p50 subunit of nuclear transcription factor NF-kB, p52 — p52 subunit of nuclear transcription factor NF-kB, p65 — p65 subunit of nuclear transcription factor NF-kB, JAK1 — Janus kinase 1, JAK2 — Janus kinase 2, JAK3 — Janus kinase 3, SOCS2 — cytokine signaling suppressor 2

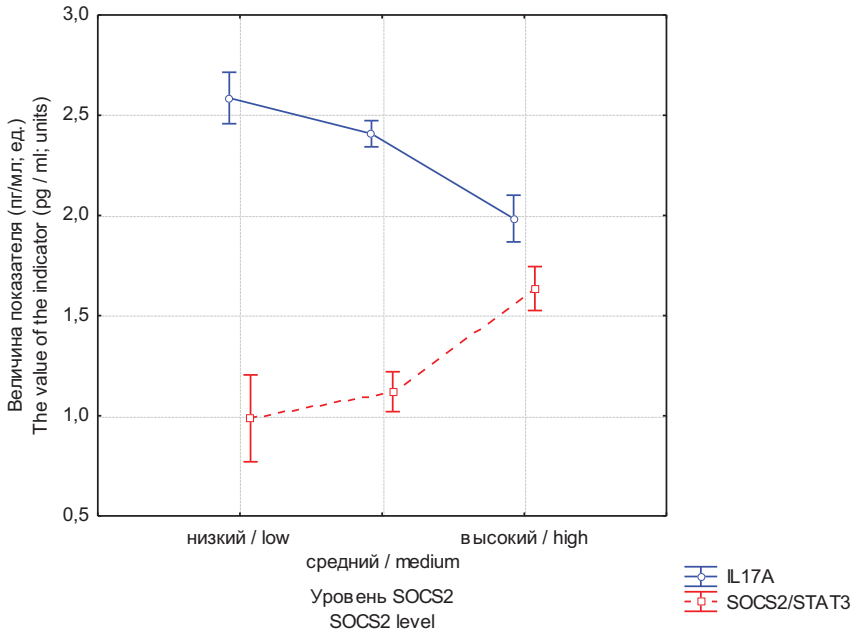

Figure 1. *Dynamics of SOCS2 / STAT3 ratio and IL-17A production depending on the content of SOCS2 protein in MNCs*

Figure 1 demonstrates changes in the SOCS2 / STAT3 ratio and IL-17A production depending on the level of SOCS2 protein in cells.

Graphical analysis of changes in the SOCS2 / STAT3 ratio and IL-17A production suggests a functional relationship between them, as indicated by the mirror reflection of the graphs. The SOCS2 / STAT3 ratio under normal conditions and in apparently healthy individuals is close to 1.0 and is significantly higher in patients after CAP. An increase in this ratio of more than 1.0, accompanied by a proportional decrease in the production of IL-17A, one of the key cytokines that protect the lower respiratory tract from bacterial infection, below the level of healthy individuals, indicates the development of an immunosuppressive response in such patients. Therefore, the results of this study suggest that the SOCS2/STAT3 ratio in the range of physiological values typical for healthy individuals, i.e., 0.66–1.41, determines the optimal reactivity of ICC. An increase in this ratio is associated with the suppression of the activity of T helper cells 17.

Therefore, this suggests that the observed features of the cytokine profile in patients after pneumonia can be largely determined by changes in the level of cytokine signaling suppressors in MNC; in particular, they can be associated with an increased level of SOCS2.

Discussion

The results obtained suggest that the recovery stage of CAP progresses with underlying suppression of the activity of the monocyte and macrophage pool of immunocompetent cells, as well as T helper cells, which can be considered as signs of dysregulation in connection with excessive suppression of immune response. Clearly, one of the mechanisms of the observed phenomenon is the decreased activity of NF-κB transcription factor and certain STAT proteins. Also, the identified changes that limit the effectiveness of both innate and acquired mechanisms of infectious immunity are one of the predisposing factors for reinfection and superinfection [6, 9, 17].

Under these conditions, the ability of the suppressor of cytokine signaling proteins SOCS2 to modulate the activity of the JAK/STAT signaling pathway and NF-κB transcription factor, thereby regulating the pro-inflammatory reactivity of ICC and their sensitivity to cytokines, was demonstrated. Moreover, the anti-inflammatory effect of SOCS2, which is clearly determined by its effect on the level of certain components of NF-κB transcription factor, is combined with an immunoregulatory effect, which is expressed in the change in the phosphorylation of certain STAT factors, which, in turn, determines changes in the sensitivity of ICC to cytokines and the formation of stimuli to the differentiation and proliferation of the corresponding ICC populations, including T helper cells [9–12, 16, 17]. A relatively high level of SOCS2 is associated with decreased STAT3 phosphorylation, which is accompanied by a decrease in the production of IL-17A, which indicates the decreased activity of T helper cells 17. In turn, increased STAT4 phosphorylation determines an increase in IFNγ production and activation of type 1 T helper cells. The level of the components of NF-κB transcription factor in cells can decrease due to the stimulation of ubiquitinylation processes and their subsequent proteasomal degradation under the action of SOCS2 [18, 19]. It is apparent that the immunosuppressive effects that develop in patients after CAP and determine the decreased reactivity of their adaptive immune response can be determined by the emerging balance of SOCS2/STAT3 activity in MNC.

The effect of SOCS2 on physiological processes in MNC can be demonstrated using the diagram shown in Fig. 2.

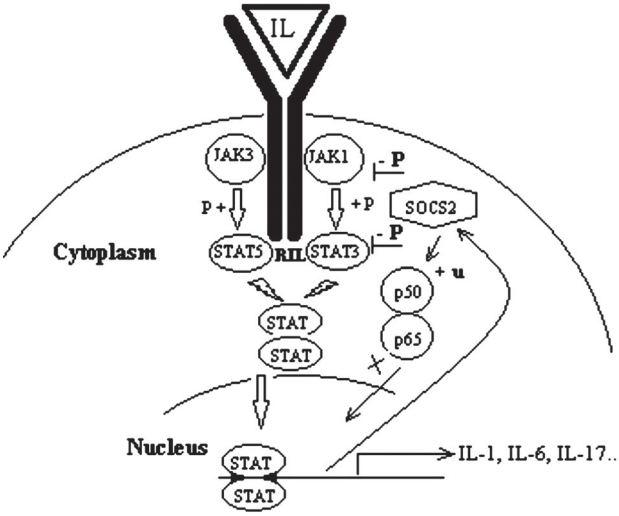

Figure 2. *Possible mechanism of immunoregulatory influence of SOCS2*

Note: IL — interleukins, RIL — receptor for type I and III interleukins, Cytoplasm — cell cytoplasm, Nucleus — cell nucleus, +P — phosphorylation, -P — dephosphorylation, + u — ubiquitinylation, x — blocking of translocation to the nucleus

The data obtained in this study, including those indicating the important role of the JAK/STAT signaling pathway and the SOCS2/STAT3 balance in the regulation of sanogenesis in CAP convalescents, enable to consider these factors as potential therapeutic targets; the impact on them can increase the activity of sanogenesis processes, as well as the restoration of impaired immunological reactivity at the stage of rehabilitation of patients after pneumonia [14, 20–22]. It is clear that the restoration of the initial reactivity of ICC in CAP convalescents determines the normal restoration of tissue repair and regeneration processes, and is also a factor preventing the development of recurrent infectious diseases, including recurrent pneumonia, as well as superinfections [17].

Conclusion

1. The recovery stage of community-acquired pneumonia progresses with underlying dysregulation of the production of pro- and anti-inflammatory cytokines, as well as impaired functional state of the JAK/STAT signaling pathway. An increased level of cytokine signaling suppressor SOCS2 in MNC in patients with pneumonia is associated with decreased production of IL-1β, IL-4, IL-5, IL-10, IL-17A, CCL5, and IFNβ in connection with increased levels of IFNα, IFNγ and IL-2. Changes in the production of these cytokines were accompanied by increased levels of STAT5B, STAT4 and p52 and decreased levels of JAK1 and STAT3.

2. Analysis of the specific features of the relationship between SOCS2 and the studied factors revealed that its high level helped limit the production of pro-inflammatory cytokines and increase the sensitivity of ICC to IL-2, as well as enhance the proliferation and differentiation of type 1 T helper cells. These effects are brought into action by increasing the phosphorylation of STAT5 and STAT4 factors and changes in the ratio of the components of NF-κB transcription factor: p50, p65, and p52. However, the overexpression of this factor is associated with the inhibition of IL-17A production, which may contribute to the weakening of the anti-infectious protection of the lower respiratory tract.

3. The suppressor of cytokine signaling proteins SOCS2 can be considered a potential therapeutic target in terms of the management of immunopathological disorders associated with the development of immunosuppression or excessive activation of the immune system.

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