

ACTIVITY OF NUCLEAR FACTOR κ B IN LYMPHOCYTE POPULATIONS OF CHILDREN WITH PSORIASISKuptsova DG¹✉, Petrichuk SV¹, Murashkin NN^{1,2,3}, Kurbatova OV¹, Radygina TV¹, Khotko AA², Ivanov RA¹¹ National Medical Research Center for Children's Health, Moscow, Russia² Central State Medical Academy of the Administrative Department of the President of the Russian Federation, Moscow, Russia³ I.M. Sechenov First Moscow State Medical University, Moscow, Russia

Alterations in intracellular signaling pathways affecting immune cell activation, proliferation and differentiation of keratinocytes in psoriasis could explain the complex pathogenesis of the disease. NF- κ B is one of the intracellular signaling pathways, which is involved in regulation of numerous pro-inflammatory genes, and affects the synthesis of pro-inflammatory cytokines directly involved in the development of psoriasis. The study was aimed to assess the number of cells with NF- κ B translocation in lymphocyte populations of children with psoriasis depending in the disease severity and therapy. A total of 130 children with psoriasis vulgaris were examined. The comparison group included 30 healthy children. The study was conducted using the imaging flow cytometry Amnis ImageStreamX system. It was found that there were significant differences in the number of cells with NF- κ B translocation in the lymphocyte populations of both children with psoriasis and comparison group. Children with psoriasis had a higher number of cells with NF- κ B translocation in the populations of T helper cells, T_{act}⁺ T_{reg}⁺, and Th17 compared to healthy children ($p < 0.05$). The number of cells with NF- κ B translocation in children with psoriasis correlated with the disease severity PASI ($R_{\text{mul}} = 0.32$) and BSA ($R_{\text{mul}} = 0.31$) scores, as well as with the disease duration ($p < 0.05$). NF- κ B determination could be considered an additional criterion for the disease severity assessment in children with psoriasis. The differences in the degree of reduction of the number of cells with NF- κ B translocation 24 h after administration of biologics (adalimumab, etanercept, ustekinumab) have been shown. Studying NF- κ B in cell populations offers the prospect of understanding pathogenetic mechanisms of inflammation and developing new therapeutic methods for psoriasis.

Keywords: children, psoriasis vulgaris, PASI, BSA, lymphocyte, NF- κ B, biologics**Finding:** the study was part of the state assignment for the Ministry of Health of the Russian Federation, № АААА-А19-119013090093-2.**Acknowledgements:** the authors wish to thank all the patients for active cooperation and express their thanks to the researchers of the Laboratory of Immunology and Virology, as well as to dermatologists and nurses at the Dermatology Department, National Medical Research Center for Children's Health, Moscow, Russia, who contributed to the study.**Author contribution:** Kuptsova DG, Petrichuk SV — study concept and design, experimental data acquisition and analysis, statistical analysis, manuscript writing and editing; Kurbatova OV, Radygina TV — experimental data acquisition, manuscript editing; Murashkin NN, Khotko AA, Ivanov RA — data analysis, manuscript editing.**Compliance with ethical standards:** the study was approved by the Ethics Committee of the National Medical Research Center for Children's Health (protocol № 2 dated February 14, 2020), conducted in accordance with the principles of the Declaration of Helsinki, and registered with ClinicalTrials.gov ID: NCT04989296. Parents of all children and adolescents enrolled submitted the informed consent to medical intervention in hospital settings, personal data processing and the use of data for scientific purposes.✉ **Correspondence should be addressed:** Darya G. Kuptsova
Lomonosovsky prospect, 2, str. 1, Moscow, 119296; dg.kuptsova@gmail.com**Received:** 11.02.2022 **Accepted:** 03.03.2022 **Published online:** 20.03.2022**DOI:** 10.24075/brsmu.2022.012АКТИВНОСТЬ ЯДЕРНОГО ФАКТОРА ТРАНСКРИПЦИИ κ B В ПОПУЛЯЦИЯХ ЛИМФОЦИТОВ У ДЕТЕЙ С ПСОРИАЗОМД. Г. Купцова¹✉, С. В. Петричук¹, Н. Н. Мурашкин^{1,2,3}, О. В. Курбатова¹, Т. В. Радыгина¹, А. А. Хотко², Р. А. Иванов¹¹ Национальный медицинский исследовательский центр здоровья детей, Москва, Россия² Центральная государственная медицинская академия Управления делами Президента РФ, Москва, Россия³ Первый Московский государственный медицинский университет имени И. М. Сеченова, Москва, Россия

Изменения в путях передачи внутриклеточных сигналов, влияющих на активацию иммунных клеток, пролиферацию и дифференцировку кератиноцитов при псориазе, могут объяснить сложный патогенез заболевания. Одним из путей передачи внутриклеточных сигналов является NF- κ B, участвующий в регуляции большого количества провоспалительных генов и влияющий на продукцию провоспалительных цитокинов, непосредственно участвующих в развитии псориаза. Целью исследования было оценить число клеток с транслокацией NF- κ B в популяциях лимфоцитов у детей с псориазом в зависимости от тяжести заболевания и проводимой терапии. Обследовано 130 детей с вульгарным псориазом. В группу сравнения вошли 30 здоровых детей. Исследование проводили методом проточной цитометрии с визуализацией Amnis ImageStreamX. Показано, что число клеток с транслокацией NF- κ B достоверно различалось в популяциях лимфоцитов как у детей с псориазом, так и в группе сравнения. У детей с псориазом выявлено повышение числа клеток с транслокацией NF- κ B в популяциях T-хелперов, T_{act}⁺ T_{reg}⁺ и Th17 по сравнению со здоровыми детьми ($p < 0,05$). Число клеток с транслокацией NF- κ B у детей с псориазом соотносится с тяжестью состояния по PASI ($R_{\text{mul}} = 0,32$), BSA ($R_{\text{mul}} = 0,31$) и длительностью заболевания ($p < 0,05$). Определение NF- κ B может быть рассмотрено как дополнительный критерий оценки тяжести состояния у детей с псориазом. Показаны различия в степени снижения числа клеток с транслокацией NF- κ B через сутки после введения биологической терапии (адалимумаб, этанерцепт, устекинумаб). Исследование активации NF- κ B в популяциях клеток открывает перспективы понимания патогенетических механизмов воспаления и разработки новых методов лечения псориаза.

Ключевые слова: дети, вульгарный псориаз, PASI, BSA, лимфоциты, NF- κ B, биологические препараты**Финансирование:** исследование выполнено в рамках государственного задания Минздрава России, № темы АААА-А19-119013090093-2.**Благодарности:** авторы выражают благодарность за активное сотрудничество всем участвовавшим пациентам и выражают признательность научным сотрудникам лаборатории иммунологии и вирусологии, а также врачам-дерматологам и медицинским сестрам отделения дерматологии ФГБУ «Национального медицинского исследовательского центра здоровья детей» г. Москва, Россия, принимавших участие в этом исследовании.**Вклад авторов:** Д. Г. Купцова, С. В. Петричук — концепция и дизайн исследования, набор экспериментальных данных и анализ результатов, статистическая обработка данных, написание и редактирование рукописи; О. В. Курбатова, Т. В. Радыгина — набор экспериментальных данных, редактирование рукописи; Н. Н. Мурашкин, А. А. Хотко, Р. А. Иванов — анализ результатов, редактирование рукописи.**Соблюдение этических стандартов:** исследование одобрено этическим комитетом ФГАУ «НМИЦ здоровья детей» (протокол № 2 от 14 февраля 2020 г.), проведено в соответствии с этическими принципами Хельсинкской декларации, зарегистрировано в ClinicalTrials.gov ID: NCT04989296. Родители всех детей и подростков, участвовавших в исследовании, подписали добровольное информированное согласие на медицинское вмешательство в стационаре, обработку персональных данных и использование данных в научных целях.✉ **Для корреспонденции:** Дарья Геннадьевна Купцова
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Psoriasis is a multifactorial immune-mediated inflammatory skin disorder with a complex pathogenesis [1–3], caused by interactions of immune system, psoriasis susceptibility loci, psoriasis autoantigens, and numerous environmental factors [4]. Psoriasis has various cutaneous clinical manifestations, based on which a number of disease forms is distinguished. Psoriasis vulgaris, being the most common form, is diagnosed in 90% cases [5, 6]. Psoriasis is characterized by hyperproliferation of keratinocytes, blood vessel dilation and leukocyte cell infiltration in skin dermis, and skin plaques [7].

It is believed that synthesis of pro-inflammatory cytokines, being the pathogenic cascade triggers, by activated T cells plays a key role in the skin inflammation associated with psoriasis [8]. Disease begins with T cell activation by unknown antigen or gene product. After the T cell activation, a number of pro-inflammatory cytokines and chemokines are secreted [9]. Cytokine action contributes to proliferation of keratinocytes, epidermal hyperplasia, neutrophil migration, enhanced T helper type 1 (Th1) cell response [10]. The increase in the number of T helper 17 cells (Th17) in blood and skin of patients with psoriasis with the progression of skin lesions was reported [11–13]. It was also found that elevated blood counts of regulatory T cells (Treg) in patients with psoriasis did not suppress inflammatory response due to the cells' functional failure [14, 15].

Complex pathogenesis of psoriasis could be explained by alterations in the intracellular signaling pathways. Impaired regulation of the signal transduction pathways affects activation, proliferation and differentiation of keratinocytes, associated with psoriasis [16, 17]. The nuclear factor κ B (NF- κ B) pathway, which has been first discovered in mature B cells by R. Sen and D. Baltimore, is one of the intracellular signaling pathways [18]. NF- κ B is capable of binding with the gene promoter encoding the immunoglobulin — light chain. NF- κ B is a family of structurally similar transcription factors, such as RelA (p65), NF- κ B1 (p50/p105), NF- κ B2 (p52/p100), c-Rel, and RelB. The NF- κ B family members function as various hetero- and homodimers (p50-p65; RelB-p100), except for RelB. The main active form of NF- κ B is a complex formed by subunit p65 and subunit p50 or p52 bound to the I- κ B inhibitor protein [19]. The advanced Amnis ImageStreamX imaging flow cytometry system makes it possible to assess the percentage of cells with NF- κ B translocation (% of activated cells with nuclear localization of NF- κ B) in various populations [20, 21].

Under physiological conditions, the NF- κ B/I- κ B complex is a self-regulating system [22]. NF- κ B activation occurs in response to the wide range of stimuli [23]. Stimulus activates the NF- κ B signaling pathway, which leads to the NF- κ B release from the inhibitor complex and NF- κ B translocation from cytoplasm into cell nucleus. NF- κ B is involved in regulation of numerous pro-inflammatory genes and affects synthesis of pro-inflammatory cytokines, such as IL1 β , IL6, IL8, TNF α , directly involved in the development of psoriasis [24–26].

Multiple studies confirmed impaired regulation of the NF- κ B signaling pathways associated with psoriasis in *in vitro* models, animal models, and skin biopsy samples, obtained from patients with psoriasis [10, 25, 26]. Furthermore, anti-TNF therapy and glucocorticoid therapy reduce the levels of active NF- κ B and related elements of the signaling pathway [27, 30]. Currently, the effects of other targeted biologics on the variation in the number of cells with NF- κ B translocation are under study, however, chronic NF- κ B pathway inhibition may reduce therapeutic efficacy in patients with immune-mediated diseases [28]. Thus, quantification of cells with NF- κ B translocation could be a promising tool for evaluation of the disease severity and therapeutic efficacy in patients with

immune-mediated diseases. The study was aimed to quantify cells with NF- κ B translocation in the lymphocyte populations of children with psoriasis depending on the disease severity and therapy.

METHODS

Clinical characteristics of patients

A total of 130 children and adolescents with psoriasis vulgaris were enrolled in the study, of them 42 patients were followed-up. The comparison group included 30 healthy children. The surveyed children's age was 1–18 years: psoriasis — Me 12.5 [8.3; 15.5], comparison group — Me 12.4 [7.4; 16.1]. There were 62 girls and 68 boys in the group with psoriasis, and 18 girls and 12 boys in the comparison group. All children were of European origin. Inclusion criteria: proven case of psoriasis vulgaris. Exclusion criteria: other forms of psoriasis, 18 years of age or more, inability to obtain blood sample.

Severity of psoriasis was assessed in all patients using the PASI (Psoriasis Area and Severity Index) and BSA (Body Surface Area, %) indices. Children with psoriasis received different pathogenetic therapy in accordance with the guidelines and psoriasis severity. Children were prescribed topical corticosteroids and emollients with keratolytic effects ($n = 41$), systemic methotrexate therapy ($n = 28$), genetically-engineered biological drugs (GEBD; $n = 61$). The effects of GEBD were assessed in 42 patients with psoriasis before and 24 hours after administration of the following drugs: adalimumab ($n = 24$), etanercept ($n = 8$), ustekinumab ($n = 10$).

Isolation of peripheral blood mononuclear cells

To isolate the peripheral blood mononuclear cells (PBMCs), the 5 mL fasting whole blood samples were used, collected in the morning into EDTA tubes. Blood samples were processed on the same day. Whole blood was diluted with warm glutamine-free RPMI 1640 medium (PanEco; Russia) in a ratio of 1 : 3, gently layered over 2 mL of the Histopaque-1.077 g/cm³ medium (Sigma-Aldrich; USA), then centrifuged at 2000 RPM for 20 min at room temperature. Buffy coat was collected into 15 mm Falcon tubes (BD; USA) and washed with RPMI medium for 8 min under the same conditions. After that supernatant was drained, and the cells were diluted with the RPMI-1640 medium to the desired volume.

Lymphocyte immunophenotyping and evaluation of NF- κ B translocation from cytoplasm into nucleus

Cell populations were isolated by step-wise gating using monoclonal antibodies (mAbs) labeled with fluorochromes (Beckman Coulter; USA): CD19-PE, CD4-PE, CD8-PE, CD (16/56)-PE, CD127-PE, CD161-PE, CD3-ECD, CD4-PB, CD25 PE-Cy7. Cells with NF- κ B translocation were quantified in lymphocyte populations with the imaging flow cytometry Amnis ImageStreamX Mk II platform (Luminex; USA) using the Amnis NF- κ B Translocation Kit (Luminex; USA), which included antibody Anti-Hu NF- κ B (p50) conjugated to Alexa Fluor 488 for detection of NF- κ B and the 7-AAD nuclear dye. The tubes, containing 100 μ L of PBMCs, were added 10 μ L of mAb and incubated for 20 min in a dark place, then NF- κ B translocation was assessed in accordance with the manufacturer's instructions. The following cell populations were assessed: CD3⁺ (T cells); CD3⁺CD4⁺ (T helper cells); CD3⁺CD8⁺ (cytotoxic T cells); CD3⁺CD19⁺ (B cells); CD3⁺CD16⁺/CD56⁺ (natural killer

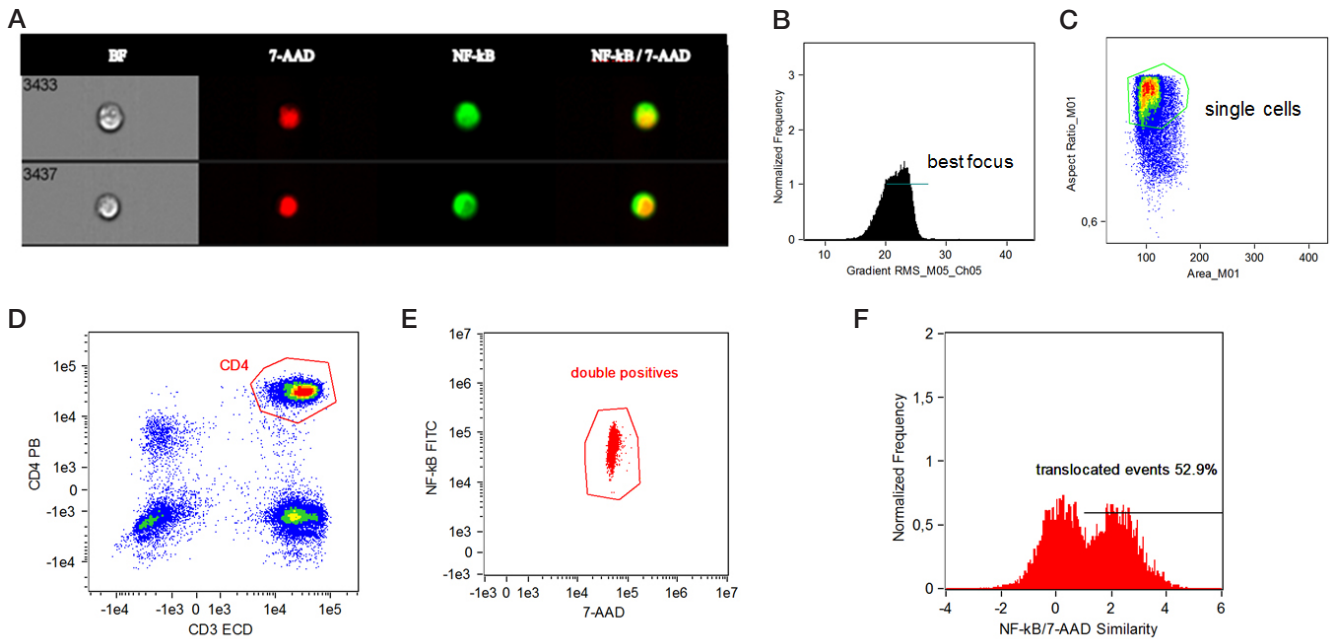


Fig. 1. Stages of analysis when quantifying cells with NF-κB translocation. **A.** Gallery of recorded cell images. **B.** Isolation of cells in good focus based on Gradient RMS. **C.** Isolation of single events. **D.** Isolation of the studied lymphocyte population, particularly CD3⁺CD4⁺ (CD3_ECD — channel 4; CD4_PB — channel 3). **E.** Isolation of double-positive cells: NF-κB+/7-AAD+ (NF-κB_FITC — channel 2; 7-AAD — channel 5). **F.** Isolation of the proportion of cells with NF-κB translocation based on Similarity>1

cells); CD3⁺CD16⁺/CD56⁺ (natural killer T cells); CD3⁺CD4⁺CD8⁻ (immature T cells); CD4⁺CD25⁺CD127^{high} (activated T helper cells, T_{act}); CD4⁺CD25⁺CD127^{low} (T_{reg}); CD4⁺CD161⁺CD3⁺ (Th17).

Data recording and analysis with the ImageStream Mk II Imaging Flow Cytometer

Cell imaging and recording were performed with the ImageStream Mk II Imaging Flow Cytometer using the INSPIRE™ software (Luminex; USA) at 40x magnification and low flow rate. Image recording involved collecting at least 20,000 events per sample in the lymphoid region for the major lymphocyte populations, and at least 40,000 events for the T_{reg}, T_{act} and Th17 populations.

The images acquired were analyzed using the IDEAS® ImageStreamX software (Luminex; USA) in accordance with the algorithm provided in Fig. 1. Data analysis started with digital pixel-based spectral compensation applied to each cell image. After compensation, single cells were analyzed with good focus, and single events were isolated (Fig. 1B, C). The percentage of the NF-κB+/7-AAD+ double-positive cells was defined based on fluorescence intensity (Fig. 1D, E). At the next stage, Similarity was assessed, which reflected the degree of the NF-κB and 7-AAD colocalization in the studied population

regardless of morphology (Fig. 1F). Similarity was calculated based on the Pearson correlation coefficient as described earlier [20, 21].

Statistical analysis

Statistical processing of the research data was performed using the Statistica 10.0 software package (StatSoft; USA). Descriptive statistics of quantitative indicators was presented as median (lower and upper quartiles), Me (Q_{0.25}; Q_{0.75}). The nonparametric Mann–Whitney U test was used to assess the significance of differences between groups. The stepwise multiple regression method with exceptions was used to obtain regression equations. The differences were considered significant at *p* < 0.05.

RESULTS

Assessment of lymphocyte populations with NF-κB translocation in children with psoriasis and comparison group

There was a significant increase in the percentage of cells with NF-κB translocation in the populations of T helper cells (*p* = 0.001),

Table 1. Number of cells with NF-κB translocation in children with psoriasis and comparison group

Population	Patients with psoriasis (n = 130)	Healthy children (n = 30)	<i>p</i>
B cells	48.3 [38;64]	58.4 [40;72]	0.322
T cells	19.9 [17;26]	18.0 [16;21]	0.113
T helper cells	20.1 [17;26]	18.4 [14;20]	0.001
Cytotoxic T cells	18.0 [15;24]	17.2 [15;23]	0.402
Immature T cells	24.4 [18;36]	23.7 [19;33]	0.473
NK cells	28.0 [19;39]	29.9 [18;42]	0.829
NKT cells	22.3 [16;34]	20.1 [17;25]	0.315
Activated T helper cells	18.7 [15;24]	15.2 [14;18]	0.012
Regulatory T cells	23.0 [19;32]	20.3 [16;26]	0.032
T helper 17 cells	20.7 [17;27]	19.6 [17;23]	0.034

Note: *p* — significance of differences between groups, Mann–Whitney U test.

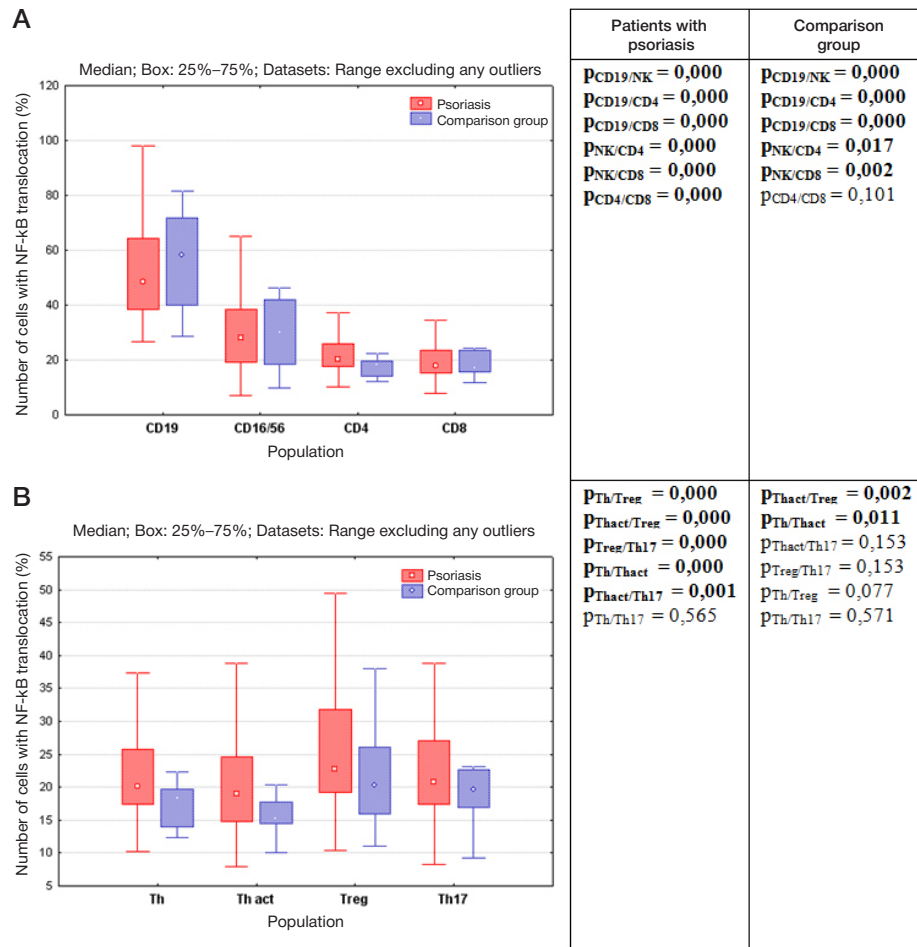


Fig. 2. Number of major (A) and minor (B) populations of lymphocytes and NK cells with NF-κB translocation in children with psoriasis and comparison group. *p* — significance of differences between populations, Wilcoxon signed rank test for dependent variables

T_{act} ($p = 0.012$), T_{reg} ($p = 0.032$), and Th17 ($p = 0.034$) in children with psoriasis compared to healthy children (Table 1). In children with psoriasis, an upward trend was observed in the percentage of cells with NF-κB translocation in the populations of T cells, cytotoxic T cells, and natural killer T (NKT) cells, however, in the populations of B cells and natural killer (NK) cells there was a downward trend. The largest proportion of cells with NF-κB translocation was revealed in B cell populations of both children with psoriasis and comparison group (Table 1).

Analysis revealed differences in the percentage of cells with NF-κB translocation in the studied cell populations of surveyed children. The number of cells with NF-κB translocation was significantly ($p = 0.000$) higher in B cell population compared to NK cells, T helper cells, cytotoxic T cells, and in the NK cell population it was significantly ($p = 0.000$) higher compared to the populations of T helper cells and cytotoxic T cells in both children with psoriasis and comparison group; moreover, it was 2.5 times higher compared to other cell populations (Table 1; Fig. 2A). In the group

Table 2. Correlations of the number of cells with NF-κB translocation in lymphocyte populations with the patients' age and the duration of psoriasis

Population	Psoriasis (n = 130)				Comparison group (n = 30)	
	Age		Disease duration		Age	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
B cells	0.09	0.299	-0.02	0.796	0.25	0.182
T cells	0.21	0.016	0.21	0.22	0.28	0.142
T helper cells	0.22	0.011	0.21	0.022	0.25	0.194
Cytotoxic T cells	0.19	0.031	0.19	0.003	0.32	0.09
Immature T cells	0.2	0.028	0.21	0.021	0.24	0.214
NK cells	0.24	0.006	0.27	0.002	0.11	0.577
NKT cells	0.16	0.071	0.15	0.083	0.41	0.052
Activated T helper cells	0.21	0.023	0.16	0.109	0.16	0.433
Regulatory T cells	0.2	0.031	0.15	0.116	0.18	0.383
T helper 17 cells	0.23	0.015	0.21	0.021	0.46	0.015

Note: *r* — Pearson correlation coefficient, *p* — probability of non-zero regression coefficients.

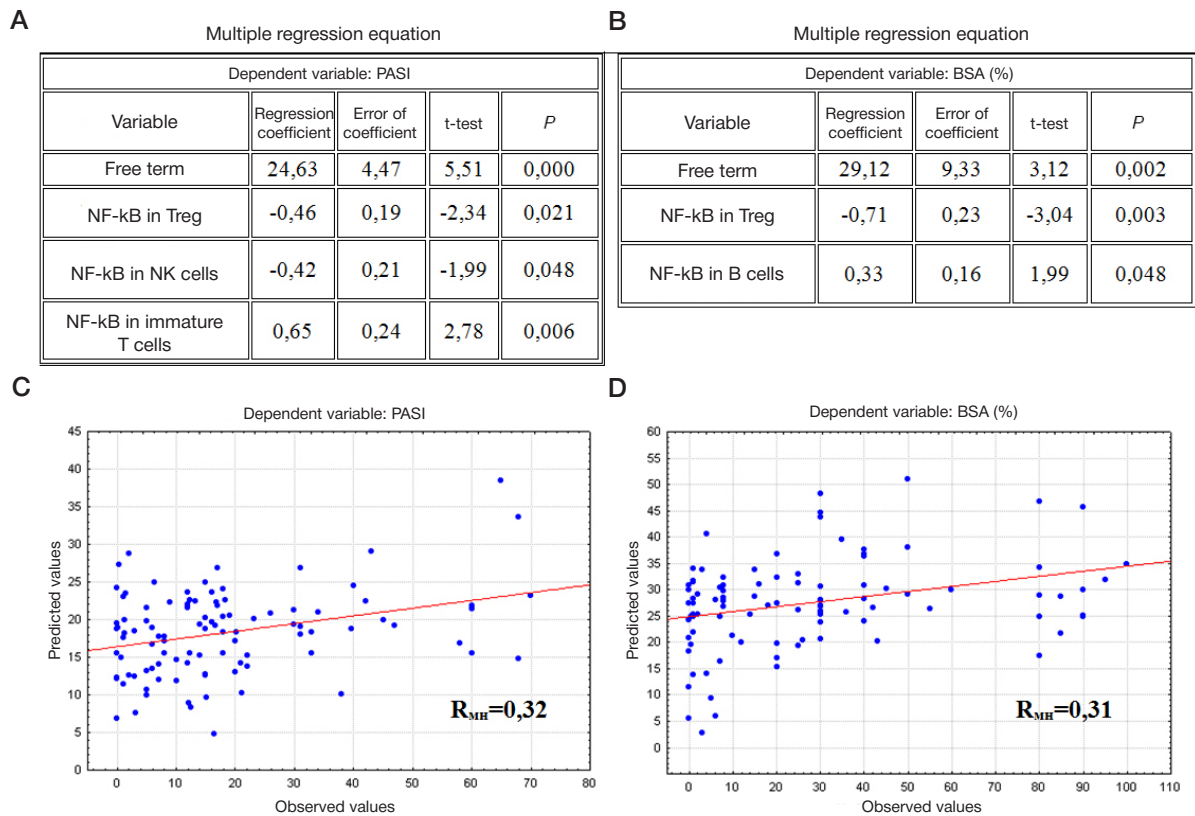


Fig. 3. Multiple regression equation for the relationship between PASI (A) and BSA (B) scores and the number of cells with NF-κB translocation in children with psoriasis, and the relationship between the observed and predicted values calculated using the regression equation for PASI (C) and BSA (D) indices. Regression equations were constructed using the stepwise multiple regression method with exceptions. R_{mir} — coefficient of multiple correlation, R ; p — significance of non-zero regression coefficients

of healthy children, the number of cells with NF-κB translocation in the populations of T helper cells and cytotoxic T cells was the same ($p = 0.101$). In contrast to healthy children, the increased percentage of cells with NF-κB translocation in the population of T helper cells compared to cytotoxic T cells was revealed in patients with psoriasis ($p = 0.000$; Fig. 2A).

In the group of healthy children, it was found that the average number of cells with NF-κB translocation in T cell populations, including T_{reg} , Th17, and T_{act} , was 15κ20%. The largest proportion of cells with NF-κB translocation was found in the T_{reg} population (20% of cells), and the lowest proportion was observed in the T_{act} population (15% of cells; $p = 0,011$), the difference was considered significant ($p = 0.002$; Fig. 2B).

In children with psoriasis, the number of cells with NF-κB translocation in the T_{reg} , Th17, and T_{act} populations was significantly higher compared to the comparison group (Table 1). In healthy children, the highest percentage of cells with NF-κB translocation was also found in the T_{reg} population (23% of cells), and was significantly higher than in the populations of T helper cells, Th17, and T_{act} ($p = 0.000$; Fig. 2B). The number of T_{act} with NF-κB translocation was significantly lower compared to the T helper cell population, just like in the group of healthy children ($p = 0.000$; Fig. 2B). In contrast to healthy children, the number of Th17 with NF-κB translocation was significantly higher compared to the T_{act} population ($p = 0.001$).

Table 3. Number of cells with NF-κB translocation in children with psoriasis before and 24 h after administration of GEBD

Population	Number of cells with NF-κB translocation								
	Adalimumab (n = 24)			Etanercept (n = 8)			Ustekinumab (n = 10)		
	before	after	<i>p</i>	before	after	<i>p</i>	before	after	<i>p</i>
B cells	46.0 [39; 65]	38.2 [33; 45]	0.002	36.7 [33; 42]	28.0 [27; 31]	0.004	51.7 [35; 71]	44.8 [32; 51]	0.217
T cells	20.2 [17; 25]	19.5 [15; 21]	0.12	17.0 [16; 20]	17.6 [15; 19]	0.721	24.9 [18; 36]	18.2 [16; 25]	0.105
T helper cells	20.2 [18; 24]	18.3 [16; 21]	0.025	19.0 [18; 21]	17.5 [16; 19]	0.049	24.8 [19; 32]	19.6 [16; 25]	0.191
Cytotoxic T cells	19.2 [16; 24]	17.2 [15; 18]	0.021	16.30 [16; 18]	16.6 [15; 18]	0.99	23.9 [19; 35]	16.6 [14; 25]	0.052
Immature T cells	22.5 [16; 31]	21.2 [20; 38]	0.814	21.2 [19; 24]	19.2 [17; 23]	0.161	36.2 [30; 48]	21.4 [14; 30]	0.009
NK cells	27.7 [18; 32]	26.2 [19; 29]	0.574	30.6 [29; 36]	30.7 [27; 33]	0.721	37.9 [35; 42]	25.3 [17; 29]	0.018
NKT cells	21.6 [15; 27]	19.7 [15; 24]	0.418	20.9 [18; 23]	18.4 [17; 21]	0.131	30.0 [26; 44]	24.7 [13; 31]	0.052
Activated T helper cells	19.1 [16; 21]	17.1 [14; 20]	0.165	19.7 [16; 24]	14.3 [14; 16]	0.007	24.4 [17; 34]	16.4 [13; 24]	0.042
Regulatory T cells	19.1 [16; 21]	20.5 [19; 23]	0.005	23.2 [19; 28]	19.4 [19; 21]	0.105	34.9 [22; 48]	21.5 [15; 29]	0.018
T helper 17 cells	22.4 [18; 23]	18.5 [16; 21]	0.032	18.6 [18; 20]	17.2 [15; 18]	0.021	27.3 [27; 39]	19.1 [14; 25]	0.011

Note: p — significance of differences in the numbers of cells with NF-κB translocation obtained before and 24 h after administration of GEBD, Mann–Whitney U test.

Correlations of the number of cells with NF- κ B translocation with the patients' age and the duration of psoriasis

Analysis of the relationship between the number of cells with NF- κ B translocation and the healthy children's age revealed no correlations in all cell populations, except for T helper 17 cells, the number of which increased with age (Table 2). In children with psoriasis, the proportion of cells with NF- κ B translocation increased with age in all populations, except for B cells and NKT cells. With increased disease duration, the number of cells with NF- κ B translocation significantly increased in the populations of T helper cells, cytotoxic T cells, immature T cells, NK cells and Th17 (Table 2). In children with psoriasis, correlation between the patient's age and the disease duration was revealed ($p = 0.000$): the older the child, the longer he was ill.

Relationship between the content of cells with NF- κ B translocation and psoriasis severity in children

PASI and BSA indices were evaluated in the surveyed children with psoriasis. The psoriasis severity PASI scores in children varied between 0–70% (Me 13.3 [5; 22]), and BSA scores varied between 0–100% (Me 20 [4; 40]).

Multiple correlation analysis of the relationship between the psoriasis severity and the number of cells with NF- κ B translocation showed the increase in the PASI score with the decrease in the number of T_{reg} and NK cells with NF- κ B translocation and the increase in the proportion of immature T cells with translocation ($R_{mul} = 0.32$; Fig. 3A). The increased BSA scores are associated with the decreased number of cells with NF- κ B translocation in the T_{reg} population and increased number in B cell population ($R_{mul} = 0.31$; Fig. 3B).

Assessing the number of cells with NF- κ B translocation in children with psoriasis receiving various types of therapy

Comparison of the proportion of cells with NF- κ B translocation in lymphocyte populations of children with psoriasis receiving various types of therapy revealed no significant differences between groups of children receiving different therapy. However, maximum deviation from healthy children was observed in the group of children, who received biological therapy. The number of cells with NF- κ B translocation in the T helper cell populations of children, who received topical therapy, increased by 6%, in the group of children, who received methotrexate, it increased by 10%, and in the group of children, who received biologics, by 11.5% compared to healthy children. The number of cells with NF- κ B translocation in B cell population decreased by 17% in patients, who received topical therapy, by 4% in patients, who received methotrexate, and by 29% in children, who received biologics, compared to healthy children.

To assess the effects of GEBD on the number of cells with NF- κ B translocation, we examined 42 children with psoriasis before and 24 h after administration of adalimumab, etanercept, ustekinumab. A significantly decreased number of cells with NF- κ B translocation in the populations of B cells, T helper cells, cytotoxic T cells, T_{reg} , and Th17 was observed 24 h after administration of adalimumab (Table 3).

The significantly decreased number of cells with NF- κ B translocation was revealed in the populations of B cells, T helper cells, T_{act} and Th17 after administration of etanercept. The significantly decreased number of NF- κ B cells was observed in the populations of NK cells, immature T cells, T_{act} , T_{reg} , and Th17 after administration of ustekinumab (Table 3).

It is interesting to note that biologics, adalimumab and etanercept, significantly decreased the number of B cells with NF- κ B translocation after 24 h: by 20.4 and 31%, respectively. However, administration of ustekinumab resulted in the insignificantly decreased number (Table 3). The same downward trend was observed in the T helper cell population after administration of adalimumab (by 10.4%) and etanercept (by 8.6%). Significant alterations in the number of cytotoxic lymphocytes with NF- κ B translocation were observed after administration of adalimumab (Table 3).

The number of Tact with NF- κ B translocation significantly decreased 24 h after administration of etanercept (by 37.8%) and ustekinumab (by 48.7%) (Table 3). Only the administration of ustekinumab resulted in the decrease in the number of T_{reg} with NF- κ B translocation by 62%, however, administration of adalimumab resulted in the significant decrease in their number by 6%. Administration of all biologics resulted in the decreased number of Th17 with NF- κ B translocation: the maximum decrease was observed after administration of ustekinumab (by 42.9%), and administration of adalimumab and etanercept resulted in the decrease by 21.1 and 8%, respectively (Table 3).

Furthermore, only administration of ustekinumab decreased the number of cells with NF- κ B translocation in the population of NK cells by 49.8% and in the population of immature T cells by 69%.

DISCUSSION

Alterations in signaling pathways, such as NF- κ B, JAK-STAT, Janus kinase (JAK)/signal transducer pathway, Akt and Wnt signaling pathways, are observed in individuals with psoriasis [16, 17]. The number of cells with NF- κ B translocation in children with psoriasis was assessed using the ImageStreamX advanced technique. Our findings about the number of cells with NF- κ B translocation are in line with the data reported by other researchers: the maximum number of cells with NF- κ B translocation has been revealed in B cell population [18].

Our study has shown that in children with psoriasis, the number of cells with NF- κ B translocation in the populations of T helper cells, T_{reg} , T_{act} , and Th17 is increased compared to healthy children. That could explain the effects of the NF- κ B signaling pathway activation in these cells, which are responsible for production of pro-inflammatory cytokines directly involved in pathogenesis of psoriasis [24–26].

There was a direct correlation between the number of cells with NF- κ B translocation and the children's age in children with psoriasis; the proportion of such cells in healthy children did not change with age. Probably, our findings about the relationship between the number of cells with NF- κ B translocation and age in children with psoriasis are attributable to the correlation between the duration of psoriasis and the surveyed children's age.

The number of T_{reg} with NF- κ B translocation decrease with the increase in the psoriasis severity in children according to PASI and BSA, which is in line with the other researchers' report of the decreased T_{reg} functional activity in psoriasis [27]. This fact confirms the importance of assessing both T_{reg} number and functional activity [14, 15]. As one of the factors involved in the cell functional activity regulation, NF- κ B determination could be considered an additional criterion for the disease severity evaluation in patients with psoriasis.

Our findings are in line with the data on the NF- κ B activation reduction by anti-TNF therapy (adalimumab) in adults with psoriasis [28–30]. The maximum decrease in the number of cells with NF- κ B translocation in the populations of NK cells, immature T cells, T_{act} , T_{reg} , and Th17 of children with

psoriasis was detected when using ustekinumab. The use of TNF α and IL12/23 blockers reduces the number of cells with NF- κ B translocation to varying degrees, depending on the cell population and therapy applied. Taking into account the findings and the minimally invasive method of assessing the number of cells with NF- κ B translocation in the whole blood of children with psoriasis, we consider this line of research to be perspective for diagnosis of the disease severity and predicting therapeutic efficacy.

CONCLUSIONS

Significant differences between lymphocyte populations by the number of cells with NF- κ B translocation were revealed

in both children with psoriasis and comparison group. The increased number of cells with NF- κ B translocation in the populations of T helper cells, T_{act}⁺, T_{reg}⁺, and Th17 in children with psoriasis compared to healthy children was observed. The number of cells with NF- κ B translocation correlates with the disease severity and duration. NF- κ B determination could be considered an additional criterion for evaluation of the disease severity in children with psoriasis. The number of cells with NF- κ B translocation in lymphocyte populations decreases 24 h after administration of GEBD and depends on the antipsoriatic therapy targets. Studying NF- κ B activation in cell populations offers the prospect of understanding pathogenetic mechanisms of inflammation and developing new treatment methods for psoriasis.

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