

Metabolism of lymphocyte populations in healthy children and patients with immune-dependent diseases

SCO — краткое сообщение

<https://doi.org/10.53529/2500-1175-2023-2-50-53>

S. V. Petrichuk, O. V. Kurbatova, D. G. Kuptsova, T. V. Radygina, E. V. Freidlin

National Medical Research Center for Children's Health, Moscow, 119296, Lomonosovsky prospect, 2, building 1, Russia

Keywords: immuno metabolism, lymphocytes, flow cytometry, mitochondrial enzymes, children, inflammatory bowel diseases, psoriasis.

For citation: Petrichuk SV, Kurbatova OV, Kuptsova DG, Radygina TV, Freidlin EV. Metabolism of lymphocyte populations in healthy children and patients with immune-dependent diseases. *Allergology and immunology in pediatrics*. 2023; 2: 50–53. <https://doi.org/10.53529/2500-1175-2023-2-50-53>

Метаболизм популяций лимфоцитов у здоровых детей и пациентов с иммунозависимыми заболеваниями

<https://doi.org/10.53529/2500-1175-2023-2-50-53>

С. В. Петричук, О. В. Курбатова, Д. Г. Купцова, Т. В. Радыгина, Е. В. Фрейдлин

ФГАУ «Национальный медицинский исследовательский центр здоровья детей» Министерства здравоохранения РФ, г. Москва, 119991, Ломоносовский пр., 2, стр. 1, Россия

Ключевые слова: иммунометаболизм, лимфоциты, проточная цитометрия, митохондриальные ферменты, дети, воспалительные заболевания кишечника, псориаз.

Для цитирования: Петричук СВ, Курбатова ОВ, Купцова ДГ, Радыгина ТВ, Фрейдлин ЕВ. Метаболизм популяций лимфоцитов у здоровых детей и пациентов с иммунозависимыми заболеваниями. *Аллергология и иммунология в педиатрии*. 2023; 2: 50–53. <https://doi.org/10.53529/2500-1175-2023-2-50-53>

Immunometabolism (ИМ) is a new field of studying metabolic processes in cells of innate and adaptive immunity. Processes of metabolic reprogramming of anaerobic glycolysis, oxidative phosphorylation (OXPHOS) and synthesis of metabolites when activating immune cells are important in regulating homeostasis, activation, proliferation and differentiation of cells [1]. Metabolic changes in response to various stimulatory signals are crucial in infection, inflammation, cancer, autoimmune disease and metabolic disorders [1]. It is known that each stage of the immune response is characterized by a specific metabolic profile of T- and B-lymphocytes [2, 3]. OXPHOS processes are prevalent in naive T-cells, processes of glycolysis and glutaminolysis predominate in proliferating cells. Differentiation of T-lymphocytes is connected with activation of different metabolic pathways. It is glycolysis for Th1, Th2 and Th17 cells, fatty acid oxidation cycle and OXPHOS [2, 4] for regulatory T-cells (Treg). There are different methods to evaluate metabolic processes in cells, such as flow cytometry, mass spectrometry, Seahorse technology, etc. [5]. There

is a need to develop the diagnostic method of metabolism evaluation in clinical practice for assessing patient's condition with immune-dependent (IDD) and metabolic diseases.

Objective — to evaluate the activity of mitochondrial dehydrogenases in the general and small lymphocyte populations in healthy children and patients with immunosuppressive diseases.

Materials and methods. 96 healthy children, 30 children with atopic dermatitis (AtD) of severe course, 140 patients with inflammatory bowel diseases (IBD), 210 children with psoriasis vulgaris, aged 6-18 years, were examined. Disease severity in children with IBD was assessed by PCDAI and PUCAI, with psoriasis — PASI. The children with IBD and psoriasis were examined before and in the context of biological therapy: for IBD — anti-TNF drugs (infliximab, adalimumab), for psoriasis — anti-TNF (etanercept, adalimumab) and anti-IL12/23 (ustekinumab). The study was approved by the Local Ethics Committee (protocol № 6 of 11.06.2019), written informed parental consent was obtained for all the children.

Mitochondrial dehydrogenase activity was measured in lymphocyte populations: succinate dehydrogenase (SDH) and glycerol-3-phosphate dehydrogenase (GPDH) by immunocytochemistry (ICC), using flow cytometry. SDH is the main enzyme of the Krebs cycle and OXPHOS II stage, closely linked to the inner membrane of mitochondria. In the citric acid cycle SDH catalyzes the oxidation of succinic fumaric acid and allows to judge the functional mitochondrial activity with high certainty. Conjugation of glycolysis and the Krebs cycle was determined by GPDH — enzyme, reflecting the work of glycerophosphate shuttle mechanism on transporting electronic equivalents from the cytoplasm in the mitochondria as well as phospholipid metabolism. ICC method combines lymphocyte immunophenotyping and quantitative cytochemical analysis, allowing to determine the activity of mitochondrial enzymes in allocated lymphocyte populations [6]. The reaction is conducted on permeabilised cells of lymphoconcentrate. Cytofix/Cytoperm (BD, USA) set was used for cell fixation and permeability. Enzyme activity determination was performed according to developer recommendation, using the set to detect SDH and GPDH (NRC “Kurchatov Institute” — IREA, Russia). Enzyme activity was evaluated by side scatter coefficient (SSC) after and before the reaction, multiplied by 100. The activity of dehydrogenases was defined in the following lymphocyte population in CD45⁺: T-lymphocytes (CD3⁺), B-lymphocytes (CD3⁻CD19⁺), NK-cells (CD3⁻CD16⁺CD56⁺), T-helpers (CD3⁺CD4⁺), cytotoxic T-lymphocytes (CD3⁺CD8⁺), activated T-lymphocytes (CD3⁺HLA-DR⁺), Th17-cells (Th17, CD3⁺CD4⁺CD161⁺), regulatory T-lymphocytes (Treg, CD3⁺CD4⁺CD127^{low}), activated T-helpers (Thact — CD3⁺CD4⁺CD127^{high}), Th2-lymphocytes (CD3⁺CD4⁺CD294⁺), B1-lymphocytes (CD3⁻CD19⁺CD5⁺), B2-lymphocytes (CD3⁻CD19⁺CD5⁻). The study was conducted on the flow cytometer CYTOMICS FC500, using monoclonal antibodies, produced by BeckmanCoulter (USA). Statistical calculations were carried out with Statistica 10.0 program (StatSoft, USA). Descriptive statistics are presented in the form of Me [Q_{0,25}; Q_{0,75}]. Differences were considered statistically significant at $p < 0,05$.

Results and discussion. The study has found that enzyme activity depended on cell population, and in patients with IDD it reflected the severity of the condition, the disease phase and effectiveness of

the therapy. In healthy children the greatest SDH activity was detected in T-lymphocyte population and was 193 [183; 201] c.u. (CD4 — 192 [181; 202]; CD8 — 198 [185–205]), the lowest — in B-lymphocyte population (149 [138; 160]), in NK-cell population — 182 [174; 198] c.u. SDH activity analysis in small populations revealed the greatest activity in Treg population — 203 [189; 213] c.u., the lowest in B2-lymphocyte population — 145 [134; 156] c.u. It was found that GPDH enzyme activity, by which glycolysis intensity in cells can be indirectly judged, is lower than SDH activity in all general lymphocyte populations; the ratio of GPDH/SDH enzymes for T-lymphocytes was 0,78 [0,77; 0,87], for B-lymphocytes — 0,92 [0,91; 0,95], for NK-cells — 0,86 [0,85; 0,96]. The highest level of GPDH activity was found in NK-cell population (175 [148; 183]) that is much higher than in B-lymphocytes (152 [137; 156]; $p = 0,002$). Thus, the obtained results in healthy children confirms the known data [7, 8] on a higher OXPHOS level in T-cells, compared to B- lymphocytes ($p = 0,000$) and NK-cells ($p = 0,000$), moreover, in B-cell SDH activity is significantly lower than in NK-cells ($p = 0,000$). Proportion of GPDH/SDH shows that glycolysis proportion in NK-cells and B-lymphocytes is higher than in T-lymphocyte population.

Significant differences in “exacerbation-remission” conditions were obtained in evaluating the activity of dehydrogenases in lymphocyte population in children with AtD. With exacerbation of the disease SDH activity was significantly higher than in remission, and the degree of increase depended on lymphocyte population. SDH activity in T-lymphocyte population in the exacerbation was 211 [208; 219], in the disease remission — 182 [171; 185] ($p = 0,000$); in the exacerbation B-lymphocytes: 165 [157; 175] vs 142 [137; 146] in the disease remission ($p = 0,000$), in the exacerbation in NK-cells: 203 [190; 210] vs 172 [157; 182] in the disease remission ($p = 0,000$). The comparison of SDH activity indicators in children with AtD and healthy ones showed that the children with AtD in the acute stage had significantly higher indicators for all the populations, except for Treg. In the disease remission SDH activity in T-lymphocyte subpopulations and in NK-cells was much lower, and in B-lymphocyte populations it did not differ from indicators of health children.

A prolonged inflammatory process in children with IBD leads to considerable SDH reduction in T-lymphocytes, cytotoxic T-lymphocytes, B- and NK-cells in the exacerbation as well as in the disease remission relative to the norm. The degree of SDH reduction increases with a disease duration. GPDH activity in the general lymphocyte population in children with IBD did not differ from the normative values. It was also found that the group of patients with a stable positive response to the biological therapy was characterized by an initially higher SDH activity in Treg population, compared to the patients with unstable effect of anti-TNF therapy (211 [195; 220] vs 198 [186; 210], $p = 0,002$). ROC-analysis for SDH activity in Treg population showed excellent quality of prognostic separation model (AUC 0,820) at SDH threshold of 207 c.u. (sensitivity 67 %, specificity 88 %).

The children with vulgar psoriasis experienced significant reduction in SDH activity ($p = 0,002$) and GPDH ($p = 0,007$) in the general lymphocyte population on the level of healthy children. SDH reduction was observed in all the populations, except from activated T-helpers and B2-lymphocytes, and decrease in GPDH activity — in B-lymphocytes and NK-cells. The study showed that the level of enzyme activity reflects the treatment effectiveness. Thus, in

biological therapy while reaching PASI 75 (good effect) relative to the children with insufficient effect, the children with psoriasis experienced much higher levels of SDH activity in Treg (197 [186; 217] vs 188 [178; 201], $p = 0,037$) and GPDH in Treg (178 [176; 187] vs 173 [150; 177], $p = 0,031$), and also GPDH activity in T-lymphocytes (170 [162; 176] vs 159 [142; 169], $p = 0,001$).

Therefore, lymphocyte populations are characterized by a different activity of mitochondrial dehydrogenases both in healthy children and patients with IDD. The highest SDH activity was identified in T-lymphocyte population and Treg, the lowest — in B-lymphocytes. The highest level of GPDH activity was found in NK-cell population. Metabolic lymphocyte profile reflects the severity of the condition and the therapy effectiveness. Treg metabolism is found to be an informative parameter to assess and forecast the effectiveness of biological therapy in children with IBD and psoriasis.

Conclusion. Flow cytometer allowed to evaluate the activity of SDH and GPDH mitochondrial dehydrogenases in lymphocyte populations. The intensity of lymphocyte metabolism reflects the activity of the inflammatory process and the therapy effectiveness in children with immune-dependent diseases.

REFERENCES / ЛИТЕРАТУРА

1. Chou WC, Rampanelli E, Li X, Ting JP. Impact of intracellular innate immune receptors on immunometabolism. *Cell Mol Immunol.* 2022; 19 (3): 337–351. <https://doi.org/10.1038/s41423-021-00780-y>.
2. Geltink RIK, Kyle RL, Pearce EL. Unraveling the Complex Interplay Between T Cell Metabolism and Function. *Annu Rev Immunol.* 2018; 36: 461–488. <https://doi.org/10.1146/annurev-immunol-042617-053019>.
3. Fu Y, Wang L, Yu B, Xu D, Chu Y. Immunometabolism shapes B cell fate and functions. *Immunology.* 2022; 166 (4): 444–457. <https://doi.org/10.1111/imm.13499>.
4. Budikhina AS, Pashenkov MV. The role of glycolysis in immune response. *Immunologiya.* 2021; 42 (1): 5–20. (In Russ.). <https://doi.org/10.33029/0206-4952-2021-42-1-5-20>.
5. Purohit V, Wagner A, Yosef N, Kuchroo VK. Systems-based approaches to study immunometabolism. *Cell Mol Immunol.* 2022; 19 (3): 409–420. <https://doi.org/10.1038/s41423-021-00783-9>.
6. Petrichuk SV, Izmailova TD, Radygina TV. Method for measuring the mitochondrial activity of lymphocytes // Patent for invention RU 2302635 C1, 10.07.2007. Application No. 2005141145/15 dated 12/28/2005. (In Russ.).
7. Vivas-García Y, Efeyan A. The metabolic plasticity of B cells. *Front Mol Biosci.* 2022; 9: 991188. Published 2022 Sep 23. <https://doi.org/10.3389/fmolb.2022.991188>.
8. Li F, Liu H, Zhang D, Ma Y, Zhu B. Metabolic plasticity and regulation of T cell exhaustion. *Immunology.* 2022; 167 (4): 482–494. <https://doi.org/10.1111/imm.13575>.