CONTENT OF CD4+ CELLS EXPRESSING CD39/CD73 ECTONUCLEOTIDASES IN CHILDREN WITH INFLAMMATORY BOWEL DISEASES

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The regulation of TNF inhibitor therapy-associated immune responses in inflammatory bowel diseases (IBD) in children remains an urgent problem. The study aimed at analyzing the expression of CD39/CD73 endonucleotidases by different subsets of peripheral blood T cells in children with IBD including Crohn's disease (n = 34) and ulcerative colitis (n = 33) having received TNF inhibitors in comparison with conditionally healthy children (n = 45). Lymphocyte subsets including regulatory T cells (Treg, $CD4^+CD127^{low}CD25^{high}$), activated T cells (Tact, $CD4^+CD25^+CD127^{high}$) and Th17 cells ($CD4^+CD161^+CD3^+$) were studied by flow cytometry. The results are presented as medians (Me) and quartiles (Q_{25}–Q_{75}). In children with IBD the highest and the lowest relative counts of $CD39^+$ cells were found in Treg and Tact subsets — 31% (15–38) and 4% (1–7), respectively. The highest relative counts of $CD73^+$ cells were found in Tact — 13% (8–21). The CD39 and CD73 expression ratio in patients with IBD, and in the control group as well, depended on particular subset. CD39 expression in Treg, Tact and Th17 of patients with IBD was not age-dependent. Patients with acute Crohn's disease revealed decreased expression of CD39 in Treg compared with the control group (12% (9–23) vs 35% (28–39), respectively; $p = 10^{-6}$). Patients with Crohn's disease in remission revealed increased expression of CD39 in Treg compared with the acute of the disease (31% (27–40) vs 12% (9–23); $p = 9.4 \times 10^{-5}$). Patients with Crohn's disease in remission revealed no significant differences with the control group apart from reduced expression of CD73 by Treg in Crohn's disease. The results indicate significant association of CD39 and CD73 expression levels in particular subsets of $CD4^+$ cells with the phase of the disease (acute vs remission) and, accordingly, with the anti-TNF regimen efficacy.

Keywords: lymphocyte subsets, $CD4^+$ subsets, Treg, Th17, Tact, CD39, CD73, children, inflammatory bowel diseases

Funding: the study was carried out on state assignment of the Ministry of Health of Russia, number AAAA-A19-119013090093-2.

Acknowledgement: the authors thank all patients for participation and acknowledge the Gastroenterology Department with Hepatology Group of the National Medical Research Center for Children's Health and its collaboration.

Compliance with ethical standards: the study was approved by the Ethics Committee of the National Medical Research Center for Children's Health, Moscow (protocol № 6 dated June 11, 2019). The informed consent was submitted by all study participants.

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Received: 22.06.2022 **Accepted:** 16.07.2022 **Published online:** 30.07.2022

DOI: 10.24075/brsmu.2022.039

СОДЕРЖАНИЕ CD4+-КЛЕТОК С ЭКСПРЕССИЕЙ ЭКТОНУКЛЕОТИДАЗ CD39/CD73 У ДЕТЕЙ С ВОСПАЛИТЕЛЬНЫМИ ЗАБОЛЕВАНИЯМИ КИШЕЧНИКА

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Изучение регуляции иммунного ответа на фоне терапии блокаторами TNF при воспалительных заболеваниях кишечника (ВЗК) у детей остается актуальной проблемой. Целью исследования было изучить экспрессию CD39/CD73 в субпопуляциях лимфоцитов (регуляторных Т-клеток (Treg) — $CD4^+CD127^{low}CD25^{high}$; активированных Т-клеток (Tact) — $CD4^+CD25^+CD127^{high}$; Th17-лимфоцитов — $CD4^+CD161^+CD3^+$) периферической крови у детей с ВЗК (с болезнью Крона (БК), n = 34; с язвенным колитом — n = 33), принимавших блокаторы TNF, и у 45 условно здоровых детей. Результаты представлены в виде медианы (Me) и квартилей (Q_{25}–Q_{75}). С помощью многоцветной цитометрии показано, что у детей с ВЗК наибольшее количество $CD39^+$ выявлено в популяции Treg — 31% (15–38), наименьшее — в Tact 4% (1–7), а наибольшее количество $CD73^+$ — в Tact 13% (8–21). Соотношение экспрессии CD39 и CD73 у пациентов с ВЗК, так же как и в группе сравнения, зависело от популяции клеток. Экспрессия CD39 в Treg, Tact и Th17 у пациентов с ВЗК не зависела от возраста ребенка. В группе детей с БК в обострении относительно группы сравнения получено снижение экспрессии CD39 в Treg (12% (9–23) против 35% (28–39), p = 0,000001). У детей в ремиссии БК экспрессия CD39 в Treg достоверно выше, чем при обострении (31% (27–40) против 12% (9–23); p = 0,000094)). Между группой пациентов в ремиссии и группой сравнения достоверных различий выявлено не было, за исключением снижения экспрессии CD73 в Treg при БК. Полученные результаты показывают, что экспрессия CD39 и CD73 в популяциях $CD4^+$-лимфоцитов в значительной степени связана с течением заболевания, с обострением или ремиссией, и, соответственно, с эффективностью проводимой анти-TNF-терапии.

Ключевые слова: популяции лимфоцитов, $CD4^+$-лимфоциты, Treg, Th17, Tact, CD39, CD73, ВЗК, дети

Финансирование: исследование проведено в рамках государственного задания Минздрава России, № AAAA-A19-119013090093-2.

Благодарности: авторы выражают благодарность всем пациентам, участвовавшим в исследовании, а также признательность за сотрудничество коллегам из гастроэнтерологического отделения с гепатологической группой национального медицинского исследовательского центра здоровья детей.

Соблюдение этических стандартов: исследование одобрено этическим комитетом ФГАУ «НМИЦ здоровья детей» МЗ РФ (протокол № 6 от 11 июня 2019 г.). Для всех участников исследования было получено информированное согласие родителей в соответствии с принципами Хельсинкской декларации.

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Статья получена: 22.06.2022 **Статья принята к печати:** 16.07.2022 **Опубликована онлайн:** 30.07.2022

DOI: 10.24075/vrgmu.2022.039

Advanced understanding of pathogenic processes in the inflammatory bowel diseases (IBD) together with the search for new approaches in predicting patient's responses to therapy is an urgent pediatric problem. The prevalence of IBD in Russia is 5.1 : 100,000 [1]. The 30% increase in the incidence of IBD among children observed over the past decade and the emergence of severe forms of the disease in early childhood are of particular concern to pediatricians [2, 3]. The onset of IBD has

been associated with a combination of several adverse factors including genetic predisposition, immune system dysfunction, abnormal gut microbiota and the harmful environmental factor exposure [4]. Among those, immune dysregulations are considered the most impactful. In particular, the dynamics of certain subsets of T lymphocytes including T helper 17 cells (Th17) and regulatory T cells (Treg) correlates with the severity of pathological process and therapy efficacy in IBD [5, 6]. As naïve T cells differentiate into Th1, Th2, Th17 and Treg cells under the action of specific combinations of cytokines produced by antigen-presenting cells, Th17 and Treg use a common TGFβ-mediated signaling pathway. In the presence of IL6, IL21 and TGFβ, naïve $CD4^+$ T cells differentiate into Th17, whereas in the absence of pro-inflammatory cytokines they differentiate into Treg cells. Abnormal balance between Th17 and Treg leads to autoimmune disorders including IBD [7, 8].

The past decade was marked by intensive research on the purinergic system and its immediate involvement in immune functionalities. The purinergic signaling hypothesis was proposed by Geoffrey Burnstock back in 1972 [9]. Its modern understanding implicates the extracellular adenosine triphosphoric acid (eATP) as a pro-inflammatory mediator participating in the regulation of cell metabolism, cell migration, cell proliferation and apoptosis through signaling pathways triggered by P2Y and P2X receptors [10, 11]. The ectonucleotidase enzymes CD39 (a.k.a. ecto-nucleoside triphosphate diphosphohydrolase 1, E-NTPDase1) and CD73 (a.k.a. ecto-5'-nucleotidase, Ecto5'NTase) enable sequential dephosphorylation of ATP to adenosine which exerts anti-inflammatory properties [11]. A quantitative imbalance between eATP and adenosine may trigger inflammation [12]. The CD39 ectonucleotidase plays an important regulatory role in bowel inflammation: high expression of CD39 in circulating Tregs correlates with clinical and endoscopic remission in patients with IBD [13, 14]. It has been also demonstrated that single nucleotide polymorphisms associated with decreased expression of CD39 increase the risks of Crohn's disease [15].

Ectonucleotidases are expressed by different lymphocyte subsets including Treg and Th17 cells. About 90% of the $Foxp3^+$ Treg cells exhibit CD39 on their surface [16] and the non-uniformity of Treg cells in terms of CD39 expression is relevant. Although both $CD4^+CD25^{high}CD39^+$ and $CD4^+CD25^{high}CD39^-$ Treg cells suppress proliferation and IFNγ production by effector T cells in multiple sclerosis [17], Treg cells with $CD4^+CD25^{high}FoxP3^+CD39^+$ phenotype suppress IL17 production, while $CD4^+CD25^{high}CD39^-$ Treg cells produce IL17 [17]. Importantly, Th17 cell subsets are also non-uniform in terms of CD39 expression and apart from the majority of pro-inflammatory Th17 there is a minor pool of suppressor Th17 lymphocytes (supTh17) expressing high levels of CD39 and facilitating adenosine production. These supTh17 cells are found in peripheral blood and lamina propria of the intestinal

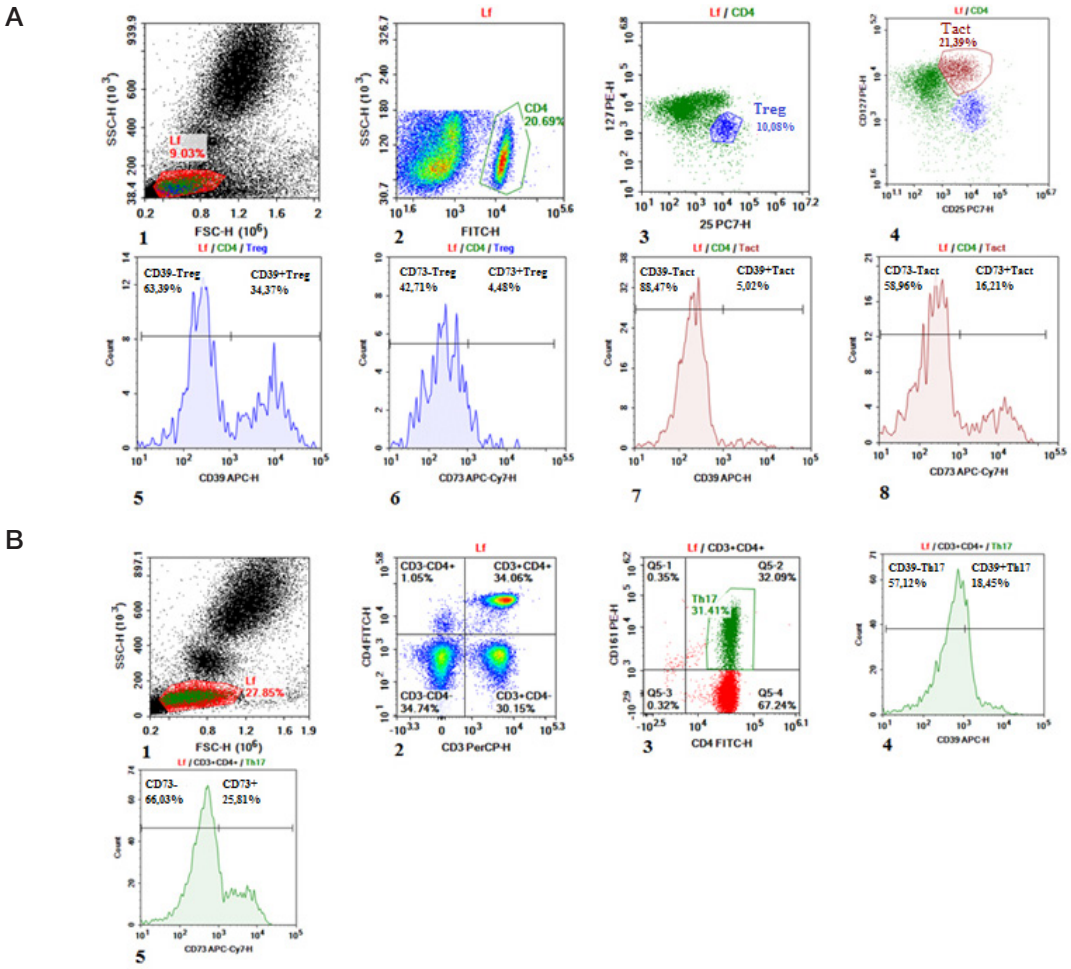

Fig. 1. Stepwise gating strategies for Treg, Tact and Th17 expressing CD39 and CD73. **A**. Stepwise gating for Treg and Tact: 1 — discrimination of the 'lymphoid' region based on forward scatter (FSC) and side scatter (SSC) parameters; 2 — discrimination of CD4-positive lymphocytes; 3 — discrimination of Treg as $CD4^+CD127^{low}CD25^{high}$; 4 — discrimination of Tact as $CD4^+CD25^+CD127^{high}$; 5 — determination of $CD39^+$ Treg; 6 — determination of $CD73^+$ Treg; 7 — determination of $CD39^+$ Tact; 8 — determination of $CD73^+$ Tact. **B**. Stepwise gating for Th17: 1 — discrimination of the 'lymphoid' region based on forward scatter (FSC) and side scatter (SSC) parameters; 2 — discrimination of $CD3^+CD4^+$ double-positive set; 3 — discrimination of Th17 subset; 4 — determination of $CD39^+$ Th17; 5 — determination of $CD73^+$ Th17

Table 1. Relative counts of CD39 and CD73 ectonucleotidase-expressing cells (positivity rates) in CD4+ T lymphocyte subsets of patients with IBD and comparison group

Enzyme / Indicators	Patients with IBD (*n* = 67)				Comparison group (*n* = 45)				*p*
	CD39		CD73		CD39		CD73		
	Me ($Q_{0.25}$–$Q_{0.75}$)	Min–Max	Me ($Q_{0.25}$–$Q_{0.75}$)	Min–Max	Me ($Q_{0.25}$–$Q_{0.75}$)	Min–Max	Me ($Q_{0.25}$–$Q_{0.75}$)	Min–Max	
Treg, %	31.2 (14.8–37.8)	6–58	5.0 (3.0–8.3)	0–15	35 (27.8–39.4)	19–49	8.1 (6.9–12.2)	2–39	*p*39 = 0.023 *p*73 = 0.000
Tact, %	3.9 (1.1–6.7)	0–14	12.9 (8.0–21.1)	2–30	5 (4.4–7.4)	3–11	17.6 (11.9–21.5)	8–35	*p*39 = 0.001 *p*73 = 0.021
Th17,%	10.4 (5.2–16.8)	0–29	7.7 (4.7–11.4)	1–26	9.6 (8.6–12.1)	6–24	10.2 (7.3–14.4)	3–33	*p*39 = 0.771 *p*73 = 0.007

Note: *p*39 — levels of significance for the differences in CD39 positivity rates between IBD and comparison group; *p*73 — levels of significance for the differences in CD73 positivity rates between IBD and comparison group

mucosa in healthy individuals and their numbers are reduced significantly in Crohn's disease, which indicates their relevance to the bowel inflammation control [18, 19]. Noteworthy, the majority of studies on the abnormal purinergic signaling in IBD enrolled adult patients [12–14].

In this regard, we aimed at a pilot quantitative evaluation of CD39/CD73 ectonucleotidase-expressing cells in CD4+ lymphocyte subsets of children with IBD compared with healthy controls.

METHODS

The study enrolled 67 pediatric patients with IBD (34 pts with Crohn's disease and 33 pts with ulcerative colitis) aged 3.4–18 years and having received TNF inhibitor therapy. The patients were assigned to four groups in accordance with the course (phase) of the disease: group 1 — acute Crohn's disease (*n* = 18), group 2 — Crohn's disease in remission (*n* = 16), group 3 — acute ulcerative colitis (*n* = 22), group 4 — ulcerative colitis in remission (*n* = 11). The assignment was based on the Pediatric Crohn's Disease Activity Index (PCDAI) for Crohn's disease (≤ 10 — remission, > 10 — acute) and the Pediatric Ulcerative Colitis Activity Index (PUCAI) for ulcerative colitis (≤ 10 — remission, > 10 — acute). The comparison group (group 5, *n* = 45) enrolled conditionally healthy children aged 3.7–17.5 years. Inclusion criteria for group 5 were as follows: all standard clinical and biochemical laboratory indicators within reference values; no acute or aggravating chronic conditions; no traumatic injury; no history of autoimmune, oncological or mental diseases. Venous blood samples for immunological tests were collected from cubital vein, fasting, in BDVacutainer® tubes with K2EDTA as an anticoagulant. Erythrocytes were lysed with BD FACS™ Lysing Solution (BD Biosciences; USA) for 10–12 min at room temperature in the dark. Immunophenotyping of lymphocytes for Th17, Treg and Tact subset markers and CD39/CD73 cell surface enzymes was carried out by laser flow cytometry (Novocyte, ACEA Biosciences; USA) using monoclonal antibodies conjugated with different fluorochromes: CD4-FITC (cat. A07750, Beckman Coulter; USA), CD127-PE (cat. IM 10980U, Beckman Coulter), CD25-PC7 (cat. A52882, Beckman Coulter), CD161-PE (cat. IM 3450, Beckman Coulter), CD3-PC5 (cat. A07749, Beckman Coulter), CD39-APC-Cy7 (Clone A1, cat. RT2241130, Sony Biotechnology; USA), CD73-APC-Cy7 (Clone AD2, cat. RT2320110, Sony Biotechnology). The measurements of CD39- and CD73-expressing fractions in Treg ($CD4^+CD127^{low}CD25^{high}$), Tact ($CD4^+CD25^+CD127^{high}$) and Th17 ($CD4^+CD161^+CD3^+$) subsets were carried out using a stepwise gating procedure (Fig. 1).

Statistical processing of the data was carried out in Statistica 10.0 (StatSoft; USA). The descriptive statistics for quantitative variables are given in the median (lower and upper quartiles) — Me ($Q_{0.25}$–$Q_{0.75}$), minimum/maximum (Min/Max) format. Between-the-group differences were evaluated for significance using the nonparametric Mann–Whitney U test.

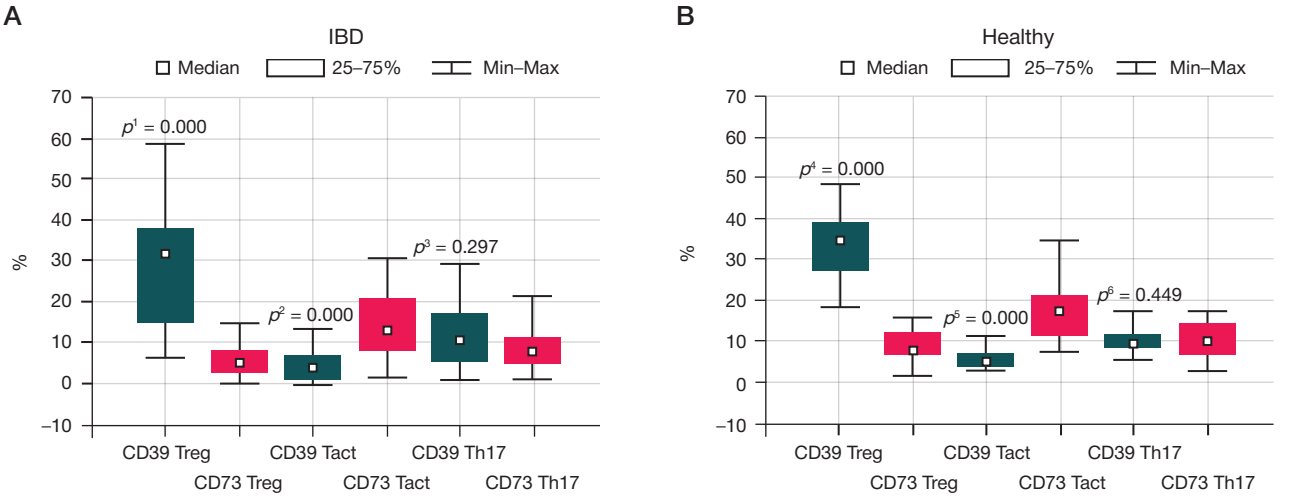

Fig. 2. Proportions of cells expressing CD39 and CD73 among CD4+ lymphocytes in patients with IBD and healthy individuals (comparison group). **A**. p^1 — level of significance for the difference between CD39+ Treg and CD73+ Treg; p^2 — level of significance for the difference between CD39+ Tact and CD73+ Tact; p^3 — level of significance for the difference between CD39+ Th17 and CD73+ Th17. **B**. p^4 — level of significance for the difference between CD39+ Treg and CD73+ Treg; p^5 — level of significance for the difference between CD39+ Tact and CD73+ Tact; p^6 — level of significance for the difference between CD39+ Th17 and CD73+ Th17

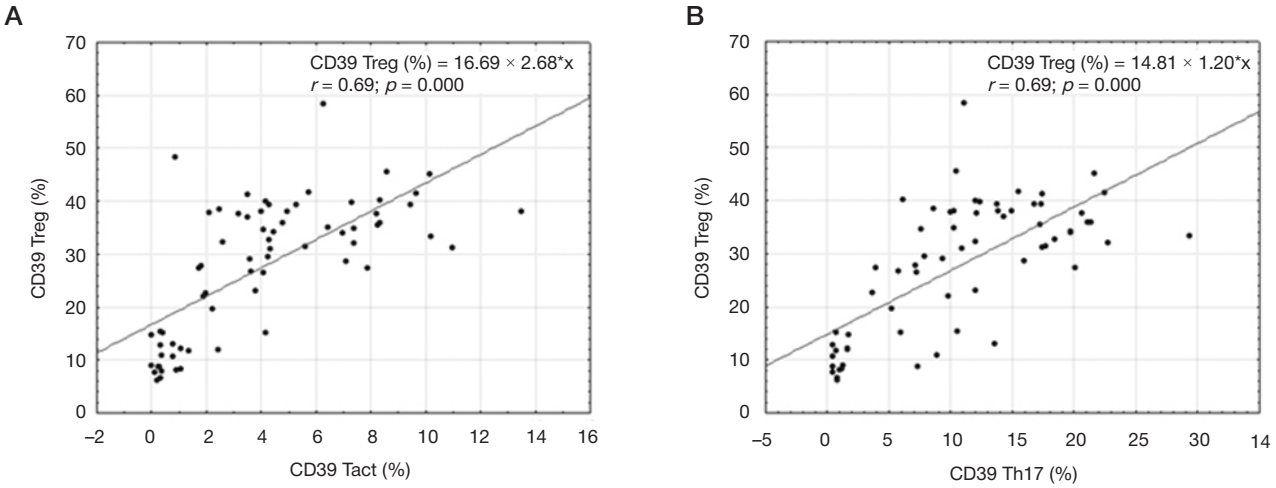

Fig. 3. Correlations of CD39⁺ content in Treg vs Tact and Th17 (respectively, **A** and **B**) in pediatric patients with IBD

The distributions were compared by Pearson's chi-square test (χ^2). The differences were considered statistically significant at $p < 0.05$.

RESULTS

CD39 and CD73 positivity rates in IBD vs healthy controls

The flow cytometry assay of CD39 and CD73 positivity for different subsets of CD4⁺ T cells in pediatric patients with IBD revealed the highest content of CD39⁺ cells among Treg (6–58% of Treg) and the lowest content of CD39⁺ cells among Tact (0–14% of Tact). By contrast, CD73 positivity was the highest in Tact (2–30% of Tact, Table1). The comparison group showed similar subset-specific positivity ratios (Table 1).

Indeed, Treg expressed CD39 at significantly higher rates than CD73 ($p = 0.000$) and Tact expressed CD39 at significantly lower rates than CD73 ($p = 0.000$) (Fig. 2A). In Th17 lymphocytes, positivity rates for the two markers were similar (Table 1; Fig. 2B).

Patients with IBD revealed significantly reduced CD39 and CD73 positivity rates in Treg and Tact compared with the controls (Table 1). As for Th17, CD73 positivity rates were significantly reduced in IBD, whereas CD39 positivity rates in IBD and the controls were similar.

The correlation analysis revealed significant positive correlations between CD39⁺ Treg and CD39⁺ Tact ($r = 0.69$; $p = 0.000$; Fig. 3A) and CD39⁺ Treg and CD39⁺ Th17 ($r = 0.69$; $p = 0.000$; Fig. 3B) in patients with IBD. Similar trends were observed for CD73: CD73⁺ Treg correlated with CD73⁺ Tact ($r = 0.45$; $p = 0.000$) and CD73⁺ Treg correlated with CD73⁺ Th17 ($r = 0.46$; $p = 0.000$).

Table 2. Relative counts of CD39 and CD73 ectonucleotidase-expressing cells (positivity rates) in CD4⁺ T lymphocyte subsets of patients with Crohn's disease and ulcerative colitis at different stages and the comparison group

	CD acute	CD remission	UC acute	UC remission	Healthy children	CD	UC
Indicator	Group 1 ($n = 18$) Me ($Q_{0.25}$–$Q_{0.75}$)	Group 2 ($n = 16$) Me ($Q_{0.25}$–$Q_{0.75}$)	Group 3 ($n = 22$) Me ($Q_{0.25}$–$Q_{0.75}$)	Group 4 ($n = 11$) Me ($Q_{0.25}$–$Q_{0.75}$)	Group 5 ($n = 45$) Me ($Q_{0.25}$–$Q_{0.75}$)	p_{12}	p_{34}
CD39 Treg, %	12.48 (8.64–22.53)	30.71 (26.62–39.56)	32.77 (14.60–39.19)	35.51 (33.91–37.70)	35 (27.8–39.4)	0	0.355
p	0	0.556	0.198	0.627			
CD73 Treg, %	3.90 (3.25–5.91)	5.87 (2.50–8.86)	4.80 (3.20–7.00)	5.45 (1.97–12.68)	8.1 (6.9–12.2)	0.606	0.836
p	0	0.022	0.001	0.072			
CD39 Tact, %	0.59 (0.30–3.20)	4.18 (2.92–7.35)	4.08 (1.36–6.05)	7.00 (2.50–8.28)	5 (4.4–7.4)	0.001	0.204
p	0	0.158	0.025	0.76			
CD73 Tact, %	11.50 (8.04–15.18)	13.07 (8.26–23.47)	13.10 (8.00–18.23)	16.28 (8.78–21.60)	17.6 (11.9–21.5)	0.423	0.585
p	0.014	0.325	0.066	0.427			
CD39 Th17, %	3.71 (0.71–12.10)	9.60 (8.54–10. 3)	14.08 (1.72–17.31)	17.26 (8.60–20.64)	9.6 (8.6–12.1)	0.092	0.462
p	0.014	0.486	0.062	0.059			
CD73 Th17, %	6.30 (4.32–10. 20)	8.22 (3.50–16.54)	6.50 (5.40–9.05)	8.30 (3.00–18.70)	10.2 (7.3–14.4)	0.224	0.418
p	0.008	0.489	0.002	0.569			

Note: p_{12} — levels of significance for the differences in CD39/CD73 positivity rates between groups 1 and 2; p_{34} — levels of significance for the differences in CD39/CD73 positivity rates between groups 3 and 4; *p* — levels of significance for the differences in CD39/CD73 positivity rates in Crohn's disease, ulcerative colitis and comparison groups; CD — Crohn's disease; UC — ulcerative colitis

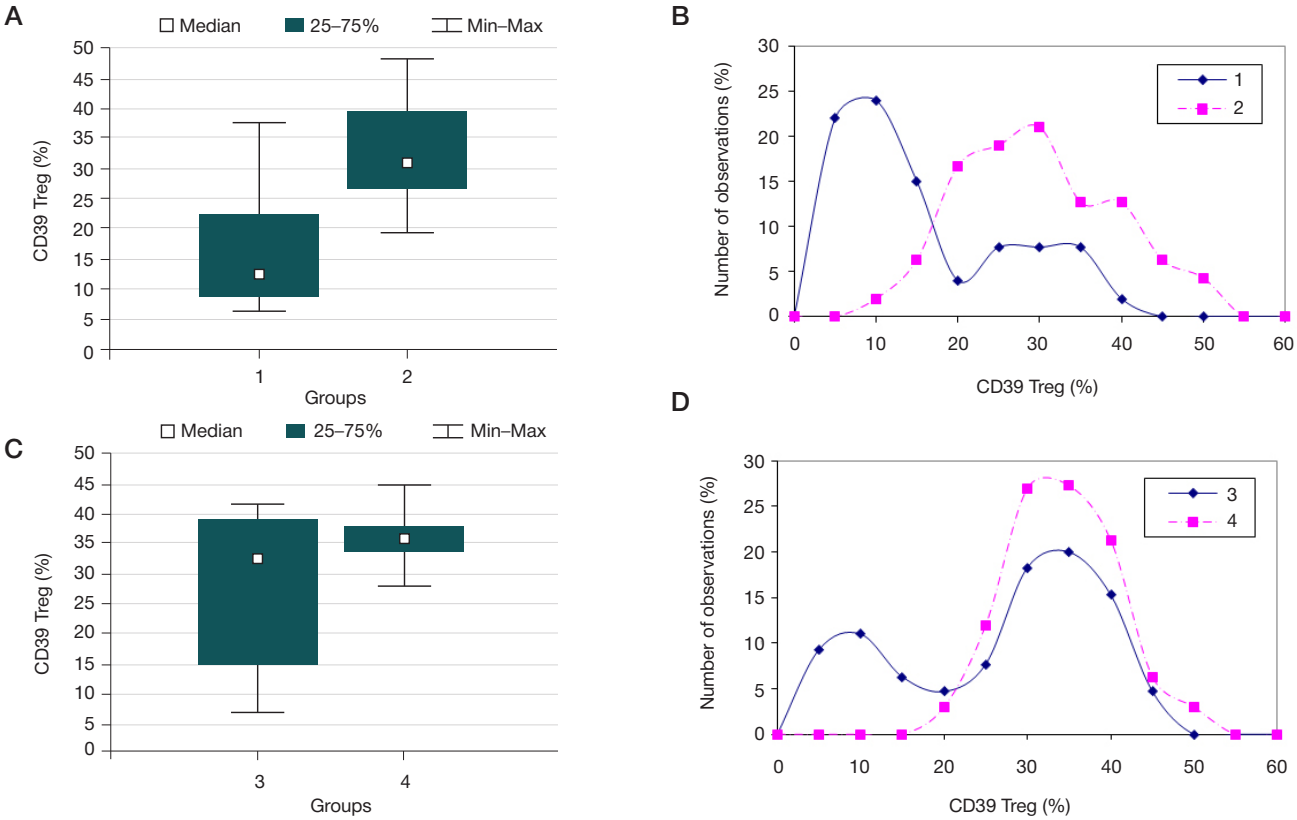

Fig. 4. CD39⁺ Treg content distributions for Crohn's disease groups 1 and 2 (respectively, **A** and **B**) and ulcerative colitis groups 3 and 4 (respectively, **C** and **D**)

The data indicate that CD39 positivity rates in Treg, Tact and Th17 in patients with IBD are age-independent. For the comparison group, we observed an inverse correlation of CD39 positivity rate in Th17 with the age (r = –0.39; p = 0.009). Of note, patients with IBD and conditionally healthy children (comparison group) were of matching age (p = 0.435).

By contrast, CD73 positivity rates in Th17 tended to increase with the age in patients with IBD and healthy controls similarly (r = 0.43; p = 0.000).

The time since diagnosis in patients with IBD varied widely within 5 months to 7.7 years, and the therapy lasted 2–288 weeks. We identified no correlations of CD39/CD73 positivity rates with the time lapse since diagnosis or on therapy.

CD39/CD73 expression in CD4⁺ cells in groups of children with acute IBD and remission

We further compared CD39 and CD73 positivity rates of CD4⁺ lymphocyte subpopulations in patients with Crohn's disease and ulcerative colitis acute disease (groups 1 and 3) or remission (groups 2 and 4) mutually or against the conditionally healthy children (group 5, comparison group) (Table 2). Patients with Crohn's disease revealed significantly reduced CD39 positivity rates among Treg and Tact in group 1 (acute) compared with group 2 (remission). Moreover, group 1 (Crohn's disease, acute) revealed significantly decreased CD39 and CD73 positivity rates in Treg, Tact and Th17 subsets compared with group 5 (healthy children) (Table 2). Comparison of patients with Crohn's disease in remission (group 2) and conditionally healthy children (group 5) revealed no significant differences for the studied markers apart from reduced CD73 positivity rates in Treg (Table 2).

Patients with acute ulcerative colitis (group 3) revealed significantly reduced CD39 positivity rates in Tact, as well as significantly reduced CD73 positivity rates in Treg and Th17, compared with conditionally healthy children (group 5) (Table 2). Comparison of patients with ulcerative colitis in remission (group 4) and group 5 revealed no significant differences for the studied markers.

In addition, significant differences in CD39⁺ Treg index were revealed among patients with Crohn's disease depending on the phase (group 1 vs group 2) (Fig. 4A). The lack of corresponding differences in the same index for ulcerative colitis (group 3 vs group 4) may be related to its broad variation in these groups (Fig. 4C). The plots in Figs. 4B and 4D show the CD39⁺ Treg index distribution densities for patients with Crohn's disease (groups 1 and 2) and ulcerative colitis (groups 3 and 4), with both high and low values identified in each group. The plots reveal a clear threshold value of 20% to differentiate between the phases of Crohn's disease on the basis of CD39⁺ Treg index. Thus, 78% of group 1 had CD39⁺ Treg index within 20% (Fig. 4C), whereas in group 2 (remission) the index exceeded 20% in 82% of the patients (Fig. 4C). In the ulcerative colitis groups 3 and 4, the CD39⁺ Treg index exceeded 20% in, respectively, 67% and 100% of the cases (Fig. 4D).

DISCUSSION

A study enrolling adult healthy donors revealed a broad variation of CD39 positivity rates in Treg (2–60%) affecting the ability of these cells to hydrolyze ATP [20]. Treg cells expressing higher levels of CD39 hydrolyze ATP more efficiently. Here we show that in healthy children CD39⁺ Treg indexes vary within 19–49%. Our estimates of the content of ectonucleotidase-expressing cells among CD4⁺ lymphocyte subsets in pediatric IBD treated with TNF blockers revealed higher CD39⁺ indexes during remission consistently with published evidence [13].

Administration of anti-TNF regimens in adult patients has been previously shown to promote CD39 expression in Treg [21]. In our setting, a group of patients with Crohn's disease (acute) had significantly lower CD39⁺ Treg index compared with the

same disease in remission. A comparison between patients with Crohn's disease (acute) and ulcerative colitis (acute) revealed higher incidence of reduced CD39 expression by Treg among the former.

One study dealing with altered functional activity of CD39 in Treg in autoimmune hepatitis revealed reduced levels of ATP hydrolysis and adenosine formation [22]. By inference, a similar reduction in the ATP hydrolysis efficiency can be assumed in IBD on the basis of reduced CD39 positivity. Occasional presentation of elevated $CD39^+$ Treg indexes in acute Crohn's disease can be related to modest effects of anti-TNF therapy in counteracting the disease. The increased relative counts of Tregs exhibiting CD39 ectonucleotidase on their surface under these conditions are likely to be compensatory and reflect insufficient activity of the enzyme.

Our results also indicate significantly reduced counts of supTh17 lymphocytes, endowed with immunosuppressive properties, in pediatric patients with Crohn's disease (acute) compared with conditionally healthy children. In this regard, supTh17 can be considered as a candidate sensor subset for transitions from acute disease to remission and vice versa. Of note, in children with and without IBD this population dwindles with the age. It can be assumed that the age-related decline of supTh17 contributes to increased risks of pronounced inflammatory reactions.

CD73 plays an important role in the intestinal homeostasis [23–25] and its dysfunction leads to damaging inflammatory processes in the colon as demonstrated using a knockout mouse model [26]. Here we show a reduction in CD73 positivity rates among Treg, Tact and Th17 in children with IBD compared with conditionally healthy peers. CD73 is known to exert its enzymatic activity (AMP conversion to adenosine) as a membrane-anchored protein but also in soluble form [27]. It is possible therefore that a comprehensive assessment of the patient's condition using this marker should also involve the activity of its soluble form, which reportedly correlates with the inflammation severity [28].

CONCLUSIONS

The results indicate that the content of cells expressing CD39/CD73 ectonucleotidases among $CD4^+$ cells depends on the subset (Treg, Tact or Th17) and phase of the disease (acute or remission), the latter being dependent on the success of anti-TNF therapy. Most of the children in remission present with high numbers of $CD4^+$ lymphocytes expressing ectonucleotidase, which helps reducing the inflammation by converting ATP to adenosine. At the same time, high content of CD39-expressing cells among the studied subsets may also accompany the acute stage of the disease, which is probably related to diverse activities of the enzyme. We believe that further elucidation of the functional activity of CD39 and CD73 ectonucleotidases complemented by other quantitative indicators can be informative for understanding and predicting the efficacy of anti-TNF regimens in IBD.

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