ACTIVATION OF CD4+CD39+ T CELLS IN COLORECTAL CANCER

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Pathogenesis of colorectal cancer (CRC) is accompanied by significant changes in the immune system. However, the role of the adenosine-A2AR-mediated immunosuppressive pathway in oncogenesis and more specifically, the expression of ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1, also known as CD39) remains unclear. The aim of this work was to study the role of CD4+ T cells, most importantly CD39-expressing regulatory T cells (Tregs) in the formation of immune suppression in CRC and in patients with acute pancreatitis (AP). Expression of CD39 by peripheral blood lymphocytes and tumor-infiltrating lymphocytes (TILs) was measured by flow cytometry. The levels of *CD39* messenger RNA (mRNA) in the peripheral blood leukocytes were determined by real-time PCR. Our study reveals that patients with CRC accumulate peripheral CD4+CD39+ cells in the advanced stages of the disease. The proportion of CD39-expressing CD4+ T cells in the total pool of TILs was higher than in the peripheral blood of the same patients. Tregs of both peripheral blood and tumor specimens of CRC patients showed increased CD39 expression. We have found reliable correlations between the levels of CD4+CD39+ T cells and the parameters of cell-mediated immunity in CRC patients. Also, *CD39* mRNA levels gradually increased during CRC progression. In contrast, patients with AP have the same levels of *CD39* mRNA and peripheral blood CD4+CD39+ T cells as the controls. Finally, we conclude that activation of CD4+CD39+ T cells has an important role in oncogenesis and needs to be studied further.

Keywords: colorectal cancer, ectonucleotidase CD39, Treg cells, transcription factor FOXP3

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АКТИВАЦИЯ CD4+CD39+ Т-КЛЕТОК ПРИ КОЛОРЕКТАЛЬНОМ РАКЕ

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Патогенез колоректального рака (КРР) сопровождается значительными изменениями в состоянии иммунной системы. Однако роль аденозин-A2AR-опосредованного иммуносупрессорного механизма и в частности экспрессии молекулы эктонуклеозидтрифосфатдифосфогидролазы-1 (ENTPD1), или CD39, в его развитии до конца не изучена. Целью работы было исследование роли CD4+ Т-клеток, прежде всего экспрессирующих CD39 регуляторных Т-лимфоцитов (T_{reg}), в формировании иммунной супрессии при КРР, а также у больных острым панкреатитом (ОП). Экспрессию молекул лимфоцитами крови и опухоль-инфильтрирующими лимфоцитами (ОИЛ) анализировали методом проточной цитометрии. Содержание матричной РНК (мРНК) *CD39* в лейкоцитах периферической крови определяли методом полимеразной цепной реакции (ПЦР) в реальном времени. В результате исследования показано, что у больных КРР накопление периферических CD4+CD39+ клеток происходит на поздних стадиях развития опухоли. Среди ОИЛ количество CD4+ Т-клеток, экспрессирующих молекулу CD39, выше, чем в крови тех же больных. Значительно повышен уровень экспрессии этой молекулы у регуляторных Т-клеток (T_{reg}) больных КРР как на периферии, так и среди ОИЛ. Установлены достоверные связи между содержанием CD4+CD39+ Т-клеток и показателями клеточного иммунитета больных КРР. Обнаружено, что уровень мРНК гена *CD39* также увеличивался в процессе развития КРР. У больных ОП, напротив, содержание мРНК гена *CD39* оставалось на уровне контроля, так же как и количество CD4+CD39+ Т-клеток в периферической крови. Таким образом, можно заключить, что активация CD4+CD39+ Т-клеток в процессе канцерогенеза играет важную роль и требует дальнейшего изучения.

Ключевые слова: колоректальный рак, эктонуклеотидаза CD39, T_{reg}-клетки, транскрипционный фактор FOXP3

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Colorectal cancer (CRC) is one of the most common malignancies and causes of death in Russia [1] and across the world [2, 3]. The number of patients with primary CRC is constantly growing; interestingly, its incidence is much higher in industrial Europe and North America than in the developing countries of Africa, Asia and Latin America [2]. CRC formation is closely associated with the mechanisms regulating the immune response and is accompanied by the infiltration of immunocompetent cells into the tumor [3–5]. The role of chronic inflammation in promoting CRC is being actively discussed at the moment, as patients with inflammatory intestinal conditions turn to be at a higher risk of developing CRC. According to some reports, anti-inflammatory therapies reduce the risk of gastrointestinal cancer [7, 8].

At present, cancer immunology research is pretty much focused on ectonucleoside triphosphate diphosphohydrolase-1 (ENTPD1, CD39). Together, CD39 and CD73 (ecto-5′-nucleotidase, NT5E) participate in the production of extracellular adenosine. Synthesis of extracellular purine nucleosides plays a crucial role in the regulation of inflammation and tissue homeostasis. Immunocompetent cells receive the adenosine signal through A2AR, one of four adenosine G-protein-coupled receptors. Stimulation of A2AR in lymphocytes causes a decline in IL2 secretion and proliferative activity of native $CD4^+$ T cells, leads to a cutdown in the production of IFNγ and IL4 by T helper cells and results in the increased expression of CTLA-4, PD1 and CD-40L molecules [8]. This mechanism of immune suppression, involving adenosine-A2AR interactions, can protect healthy tissues against damage induced by inflammation. However, the adenosine-A2AR signaling pathway is also activated in malignant tissues, especially in response to hypoxia, allowing cancer cells to evade recognition by the immune system and, therefore, escape elimination [9]. The role of this mechanism in oncogenesis has been demonstrated in A2AR-deficient mice that rejected immunogenic tumors [10] and also in mice with knocked-out *CD39* and *CD73* that acquired different cancer-resistant phenotypes [11, 12].

Expression of CD73 in malignant tissues is well described. CD73 is known to be expressed by endothelial, stromal and tumor cells [13]. Less is known, however, about the expression of the membrane marker CD39 in the microenvironment of the tumor. Presumably, regulatory T cells (Tregs) are one of the major sources of CD39 in tumor infiltrates [14]. Using a variety of suppressive mechanisms, Tregs can block the autoimmune response and sustain immune tolerance [15, 16]. The master transcription factor for these cells responsible for their growth and suppressive function is FOXP3 [17]. The role of Tregs in cancer formation is detrimental since they contribute to the disease progression. Tregs have been shown to accumulate in the peripheral blood of cancer patients and tumor tissues [18].

At present, CRC remains one of the most common type of malignancies, but the role of the immunosuppressive adenosine-A2AR pathway in its formation is still understudied. The aim of our work was to explore the role of CD39-expressing $CD4^+$ T cells in the development of immune suppression in patients with CRC.

METHODS

Our study was conducted in 42 patients aged between 18 and 70 years (mean age of 65 ± 12.4 years) with a histologically and cytologically confirmed diagnosis of colorectal cancer. Patients with a previous history of other cancers or immunoinflammatory disorders were excluded from the study. Lymphocytes of 30 healthy donors aged 54.4 ± 20.6 years were used as a control. The CRC diagnosis was established based on clinical, laboratory, endoscopic and morphological tests. Six patients were diagnosed with stage I CRC (14.3%); 15, with stage II (37%); 12, with stage III (28.6%), and 9 patients had stage IV (20%). The patients were divided into two groups: the first group included patients with stages I and II CRC; the second consisted of patients with stages III and IV of the disease. The study was approved by the Ethics Committee affiliated with the Ministry of Health and Social Development of the Republic of Karelia and Petrozavodsk State University (Protocol 25 dated February 122013). We analyzed phenotypes of peripheral blood lymphocytes, as well as tumor-infiltrating lymphocytes (TILs) isolated from the clinical specimens of tumor tissue (n = 5) obtained from the patients with stage III CRC.

Adenosine is accumulated in the extracellular matrix in response to metabolic stress and cell breakdown, i.e. in ischemia, hypoxia, inflammation and injury. Therefore, we thought it would be interesting to study activation of $CD4^+CD39^+$ cells in the context of inflammation and immune suppression that bears no connection to oncogenesis. So, we recruited a comparison group consisting of 29 patients (mean age of 44.5 ± 18 years) with acute pancreatitis (AP). The diagnosis was established based on the classification accepted at the ninth All-Russian Congress of Surgeons in 2000. The inclusion criteria applied to the comparison group were: age from 18 to 70 years and acute pancreatitis. Patients with other co-morbidities, such as cancers and autoimmune disorders, were excluded from the study. Lymphocyte profiles were analyzed prior to treatment.

TILs were isolated by enzymatic disaggregation. Freshly explanted tissues were minced, placed into the medium for enzymatic disaggregation and incubated at room temperature for 2–3 hours. The medium was prepared from RPMI-1640 (PanEco, Russia) supplemented with 10% FBS (HyClone, USA), 100 μg/ml gentamycin (Sigma, USA) and 1 mg/ml collagenase IV (PanEco, Russia). The obtained suspension was passed through sterile filters with 70- and 40-μm pores. Lymphocyte subpopulations were separated using 75% and 100% density gradients prepared from ficoll with a density of 1.077 g/cm^3 (PanEco, Russia).

Expression of the studied molecules was measured by multicolor flow cytometry using Cytomics FC500 (Beckman Coulter, USA), monoclonal antibodies against CD4-FITC, CD8-FITC, CD25-PC5, and CD127-PC7 (Beckman Coulter, France); against CD3-PE, CD16-FITC, and CD19-FITC (Sorbent, Russia); against FOXP3-PE (eBioscience, USA); against CD39 (R&DSystems, USA), and the corresponding isotope controls. Intracellular expression of FOXP3 was analyzed using fixation and permeabilization buffers by eBioscience, USA. Expression of *CD39* mRNA was measured by real-time PCR. Isolation and purification of nucleic acids were done using AxyPrep Blood Total RNA Miniprep Kit (Axygen, USA). CDNA synthesis was aided by random hexamer primers and M-MLV reverse transcriptase (Sileks, Russia). CDNA amplification and the analysis of amplification products conducted in real time were performed using a reagent mix containing the intercalating dye SYBR Green I (Evrogen, Russia) in iCycler Thermal Cycler (Bio-Rad, USA). To analyze the obtained data, we applied the $2^{-\Delta\Delta Ct}$ method, where Ct was a threshold cycle and ΔCt was the difference between the values of threshold cycles for the reference (*GAPDH*) and target (*CD39*) genes. The expression level of the studied gene was calculated relative to the controls (healthy donors). Expression of the studied gene in the controls was taken as 1. The data were processed in Statistica 6.0; significance of differences between the groups was calculated using the Mann-Whitney U-test. Differences were considered significant at $p < 0.05$. To assess correlations between the variables, we used Spearman's rank correlation coefficient. The data are presented as M ± SD. The study was carried out on the equipment of the Shared Facility of the Federal Research Center *Karelian Research Center of the Russian Academy of Sciences*.

RESULTS

In the course of this work we measured the levels of $CD4^+CD39^+$ T cells in the peripheral blood and TILs of patients with CRC. We found that the number of $CD4^+CD39^+$ T cells in the peripheral blood varied a great deal both among diseased

and healthy individuals. The patients with advanced stages of CRC had significantly more $CD4^+CD39^+$ lymphocytes than the controls ($p < 0.05$). At the same time, no significant differences in the $CD4^+CD39^+$ lymphocyte count were observed between the patients with stages I and II CRC (Fig. 1).

In the population of lymphocytes isolated from tumor specimens the number of $CD4^+CD39^+$ T cells was 4 times higher than in the peripheral blood of the same patients (Fig. 2A).

The proportion of $CD4^+CD39^+$ T cells was increased in the subpopulation of $CD4^+$ T lymphocytes (Fig. 2B). $CD39^+$ TILs also expanded in the subset of T cells that did not have the CD4 marker on their surface (Fig. 2C). At the same time, there were more $CD4^-CD39^+$ cells in the tumor tissue specimens than there were $CD4^+CD39^+$ ($p < 0.05$). This trend was not observed for the peripheral blood lymphocytes.

Previously, we studied a population profile of peripheral blood lymphocytes in patients with CRC, including T cells and their subsets, B cells and natural killer (NK) cells [19]. Cytometry findings are shown in Table 1.

The patients with CRC had fewer B cells than the controls, both in the early and advanced stages of the disease ($p < 0.05$). The levels of $CD3^+$ T cells changed in stages III and IV CRC. In all CRC stages the patients had reduced levels of $CD4^+$ T helpers ($p < 0.05$) and activated $CD4^+CD25^+$ T cells ($p < 0.05$); the levels of cytotoxic $CD8^+$ lymphocytes (CTLs) were elevated ($p < 0.05$). No significant differences in the levels of NK cells were observed between the patients with CRC and the controls.

We established a few associations between the shifts in the population profile of lymphocytes and the number of $CD4^+CD39^+$ T cells in patients with CRC. Negative correlations were observed between the number of $CD3^+CD4^+$ T helpers and $CD4^+CD39^+$ T cells ($r = –0.60$, $p < 0.05$), between the number of $CD3^-CD19^+$ B cells and $CD4^+CD39^+$ T cells ($r = –0.40$, $p < 0.05$), and between the value of the immunoregulatory index (the $CD4^+$ to $CD8^+$ ratio) and the number of $CD4^+CD39^+$ T cells ($r = –0.58$, $p < 0.05$). Our findings suggest involvement of $CD4^+CD39^+$ T cells in the immune suppression during CRC formation.

Treg cells play an important role in oncogenesis. Expression of CD39 by Tregs and their participation in the synthesis of extracellular adenosine are believed to constitute one of the key mechanisms underlying the suppression of the immune response [15, 16]. In this study we measured the levels of Treg cells with the $CD4^+CD25^+CD127^{lo/–}$ phenotype. The patients with stages I and II CRC had increased levels of $CD4^+CD25^+CD127^{lo/–}$ Tregs in comparison with the healthy individuals, while in the patients with the advanced stages of CRC the number of these cells was the same as in the controls (Table 1). Tregs with the $CD4^+CD25^+CD127^{lo/–}$ and $CD4^+CD25^{hi}$ phenotypes circulating in the peripheral blood of the patients with CRC increasingly expressed CD39 (Table 2).

As shown in Table 2, CD39 expression in $CD4^+CD25^+CD127^{lo/–}$ Tregs starts to go up in the very early stages of the disease (stages I and II), which does not happen in healthy controls, and reaches its maximum in patients with advanced cancer. The same pattern is observed for $CD4^+CD25^{hi}$ Treg cells. In the cells with the $CD4^+CD25^-$ phenotype, CD39 expression is quite low and does not differ in its intensity from that observed in the controls.

In addition, in this work we studied expression of the Treg transcription factor FOXP3 and its association with CD39 expression. We managed to establish a direct correlation between the expression of CD39 and the expression of FOXP3 in $CD4^+CD25^{hi}$ T cells isolated from the peripheral blood of

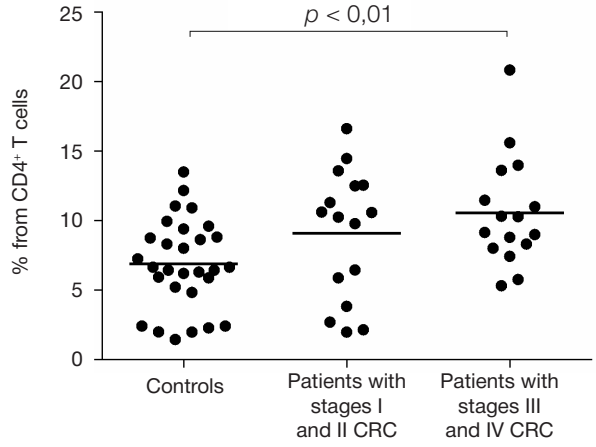

Fig. 1. Levels of $CD4^+CD39^+$ T cells in the peripheral blood of patients with CRC

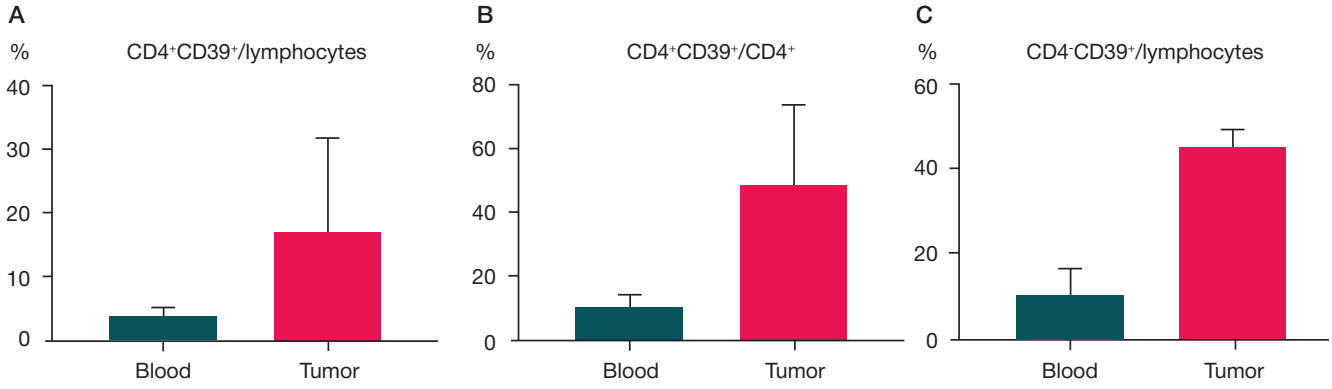

Fig. 2. Levels of CD39-expressing lymphocytes in the peripheral blood and tumor tissue of patients with CRR
Note: * — represents significant differences at $p < 0.05$.

patients with CRC ($r = 0.51$, $p < 0.05$). The subset of $CD4^+CD25^{hi}$ Tregs representing TILs also expressed CD39 more vigorously than peripheral blood lymphocytes. At the same time, almost all TILs with the $CD4^+CD25^{hi}$ phenotype expressed CD39 (Table 2). However, increased CD39 expression was also observed in non-regulatory $CD4^+CD25^-$ cells, as compared with the peripheral lymphocytes of the same patients. This suggests that cancer stimulates expression of CD39 in different subpopulations of $CD4^+$ T cells, including Tregs.

Changes in the population profile of lymphocytes and the relative number of Tregs were also evaluated in patients with AP (Table 1). The levels of T lymphocytes, $CD4^+$ T cells and activated T helpers were lower in these patients than in the controls. In contrast, CD25 expression by T helpers was higher in the patients with AP than in the controls (25.37 ± 8.6% and 18.09 ± 7.5% from the total number of $CD4^+$ T cells, respectively, $p < 0.05$). Changing proportions of different T cell subsets manifested themselves as a drop in the value of the immunoregulatory index (IRI). Unlike healthy donors, the patients with AP had elevated levels of $CD8^+$ CTLs and NK cells. Thus, the patients with AP, as well as the patients with CRC, showed signs of compromised immunity. Besides, the patients with AP had more peripheral $CD4^+CD25^+CD127^{lo/-}$ Treg cells than the controls (Table 1).

When studying the expression of the ectonucleotidase CD39, we found that in the patients with AP, $CD4^+CD39^+$ T cells made 9.16 ± 2.9% from the total count of $CD4^+$ T cells. Expression of CD39 by Treg cells in the patients with AP followed a pattern similar to that observed for the patients with CRC. CD39 was increasingly expressed in the Tregs ($p < 0.05$) of the patients with AP. Expression of CD39 in the Treg cells with the $CD4^+CD25^{hi}$ phenotype in the patients with AP was 57.98 ± 19.6%, while in $CD4^+CD25^+CD127^{lo/-}$ Tregs it amounted to 62.09 ± 16.4%. In $CD4^+CD25^-$ lymphocytes that were not Tregs CD39 expression reached 7.67 ± 4.3% and was not reliably different from the levels observed in the controls.

We also studied involvement of $CD4^+CD39^+$ T cells in the immune suppression in the patients with AP. The correlation analysis of the $CD4^+CD39^+$ T cell count, the expression of this molecule in $CD4^+CD25^+CD127^{lo/-}$ Tregs and the shifts in the population profile of lymphocytes in the patients with AP revealed no reliable associations, in contrast to the CRC situation.

Besides, we measured relative expression of *CD39* mRNA in the peripheral blood leukocytes of patients with CRC and AP. MRNA expression was 2.36 times higher in the patients with CRC than in the controls (Fig. 3). No significant differences in the expression of *CD39* transcripts were observed between the patients with AP and the controls.

DISCUSSION

Extracellular adenosine is a signal molecule that modulates many physiological processes in the body. Recently, adenosine-mediated suppression of the immune response has received a lot of attention as one of the key mechanisms helping cancer cells to evade the immune system. Adenosine is a product of adenosine monophosphate (AMP) dephosphorylation occurring in the extracellular matrix. One of the key enzymes involved in this process is the ectonucleotidase CD39; it ensures conversion of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) to AMP [8, 9].

In the course of this work we explored the role of $CD4^+$ T cells expressing CD39 in the formation of immune suppression in patients with CRC. We found that patients with CRC accumulate $CD4^+CD39^+$ cells in their peripheral blood in the advanced stages of cancer. Among TILs the number of $CD4^+$ T cells expressing CD39 was significantly higher than in the blood of the same patients. Besides, the patients demonstrated a negative correlation between the levels of $CD4^+CD39^+$ T cells and a few other parameters, such as the levels of $CD3^+CD4^+$ T helpers, $CD3^-CD19^+$ B cells and the value of the immunoregulatory index, suggesting involvement of $CD4^+CD39^+$ T cells in immunosuppression during CRC progression.

Treg cells play an important role in promoting immune suppression in nascent and progressing cancer. Recently, it has been discovered that Tregs can engage in the accumulation of

Table 1. Percentage of major lymphocyte subsets in the peripheral blood of healthy donors, patients with CRC and patients with AP from the total number of lymphocytes

Phenotypes	Controls	Patients with CRC		Patients with AP
		stages I–II	stages III–IV	
$CD3^+$ (T cells)	69.26 ± 5.3	66.96 ± 6.4	64.02 ± 6.4*	63.87 ± 7.1*
$CD3^+CD4^+$ (T helpers)	42.39 ± 6.4	32.80 ± 9.5*	35.66 ± 5.9*	34.46 ± 9.1*
$CD4^+CD25^+$ (activated T helpers)	10.74 ± 5.2	6.46 ± 2.7*	5.57 ± 1.8*	7.90 ± 3.2*
$CD3^+CD8^+$ (CTL)	21.98 ± 4.7	31.45 ± 6.1*	27.28 ± 6.5*#	28.20 ± 7.7*
$CD3^-CD19^+$ (B cells)	11.15 ± 3.0	8.13 ± 4.3*	5.99 ± 2.8*	7.90 ± 5.2
$CD3^-CD16^+$ (NK cells)	14.84 ± 5.7	13.39 ± 4.8	15.56 ± 8.5	17.65 ± 7.2*
$CD4^+/CD8^+$ (immunoregulatory index)	2.07 ± 0.5	1.12 ± 0.5*	1.40 ± 0.4*	1.36 ± 0.7*
$CD4^+CD25^+CD127^{lo/-}$ (Tregs)	4.56 ± 1.0	5.34 ± 1.9*	4.7 ± 1.4	6.83 ± 2.7*

Note: * — represents significant differences from the controls, $p < 0.05$; # — represents significant differences from patients with stages I and II CRC, $p < 0.05$.

Table 2. Expression of ectonucleotidase C39 in the subset of $CD4^+$ T cells isolated from patients with CRC presented as percentage from the total number of $CD4^+$ T cells

	$CD4^+CD25^-$	$CD4^+CD25^{hi}$	$CD4^+CD25^+CD127^{lo/-}$
Controls	5.58 ± 3.9	42.70 ± 5.8	41.25 ± 2.7
Patients with stages I and II CRC	5.97 ± 3.6	53.85 ± 3.9*	55.32 ± 4.1*
Patients with stages III and IV CRC	7.54 ± 3.3	66.14 ± 3.4**	67.87 ± 2.9**
TIL	35.76 ± 22.6#	90.06 ± 7.1#	Нет данных

Note: * — represents significant differences from the controls; ** — represents significant differences from the controls and patients with stages I and II CRC; # — represents significant differences from the peripheral blood lymphocytes of the same patients with CRC.

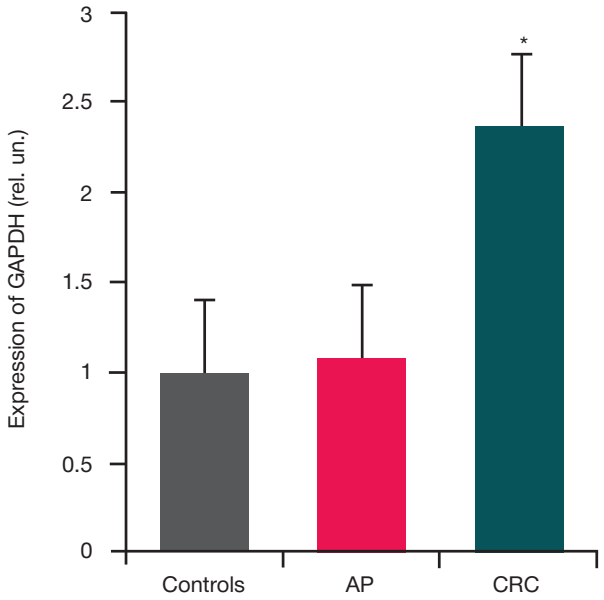

Fig. 3. Changes in the levels of *CD39* mRNA in the peripheral blood leukocytes of patients with CRC and AP (relative to *GAPDH* mRNA)
Note: * — represents significant differences from the controls at $p < 0.05$; data are presented as M ± SE.

extracellular adenosine. Unlike other T lymphocytes, these cells increasingly express CD39 required for their suppressive activity [20, 21]. It has been shown that Treg cells isolated from the blood of CD39-deficient mice exhibit a low level of suppressive activity *in vitro* and cannot prevent transplant rejection *in vivo* [22]. Murine $CD4^+CD25^+$ Tregs express on their surface both CD39 and CD73, the nucleotidase dephosphorylating AMP to adenosine. In humans, expression of CD73 by $CD4^+CD25^{hi}$ T cells is very low [23], but in the cytoplasm it becomes more vigorous than in $CD4^+CD25^-$ T lymphocytes [21]. Moreover, the cell culture of human Tregs has been shown to promote adenosine accumulation, confirming that these cells produce active CD73 molecules.

We were able to show that peripheral blood $CD4^+CD25^{hi}$ and $CD4^+CD25^+CD127^{lo/-}$ T cells increasingly express CD39 in patients with colorectal cancer. High expression of this ectonucleotidase correlated with the stage of the disease and was also noted for $CD4^+CD25^{hi}$ TILs. Given that, we conclude that TILs accumulate Tregs that increasingly express $CD39^+$ as a result of cell recruiting from the peripheral pool of lymphocytes. These Tregs constitute one of the dominating subsets of Tregs in colorectal cancer, especially in its advanced stages. Besides, $CD39^+$ Tregs exhibit a high immunosuppressive activity, which can promote cancer, along with other mechanisms of immune suppression.

Progression of severe AP is also accompanied by the changes in the reactivity of the immune system. AP is characterized by pancreatic inflammation that can affect peripancreatic tissue and lead to multiple organ failure occurring as a result of necrosis, infection or sepsis [24]. Presumably, overt immune manifestations of the systemic inflammatory response syndrome can trigger immunosuppression, leading to the inability of the body to resist microbial invasions, festering and necrotic complications [25].

Our findings about the changes in the population profile of lymphocytes and increased levels of Treg cells in patients with AP may be indicative of immune suppression occurring in such patients. Considering elevated levels of Tregs, the inflammatory nature of the disease and the increasing apoptosis of circulating lymphocytes [26], it can be assumed that immune suppression in patients with AP is a compensatory mechanism restraining the inflammatory response.

Unlike patients with CRC, patients with AP had the same levels of $CD4^+CD39^+$ cells as the controls. No correlation has been observed between the proportions of major lymphocyte subsets and the levels of $CD4^+CD39^+$ T cells. In the patients with AP, expression of CD39 by Tregs ($CD4^+CD25^{hi}$ and $CD4^+CD25^+CD127^{lo/-}$) was higher than in the controls, which may be explained by the increased presence of Tregs in the blood of these patients. It is likely that in patients with AP $CD4^+CD39^+$ T cells do not make a considerable contribution to the development of systemic immune suppression as it happens in patients with cancer. This supposition is supported by the results of the analysis of relative *CD39* mRNA levels in patients with CRC and AP. The patients with CRC have demonstrated a gradual increase in *CD39* mRNA levels in the course of the disease, which reached its maximum in the advanced stages. At the same time, the patients with AP had the same levels of *CD39* mRNA as the controls.

CONCLUSIONS

Progression of CRC is accompanied by the expansion of $CD4^+$ T cells expressing CD39; active expansion of $CD39^+$ cells is observed in the pool of TILs. These cells play an important role in the formation of immune suppression in patients with CRC. The substantial proportion of CD39-expressing cells is constituted by Treg lymphocytes. Inhibition of CD39 expression and/or restriction of Treg cell activity may be of interest for the development of new approaches to anticancer therapies. Further research is necessary to elucidate the mechanisms of adenosine-A2AR-mediated immune suppression in patients with cancer.

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