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ЗАВИСИМОСТЬ ФЕНОТИПА И ХЕМИЛЮМИНЕСЦЕНТНОЙ АКТИВНОСТИ МОНОЦИТОВ ОТ КОЛИЧЕСТВА Т-РЕГУЛЯТОРНЫХ КЛЕТОК У БОЛЬНЫХ РАКОМ ПОЧКИ

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Резюме. Целью данного исследования явилось изучение зависимости фенотипа и активности респираторного взрыва моноцитов от количества Т-регуляторных клеток (Tregs) у больных с раком почки (РП). Больные с РП (T3N0M0, светлоклеточный тип) были обследованы до хирургического лечения. Фенотип Tregs и моноцитов крови изучали методом проточной цитометрии. Исследование состояния респираторного взрыва моноцитов проводилось путем определения активности люцигенин- и люминол-зависимой спонтанной и зимозан-индуцированной хемилюминесценции. Установлено, что количество Tregs в крови больных с РП было увеличено относительно контрольных значений (у пациентов с РП – Me = 6,3%). Все обследованные пациенты были разделены на две группы в соответствии с медианой по Tregs (менее и более 6,3%). Наиболее выраженные изменения в фенотипе моноцитов и их хемилюминесцентной активности были обнаружены у больных с РП с уровнем Tregs менее 6,3%. Только эта группа пациентов имела перераспределение в субпопуляционном составе моноцитов: уменьшение относительного количества классических моноцитов и увеличение относительного содержания неклассических (провоспалительных) моноцитов. Увеличение абсолютного количества общих моноцитов и уменьшение процентного содержания HLA-DR$^+$ и HLA-DR$^+$CD64$^+$ моноцитов было обнаружено у больных РП независимо от количества Tregs в крови. Изменения в фенотипе моноцитов у больных РП сопровождались изменением состояния их респираторного взрыва. Спонтанный синтез супероксид-радикала (первичная активная форм кислорода – АФК) моноцитами у больных РП с низким уровнем Tregs в крови характеризовался более коротким временем активации НАДФН-оксидазы и более высоким уровнем ее активности, чем у пациентов с высоким содержание Tregs в крови. Индекс активации люцигенин-зависимой хемилюминесценции у больных РП был снижен, не зависел от количества Tregs в крови и определялся, по-видимому, недостаточностью метаболических резервов. Спонтанный синтез вторичных АФК в моноцитах у больных РП был снижен и не зависел от количества Tregs в крови. Индуцированный синтез вторичных АФК и индекс активации их синтеза в моноцитах были снижены только у больных РП с пониженным количеством Tregs в крови. В целом характеристики хемилюминесцентной реакции моноцитов у больных РП определяют наличие дисбаланса между синтезом первичного и вторичного АФК в моноцитах крови. Моноциты

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у больных РП с низким уровнем Tregs в крови характеризуются большей провоспалительной активностью благодаря быстрой активации и интенсивности синтеза первичных АФК.

Ключевые слова: моноциты, регуляторные Т-лимфоциты, рак почки, фенотип, хемилюминесцентная активность, респираторный взрыв

DEPENDENCE OF PHENOTYPE AND CHEMILUMINESCENT ACTIVITY OF MONOCYTES ON THE T REGULATORY CELLS CONTENT IN PATIENTS WITH KIDNEY CANCER

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Abstract. The aim of this work was to reveal the interrelations between the number of T regulatory cells (Tregs) in patients with kidney cancer (KC) and phenotype of peripheral blood monocytes and their capacities to produce ROS. Patients with KC (T3N0M0, clear cell type) were examined prior to surgical treatment. Tregs phenotype and blood monocytes were identified by flow cytometry. ROS production of purified monocytes was carried out through the determination of lucigenin- and luminol-dependent spontaneous and zymosan-induced chemiluminescence activity. It has been found that the relative number of Tregs within total lymphocyte subset in KC patients was increased if compared to control values (in KC patients – Me = 6.3%). Then the patients were divided into two groups according to the median of Tregs number (less and more than 6.3%). The most pronounced changes in the phenotype of monocytes and their chemiluminescent activity were found in KC patients with the Tregs count of less than 6.3%. Our findings suggest that low frequency of Tregs in the periphery was associated with increased relative numbers of "intermediate" and "non-classical" ("pro-inflammatory") monocytes as it was shown on the samples from patients with KC with a low level of Tregs. According to our data, both groups of KC patients had low levels of HLA-DR expression when comparing to control group. Furthermore, both groups of patients had decreased rates of HLA-DR and CD64 co-expressing cells. Changes in the phenotype of monocytes in patients with KC were closely linked with imbalance in ROS production. Thus, the monocytes spontaneous superoxide radical (primary ROS) synthesis in KC patients with a low Treg numbers were characterized by redused NADPH-oxidase activation time and increased level of its activity if compared to patients with a high Treg rates in peripheral blood. Next, the activation index for lucigenin-dependent chemiluminescence in KC patients was reduced, as well as it was independent of circulating Tregs rates and was determined apparently by the insufficiency of metabolic reserves. Similarly, spontaneous secondary ROS production by the monocytes in KC patients was lower then in healthy controls and was also independent of circulating Tregs rates. Finally, the induced secondary ROS synthesis and activation index for their synthesis in monocytes were reduced only in patients with KC with a low number of Tregs in the blood. In general, the characteristics of the chemiluminescent reaction of monocytes in patients with KC determined the imbalance in peripheral blood monocytes primary and secondary ROS production. Monocytes in patients with KC with a low number of Tregs in the blood were characterized by more pro-inflammatory activity due to the rapid activation and intensity of the synthesis of primary ROS.

Keywords: monocytes, T regulatory cells, kidney cancer, phenotype, chemiluminescent activity, respiratory burst

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Introduction

Monocytes are innate immunity cells that play an important part in cancer development and progression. During cancer, these cells were involved in systemic inflammatory reactions that develops with the progression of the tumor in an organism [8, 27]. The most recent study by Komala A.S. et al. (2018) have shown that the intensity of the inflammatory reactions as well as the number of circulating blood monocytes were increased during nasopharyngeal carcinoma progression [11]. Thus,

the inflammatory reaction was defined as one of the most important pro-tumoral factors. Accordingly, circulating monocytes and tumor-associated macrophages could perform functions that contribute to pro-tumoral activity. For example, monocyte chemoattractant protein 1 (MCP-1) was defined as one of the key factors stimulating the breast cancer development and progression [5]. MCP-1 stimulated up-regulated macrophages migration to the tumor microenvironment.

Next, monocytes were the key regulators of the immune reactions during tumor growth [15, 26]. The most important monocyte-devided immunoregulatory factors were the cytokines as well as reactive oxygen species (ROS) and nitrogen forms that modulated the functional activity of different immune cells [12, 25]. Furthermore, monocyte differentiation into tumor-associated macrophages and dendritic cells – subsets of antigen-presenting cells that were required for effective adaptive immune responses – also played an important part in cancer development and progression [10, 32, 39]. However, depending on the phenotype both macrophages and dendritic cells could take part in anti- and pro-tumorigenic processes.

Currently, monocytes are a heterogeneous population with phenotypical and functional differences. Thus, human circulating monocytes consisted at least of two subpopulations that could be identified base on surface expression of two molecules – CD14 and CD16. The cells expressing only the high affinity receptor for bacterial lipopolysaccharide and lipopolysaccharide-binding proteins ($CD14^+CD16^-$) were defined as "classical" monocytes [23, 37]. This subset exhibited high phagocytosis rate but didn't take part in augmenting of inflammation. Next, monocytes expressing both CD14 and CD16 (the low-affinity receptor for IgG – FcgRIII) were termed as "non-classical" or "pro-inflammatory" [13, 19]. It was shown that the increased number of "non-classical" monocytes was strongly associated with several infectious and inflammatory diseases [4, 20]. Part of the difficulty in monocyte nomenclature arises from the fact that "classical" monocytes under the influence of regulatory factors could differentiate into "non-classical" ones [2, 21]. The heterogeneity of monocytes was also confirmed by the presence of cells with intermediate – $CD14^{low}CD16^+$ – phenotype, thus, they were defined as "intermediate" monocytes [21, 34]. Accordingly, different subsets of peripheral blood monocytes that penetrate the site of inflammation could acquire either macrophage or dendritic cell phenotypes with different functional activities.

In its turn, cancer cells were also able to edit immune system response via downregulation of antitumor activity [6, 22, 36]. This editing is carried out through the receptor-ligand interactions as well as the synthesis of soluble factors production by tumor cells. Consistent with that evidence, not only the nearest microenvironment was edited but also the state of the immune system cells at the system level. Consequently, the phenotype and functional activity of blood monocytes was also changing with tumor progression. We have previously underlined that the number of circulating $CD14^{low}CD16^+$ monocytes were increased in patients with kidney cancer [29]. Furthermore, we demonstrated the imbalance in monocytes activation markers expression and the decrease in reactive oxygen species production within this leucocytes subset in these patients.

Finally, T regulatory cells (Tregs) are a separate fraction of $CD4^+$T cells that are able to inhibit anti-tumor immune response, implied their involvement in tumor pathogenesis and disease progression. Tregs constitutively express the α-chain of interleukin-2 receptor (CD25) and inhibit the T lymphocytes proliferation and cytokines production [24, 33].

The aim of this study was to analyze the dependence of the phenotype and the activity of the respiratory burst of monocytes on the number of Tregs in patients with kidney cancer (KC).

In the current study we report that peripheral blood monocytes phenotype as well as their ability to produce ROS was dependent on the number of Tregs in patients with KC.

Materials and methods

Study participants

Patients (n = 74, age – 40-60 years) with KC (T3N0M0, clear cell type) were examined prior to surgical treatment on the basis of the Krasnoyarsk Regional Oncology Center. The diagnosis of KC of all patients was verified histologically. Peripheral blood samples from 63 healthy volunteers with the same age range were used as a control group. All studies were performed with the informed consent of the patients and in accordance with the Helsinki Declaration of the World Association "Ethical principles of scientific medical research involving human" as amended in 2013 and "Rules of clinical practice in the Russian Federation" approved by the Order of the Ministry of Health of Russia of 19.06.2003 (No. 266).

Flow cytometry

The phenotype of Treg and blood monocytes were studied by direct immunofluorescence using monoclonal antibodies (Beckman Coulter, USA) labeled with FITC (fluorescein isothiocyanate), PE или RD1 (phycoerythrin), ECD (phycoerythrin-Texas Red-X), PC5 (phycoerythrin-cyanin 5) и PC7 (phycoerythrin-cyanin 7) in the following panels: CD62L-FITC/CD127-PE/CD3-ECD/CD25-PC5/CD4-PC7 and HLA-DR-FITC/CD14-PE/CD45-ECD/CD64-PC5/CD16-PC7. The distribution of

antibodies in fluorescence channels was carried out in accordance with the principles of panel formation for the multicolor cytofluorimetric investigations [14]. Sample preparation was performed according to the standard procedure [16]. Erythrocyte lysis was carried out by a washout-free technology using the reagent VersaLyse (Beckman Coulter, USA). The analysis of stained cells was performed on a flow cytometer Navios (Beckman Coulter, USA) of the Center of collective usage Federal Research Center "Krasnoyarsk Science Center of the Siberian Branch of the Russian Academy of Sciences". Not less than 50 000 lymphocytes or monocytes were analyzed in each sample.

Chemiluminescent activity

Blood monocytes were obtained by standard adhesion method from a suspension of mononuclear cells isolated from blood with heparin in a density gradient ficoll-urografin (ρ = 1.077) [7]. The study of the state of respiratory burst of monocytes was carried out through the determination of the activity of lucigenin- and luminol-dependent spontaneous and zymosan-induced chemiluminescence [31]. The chemiluminescent activity was evaluated for 90 minutes on a 36-channel biochemiluminescence analyzer BLM-3607 (MedBioTech Ltd, Russia). The following characteristics of the chemiluminescent reaction were identified: time to maximum (Tmax), maximum intensity (Imax) and area under chemiluminescence curve (S). The enhancement of chemiluminescence induced by zymosan was evaluated by the ratio of the induced chemiluminescence area to the spontaneous area and characterized as an activation index (Sind/Sspont).

Statistical analysis

Statistical description was performed by counting the median (Me) and the inter-quarter span in the form of 25 and 75 percentiles ($Q_{0.25}$-$Q_{0.75}$). Significance of differences between indicators was assessed by nonparametric criterion Mann-Whitney U test. Statistical analysis was performed in an application package Statistica 6.1 (StatSoft Inc.).

Results

First, we found that the number of Tregs in the blood of the KC patients was increased relative to control values (in KC patients – Me = 6.3%, $Q_{0.25}$ = 4.3% and $Q_{0.75}$ = 8.3%, p = 0.043). Based on the relative number of Tregs within total lymphocytes subset, all patients were divided into two groups according to the median of Tregs number (less and more than 6.3%). Next, our findings demonstrated that the relative and absolute number of Tregs as well as activated Tregs in patients with low number of Tregs were reduced if compared with the control indicators (Table 1). Accordingly, an increase in the number of Tregs and activated Tregs in comparison with the control values was detected in patients with high level of Tregs. The absolute number of $CD4^+$T lymphocytes in patients of this group was also increased if compared with patients with low levels of Tregs.

We further measured the relative numbers of main monocytes subsets with different patterns of CD14

TABLE 1. PERCENTAGE AND ABSOLUTE NUMBER OF T REGULATORY CELLS IN PATIENTS WITH KC, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control n = 32	Patients with KC	
		Treg < 6.3% n = 15	Treg > 6.3% n = 15
$CD3^+CD4^+$, 10^9/l	0.92 (0.56-1.16)	0.75 (0.52-0.90)	0.92 (0.68-1.11) p_2 = 0.016
$CD3^+CD4^+CD127^{low}CD25^{high}$, %	5.5 (4.1-6.7)	4.4 (2.9-5.4) p_1 = 0.002	8.4 (7.0-10.4) $p_{1.2}$ < 0.001
$CD3^+CD4^+CD127^{low}CD25^{high}$, 10^9/l	0.100 (0.070-0.140)	0.073 (0.052-0.091) p_1 < 0.001	0.151 (0.119-0.202) p_1 = 0.003 p_2<0.001
$CD3^+CD4^+CD127^{low}CD25^{high}CD62L^+$, %	3.8 (2.9-4.9)	3.0 (1.8-3.7) p_1 = 0.001	5.8 (4.5-7.4) $p_{1.2}$ < 0.001
$CD3^+CD4^+CD127^{low}CD25^{high}CD62L^+$, 10^9/l	0.071 (0.052-0.099)	0.045 (0.031-0.066) p_1 < 0.001	0.115 (0.067-0.147) p_1 = 0.021 p_2 < 0.001

Note. p_1, statistically significant differences with the control group; p_2, -//- between patients with high and low Treg.

TABLE 2. FEATURES OF THE BLOOD MONOCYTE PHENOTYPE IN PATIENTS WITH KC IN DEPENDING ON THE NUMBER OF T REGULATORY CELLS, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control n = 32	Patients with KC	
		Treg < 6.3% n = 15	Treg > 6.3% n = 15
Monocytes, 10^9/l	0.35 (0.18-0.51)	0.45 (0.35-0.64) $p_1 = 0.003$	0.52 (0.32-0.73) $p_1 < 0.001$
$CD14^+CD16^-$	62.7 (42.3-72.3)	34.7 (22.7-55.4) $p_1 = 0.014$	52.4 (21.7- 62.3)
$CD14^+CD16^+$, %	34.4 (16.2-52.2)	54.3 (46.0-70.4) $p_1 = 0.043$	40.9 (32.7- 51.6)
$CD14^{low}CD16^+$, %	5.8 (4.1-11.1)	7.1 (4.7-10.1)	6.0 (2.3-11.2)
$HLA\text{-}DR^+$, %	90.8 (84.5-95.8)	83.5 (75.6-88.2) $p_1 = 0.011$	74.9 (5.2-85.7) $p_1 = 0.004$
$CD64^+$, %	90.0 (82.9-93.6)	87.7 (78.6-94.5)	91.0 (87.3-94.9)
$HLA\text{-}DR^+CD64^+$, %	90.3 (81.5-98.9)	70.7 (56.6-81.6) $p_1 < 0.001$	76.5 (63.3-87.8) $p_1 < 0.001$

Note. As for Table 1.

and CD16 expression and showed that the percentage of $CD14^+CD16^-$ monocytes was decreased while $CD14^+CD16^+$ cells were increased in patients with a low number of Tregs compared to healthy control (Table 2). In addition, we analyzed the HLA-DR and CD64 expression within total monocyte subset. It was found out that in peripheral blood from both groups of KC patients the levels of HLA-DR-positive and $HLA\text{-}DR^+CD64^+$ were reduced if compared with control.

Additionally, the time to the maximum of the spontaneous lucigenin-dependent monocyte chemiluminescence was reduced in KC patients with a low number of Tregs in the blood in comparison with control values (Table 3). The maximum and area under the curve of this type of monocyte chemiluminescence in patients with KC in both groups were higher then in healthy subjects. However, the maximum of this chemiluminescence type with a high level of circulating Tregs was elevated less pronounced relative to control values than with a low number of them. Both groups of patients with KC exhibited decreased levels of the activation index of luminol-dependent chemiluminescence of monocytes if compared with the control group. Next, the maximum and area under the curve of spontaneous luminol-dependent chemiluminescence of monocytes in patients with KC were lower then in control values regardless the number of Tregs in circulation. Finally, KC patients with a low level of circulating Tregs exhibited low maximum and area under the curve of the zymosan-induced luminol-dependent chemiluminescence of monocytes as well as an activation index for luminol.

Discussion

The functional activity of monocytes is determined by their quantitative composition, phenotype and complex regulatory effects. Here, we describe for the first time that in patients with kidney cancer changes in Tregs frequency in circulation were closely linked with disturbances in monocytes subsets and their functional activity. The patients with a low number of Tregs had a quantitative redistribution between "classical" and "non-classical" monocytes: reducing the percentage of "classical" monocytes and increasing "non-classical". The subset of "classical" monocytes consists of large cells with expressed phagocytic properties and high levels respiratory burst enzymes. They also characterized by an increased levels of cell-surface CD36, CD62L, CD64, CCR2 and low capacity of IL-1 and TNFα production [7, 23, 37]. "Non-classical" (or "pro-inflammatory") monocytes were relatively small cells with decreased phagocytic activity and low reactive oxygen species production [13, 19, 21]. This type of cell expressed high levels of CD11c, HLA-DR, and CX3CR1, while the expression of CD62L and CD64 were low [4, 20, 34]. Thus, monocyte subsets in KC patients with low Tregs level in circulation exhibited pro-inflammatory alteration if compared with the patients showed the high level of Tregs. It

TABLE 3. CHEMILUMINESCENT ACTIVITY OF THE MONOCYTES IN PATIENTS WITH KC IN DEPENDING ON THE NUMBER OF T REGULATORY CELLS, Me ($Q_{0.25}$-$Q_{0.75}$)

Parameters	Control n = 32	Patients with KC	
		Treg < 6.3% n = 15	Treg > 6.3% n = 15
Spontaneous lucigenin-dependent chemiluminescence			
Tmax, sec.	2014 (1452-2604)	1421 (850-1892) $p_1 = 0.045$	2385 (943-2465)
Imax, r.u. × 10^3	0.67 (0.40-1.51)	8.49 (3.05-19.31) $p_1 < 0.001$	2.16 (0.94-4.38) $p_1 = 0.045$ $p_2 = 0.019$
S, r.u. × sec. × 10^6	2.83 (1.77-7.02)	27.69 (5.74-59.47) $p_1 = 0.009$	7.73 (3.58-12.51) $p_1 = 0.008$
Zymosan-induced lucigenin-dependent chemiluminescence			
Tmax, sec.	1835 (1576-2842)	2930 (1755-3357)	2086 (255-2109)
Imax, r.u. × 10^3	2.89 (1.12-8.42)	3.77 (2.70-4.60)	2.86 (1.54-3.02)
S, r.u. × sec. × 10^6	15.24 (4.11-24.55)	15.50 (8.66-18.83)	7.26 (1.01-11.49)
Sind/Sspont	3.61 (1.65-8.03)	0.93 (0.27-1.90) $p_1 = 0.012$	1.21 (0.12-2.65) $p_1 = 0.027$
Spontaneous luminol-dependent chemiluminescence			
Tmax, sec.	1810 (660-2575)	1489 (1167-2505)	1341 (151-1671)
Imax, r.u. × 10^3	6.29 (1.52-15.10)	2.51 (1.24-9.60) $p_1 = 0.039$	3.06 (1.68-4.86) $p_1 = 0.044$
S, r.u. × sec. × 10^6	24.79 (6.11-47.57)	8.88 (3.99-22.87) $p_1 = 0.028$	12.18 (5.17-17.45) $p_1 = 0.039$
Zymosan-induced luminol-dependent chemiluminescence			
Tmax, sec.	1150 (973-1808)	1178 (555-2171)	1034 (677-1507)
Imax, r.u. × 10^3	11.60 (5.85-45.20)	3.10 (2.56-6.94) $p_1 = 0.020$	9.20 (2.11-22.67) $p_2 = 0.042$
S, r.u. × sec. × 10^6	41.49 (16.18-143.49)	8.35 (3.93-22.18) $p_1 = 0.011$	33.62 (15.49-80.07) $p_2 = 0.035$
Sind/Sspont	2.89 (1.72-3.80)	0.55 (0.33-3.45) $p_1 = 0.017$	1.67 (1.05-4.16) $p_2 = 0.028$

Note. sec., seconds; r.u., relative units; p_1, statistically significant differences with the control group; p_2, -//- between patients with high and low Treg.

is known that Tregs have suppressive activity during inflammation [9, 38]. Our findings suggest that low frequency of Tregs in the periphery was associated with increased relative numbers of "intermediate" and "non-classical" monocytes as it was shown on the samples from patients with KC with a low level of Tregs. These data also indicate the inability of Tregs to effectively suppress inflammation and inhibit antigen presentation as well as all other monocytes functions. However, the revealed imbalance in monocyte subsets in patients with a low level of Tregs and the absence of significant differences between healthy control and KC patients with high rates of Tregs did not lead to any peculiarities on HLA-DR expression by monocytes. The HLA-DR belongs to major histocompatibility class II complex and takes part in antigen presentation [17]. It has been shown that monocytes with low HLA-DR expression could not effectively respond to stimulating signals, as well as exhibited low antigen presentation capacity and decreased proinflammatory cytokines production [40]. According to our data, both groups of KC patients had low levels of HLA-DR expression when comparing to control group. Furthermore, both groups of patients had decreased rates of HLA-DR and CD64 co-expressing cells. It is known that CD64 consists of a signal peptide and 3 extracellular immunoglobulin domains of type C2 forming a glycoprotein which is a high affinity IgG receptor [1, 17]. Consequently, the functional activity of monocytes in patients with kidney cancer was reduced, yet this imbalance was not associated with the number of Tregs in peripheral blood.

Chemiluminescent activity of monocytes characterizes the state of their respiratory burst [3, 30]. We used two chemiluminescent indicators: lucigenin and luminol. The lucigenin luminescence appears only after superoxide radical – the primary ROS which is formed by membrane NADPH-oxidase (Nox) – generation [18, 30]. Luminol can penetrate through the cell membrane and reveals its luminescence properties after interaction with secondary ROS such as hydroxyl radical, hydrogen peroxide, singlet oxygen, etc [30, 31]. The chemiluminescent curve is characterized by three parameters: time to attain the maximum (Tmax), the maximum intensity (Imax) and the area under the chemiluminescence curve (S). "Tmax" characterizes the time required for the maximum activation of ROS production including the period from the perception of a functional or regulatory signal to the maximum activity of enzymes of ROS synthesis (kinetic characteristic of the respiratory burst). "Imax" reflects the maximum level of ROS synthesis. While "S" integrally characterizes the level of ROS synthesis for 90 minutes of measuring chemiluminescence [30].

The special feature of lucigenin-dependent chemiluminescence of monocytes in KC patients with low Tregs count in the blood was the reduction of Tmax during spontaneous superoxide radical synthesis. Upregulation of spontaneous superoxide radical synthesis by monocytes was observed in both groups of patients with KC. However, the maximum intensity of primary ROS synthesis by monocytes was significantly higher in KC patients with low Treg numbers vs. KC patients with high level of Tregs. Next, zymosan-induced superoxide radical synthesis by monocytes in patients with KC corresponded to control indicators and didn't differ within patients groups. At the same time, the lucigenin-dependent chemiluminescence activation index was decreased in patients with KC. The activation index characterizes the level of metabolic reserves that are necessary for ROS synthesis during the functional activation of cells [30]. Accordingly, monocytes metabolic reserve in patients with kidney cancer was lower then in healthy controls and was independent of circulating Tregs rates.

Finally, our findings on luminol-dependent chemiluminescence of monocytes made it possible to establish that the spontaneous secondary ROS production in patients with kidney cancer was reduced and independent on Treg numbers. Only kidney cancer patients with the decreased number of Tregs showed low levels of induced secondary ROS synthesis as well as a lower value of the activation index by luminol-dependent chemiluminescence.

In line with these findings, we propose that blood monocytes in patients with KC had an imbalance in the synthesis of primary and secondary ROS that were related with relative numbers of Tregs in peripheral blood. In summary, monocytes from KC exhibited an increased level of primary ROS synthesis, while monocytes secondary ROS production was decreased. Monocytes from kidney cancer patients with reduced Treg levels showed pro-inflammatory shift due accelerated activation and increased level of superoxide radical synthesis. At the same time, metabolic reserves for the induced ROS synthesis in monocytes were minimal in patients with KC with the low blood Treg counts. It should also be noted that in the literature there are publications not only about the effect of Tregs on inflammatory processes but also about the effect of ROS on the proliferation of Tregs [28, 35]. Thus, the relationship between ROS production and Tregs should be analyzed regarding their mutual regulation.

Conclusion

We describe for the first time that the levels of monocyte subsets as well as the state of their respiratory explosion in KC patients was depended on the number of circulating Tregs. The most pronounced changes in

monocytes phenotype and their chemiluminescent activity were found in KC patients with the Tregs count of less than 6.3%. Only this group of patients had pronounced imbalance of monocyte subsets: a decrease in the relative number of "classical" monocytes and an increase in the relative content of "non-classical" (or "pro-inflammatory") monocytes. At the same time, an increase in the absolute number of total monocytes and a decrease in the percentages of HLA-DR$^+$ and HLA-DR$^+$CD64$^+$ monocytes were described for patients with KC in regardless of the Treg rates. It is assumed that the decrease in the effect of Tregs on monocytes in patients with KC could lead to a more pronounced participation of monocytes in inflammatory reactions that are realized during tumor progression. The imbalance of peripheral blood monocyte subsets in KC patients with a low number of Tregs as well as the migration of cells from the blood leads to a decrease in the number of monocytes expressing activation receptors. At the same time, the reduction of HLA-DR$^+$ and HLA-DR$^+$CD64$^+$ monocytes in patients with KC with a high Treg levels may have a regulatory nature and could be associated with a general inhibitory effect of tumor growth (which is implemented including through Tregs). Alterations in monocytes phenotype of in patients with KC were accompanied by shifts in their abilities to produce ROS. It has been found that monocyte spontaneous superoxide radical (primary ROS) synthesis in KC patients with a low number of Tregs was characterized by diminishment of Nox activation time and increased level of its activity vs. the patients with a high Treg rates. Monocyte total spontaneous superoxide radical production in patients with KC was higher than in healthy people and was not linked with number of Tregs in peripheral blood. Next, in both groups of KC patients the activation index for lucigenin-dependent chemiluminescence was significantly lower then in healthy control, it didn't depend on the number of blood Tregs and was determined apparently by the insufficiency of metabolic reserves. Furthermore, monocyte spontaneous secondary ROS synthesis in patients with KC was reduced and also was not related with Tregs frequency in circulation. Interestingly, the induced secondary ROS synthesis and activation index of the current assay in monocytes were reduced only in patients with KC with a low number of Tregs. In general, the characteristics of the chemiluminescent reaction of monocytes in patients with KC determined the imbalance between the synthesis of primary and secondary ROS in blood monocytes. These findings suggest that monocytes in patients with KC with a low number of Tregs in the blood were characterized by more pro-inflammatory activity due to the rapid activation and intensity of the synthesis of primary ROS. A better understanding of how various tumor growth factors influence monocyte functions are promising in the development of new effective mechanisms for stimulating immune system antitumor activity in cancer patients.

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