ТРОМБОЦИТЫ ПРИ ХРОНИЧЕСКОЙ РЕАКЦИИ «ТРАНСПЛАНТАТ ПРОТИВ ХОЗЯИНА»: СВЯЗЬ С Th1/Th2-СООТНОШЕНИЕМ

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Резюме. Как обнаружено в клинических и лабораторных исследованиях, тромбоциты не только играют ключевую роль в процессах коагуляции и тромбообразования, но и способны активно участвовать в других патофизиологических процессах, в том числе и в развитии иммунных реакций. В частности, показано, что изменения в иммунной системе, приводящие к заболеванию системной красной волчанкой (СКВ), нередко сопровождаются изменениями числа тромбоцитов и их активности в периферической крови больных СКВ, коррелирующими с выраженностью клинических проявлений болезни. В предыдущие годы нами была детально исследована одна из стандартных экспериментальных моделей СКВ, основанная на индукции хронической реакции «трансплантат против хозяина» (хРТПХ) в полуаллогенной системе DBA/2 → (C57Bl/6 x DBA/2)F_1. Однако участие тромбоцитов в этом иммунопатологическом процессе исследовано не было, и в литературе нет данных о поведении тромбоцитов при хРТПХ или о связи их с состоянием Th1/Th2-баланса, хотя по аналогии с развитием СКВ у человека можно ожидать, что и в использованной нами экспериментальной модели количество тромбоцитов изменяется в соответствии с развитием хРТПХ. Поэтому целью данной работы было определение числа тромбоцитов в крови у мышей с Th1- и Th2-зависимыми вариантами хРТПХ.

В экспериментах были использованы самки мышей линий DBA/2 и гибридов (C57Bl/6 × DBA/2) F_1. Хроническую РТПХ в полуаллогенной системе индуцировали вводя спленоциты мышей DBA/2 мышам-гибридам B6D2F_1: по 60-70 × 10^6 клеток в/в двукратно с интервалом в 6 дней. Исследуемые параметры оценивали через три месяца после начала эксперимента и формирования люпус-подобного гломерулонефрита у животных с Th2-зависимым вариантом хРТПХ.

Снижение количества эритроцитов и гемоглобина, уменьшение показателей гематокрита и параллельное увеличение количества ретикулоцитов в крови мышей с хРТПХ хорошо согласуется с ранее сделанным нами выводом о наличии у этих животных аутоиммунной гемолитической анемии. Было

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обнаружено, что в отличие от других форменных элементов крови тромбоциты существенно возрастают при развитии хРТПХ, но о вторичном тромбоцитозе в данной модели СКВ можно говорить только в отношении Th2-зависимого варианта этого процесса, в то время как в группе с Th1-зависимым вариантом хРТПХ среднее количество тромбоцитов в крови не отличается от контрольной группы.

Ключевые слова: хроническая реакция трансплантат против хозяина, системная красная волчанка, экспериментальная модель, тромбоциты, Th1/ Th2-баланс

BLOOD PLATELETS IN CHRONIC GRAFT-VERSUS-HOST DISEASE: ASSOCIATION WITH Th1/Th2 RATIO

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Abstract. As found in clinical and laboratory studies, platelets not only play a key role in the processes of coagulation and thrombosis, but are also able to actively participate in other pathophysiological processes, including the development of immune reactions. It has been shown that changes in the immune system leading to systemic lupus erythematosus (SLE) are often accompanied by changes in the number of platelets and their activity in the peripheral blood of SLE patients, which correlate with the severity of the clinical manifestations of the disease. Earlier we have studied the standard experimental model of SLE in detail, based on the induction of chronic graft-versus-host disease (cGVHD) in the semi-allogeneic system DBA/2 → (C57Bl/6 x DBA/2)F_1. However, the participation of platelets in this immunopathological process has not been studied. There are no data in the literature on the behavior of platelets in cGVHD or on their relationship with the state of Th1/Th2 balance. It can been expected that the platelet count changes according to the development of cGVHD in the used experimental model by analogy with the development of SLE in humans.

In the experiments, we used female mice of the DBA/2 strain and (C57Bl/6 × DBA/2)F_1 hybrids. Chronic GVHD in a semi-allogeneic system was induced by injecting DBA/2 mouse splenocytes into B6D2F_1 hybrid mice: 60-70 × 10^6 cells intravenously twice with an interval of 6 days. The studied parameters were evaluated three months after the start of the experiment and the formation of lupus-like glomerulonephritis in animals with Th2-dependent cGVHD variant.

A decrease in the number of erythrocytes and hemoglobin, a decrease in hematocrit and a parallel increase in the number of reticulocytes in the blood of mice with cGVHD are in good agreement with our earlier conclusion that these animals have autoimmune hemolytic anemia. It was found that, platelets increase significantly with the development of cGVHD unlike other blood cells. Secondary thrombocytosis is observed in the case of the Th2-dependent variant of cGVHD in this model of SLE, while in the group with the Th1-dependent variant of cGVHD, the average number of platelets in the blood does not differ from the control group.

Keywords: chronic graft-versus-host disease, systemic lupus erythematosus, experimental model, platelets, Th1/Th2 balance

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Introduction

The results of numerous clinical and laboratory studies convincingly indicate that platelets, blood cells that are well studied in relation to their direct participation in the processes of coagulation and thrombosis, can play a significant role in other pathophysiological processes, including the development of immune reactions [4, 7]. In particular, it has been found that changes in the immune system leading to systemic lupus erythematosus (SLE) are often accompanied by changes in the number of platelets and their activity in the peripheral blood of SLE patients, which correlate with the severity of the clinical manifestations of the disease [1, 8, 12].

In previous years, we have studied in detail one of the standard experimental models of SLE, based on the induction of chronic graft-versus-host disease (cGVHD) in the semi-allogeneic system DBA/2 → (C57Bl/6 x DBA/2)F1 [3, 5]. It was found that the immunopathological process caused by the transfer of lymphocytes from one of the parental lines to hybrid mice can proceed both according to the Th2-dependent developmental variant with the formation of lupus-like immunocomplex glomerulonephritis, and according to the Th1-dependent developmental variant – without nephritis, but with manifestations of severe immunodeficiency. As we have described earlier [5, 11], an essential pathological component of cGVHD in this system is the presence of autoimmune hemolytic anemia caused by the production of large amounts of autoantibodies to own erythrocytes by activated B cells. However, the participation of platelets in this immunopathological process has not been studied, and there are no literature data on the behavior of platelets in cGVHD or on their relationship with the state of Th1/Th2 balance.

Based on the above-mentioned data obtained in clinical studies of patients with SLE, it can be expected that in our experimental model the number of platelets changes in accordance with the development of cGVHD.

Therefore, **the aim of present work** was to determine the number of platelets in the blood of mice with Th1- and Th2-dependent developmental variants of the immunopathological process.

Materials and methods

In the experiments, we used female mice of the DBA/2 strain and (C57Bl/6 × DBA/2)F1 hybrids obtained from the SPF vivarium of the Institute of cytology and genetics SB RAS (Novosibirsk). The animals were kept under standard vivarium conditions in accordance with the rules adopted by the European Convention for the Protection of Animals Used for Experimental Purposes (Strasbourg, 1986). The study was approved by the RIFCI ethics committee (protocol No. 92 dated November 10, 2015).

Chronic GVHD in the semi-allogenic system was induced according to the standard regimen by inoculating splenocytes from DBA/2 mice to B6D2F1 hybrid mice: 60-70 × 10^6 cells intravenously twice with an interval of 6 days [6]. The outcome of the immunopathological process in individual animals was determined 3 months after the induction of cGVHD by measuring the level of protein in the urine: with proteinuria of 3 mg/mL or more, mice were classified as a Th2-dependent variant of cGVHD, leading to the formation of glomerulonephritis, in the presence of protein in urine less than 3 mg/mL – to a Th1-dependent variant of the disease.

The cellular composition of peripheral blood, hematocrit and the content of hemoglobin was assessed using a PCE-90 hematological analyzer (ERMA Inc., Japan). Statistical processing of the results was carried out by nonparametric statistics using the Mann-Whitney test.

Results and discussion

The results of determining the indicators characterizing the state of blood cells in mice with Th1- and Th2-dependent variants of the development of cGVHD are presented in Table 1.

A decrease in the number of erythrocytes and hemoglobin, a decrease in hematocrit and a parallel increase in the number of reticulocytes in the blood of mice with cGVHD is in good agreement with our earlier conclusion that these animals have autoimmune hemolytic anemia [5, 11]. In our previous studies, this conclusion was also confirmed by high Coombs test values, indicating the production of a large number of anti-erythrocyte antibodies during the development of cGVHD, and an increase in the amounts of erythroid precursors in the bone marrow, which

TABLE 1. PERIPHERAL BLOOD PARAMETERS IN MICE WITH TH1- AND TH2-DEPENDENT CGVHD VARIANTS 3 MONTHS AFTER DONOR CELL TRANSFER

Group	Leucocytes, 10^6/mL	Erythrocytes, 10^9/mL	Hemoglobin, g/100 mL	Hematocrit, %	Reticulocytes, pro mille
Control (intact BDF_1) (n = 12)	11.1	7.13	17.9	49.5	10.2
Th1-dependent variant of cGVHD (n = 11)	8.4*	7.04	16.6	46.4	15.9*
Th2-dependent variant of cGVHD (n = 13)	13.8#	5.9*#	15.0*#	40.5*#	21.3*#

Note. *, significant difference from the control group ($p < 0.01$); #, significant difference from Th1-dependent variant of cGVHD ($p < 0.02$).

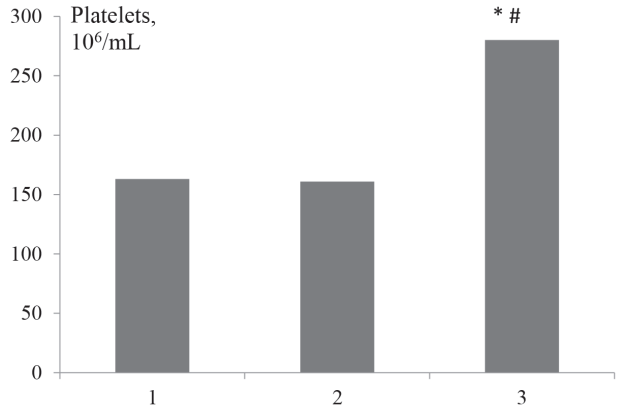

Figure 1. Number of platelets in the blood of mice with cGVHD

Note. 1, control (intact BDF_1) (n=12); 2, Th1-dependent variant of cGVHD (n = 11); 3, Th2-dependent variant of cGVHD (n = 13). *, statistically significant difference from the control group ($p < 0.001$); #, statistically significant difference from Th1-dependent variant of cGVHD ($p < 0.02$).

directly indicates the stimulation of erythropoiesis in these animals [5].

The data in Table 1 also shows that the manifestations of autoimmune hemolytic anemia are noticeably more pronounced in the group of mice with Th2-dependent variant of the development of cGVHD, compared with animals in which a relatively greater Th1 lymphocytes influence was observed on the development of the immunopathological process. This was to be expected, since it is Th2 cells that have a stimulating effect on the polyclonal activation of B lymphocytes and, accordingly, on the mass production of anti-erythrocyte autoantibodies.

Platelets increase significantly with the development of cGVHD unlike other blood cells. The data obtained in our experiments on the increased amount of platelets in the blood of experimental mice are shown in Figure1.

It can be seen that the presence of secondary thrombocytosis in cGVHD can be said only in relation to the Th2-dependent variant of this process, while in the group with Th1-dependent variant of cGVHD, the average number of platelets in the blood does not differ from the control group.

In a literature review based mainly on data from clinical studies [9], among the common causes leading to the occurrence of secondary thrombocytosis, autoimmune hemolytic anemia and chronic inflammation are indicated. It is precisely these manifestations of the pathological process that are characteristic signs of cGVHD in the case studied by us, which allows us to assume their pathogenetic role in the formation of thrombocytosis in this experimental model of SLE. As mentioned above, the anemia severity is significantly higher in the group with Th2-dependent variant of the development of cGVHD, which may be the cause of thrombocytosis in these mice.

In addition, the same group is characterized by the development of an active inflammatory process, lupus-like glomerulonephritis, which does not occur in mice with Th1-dependent cGVHD. Previously, we found that in the serum of mice with Th2-dependent variant of cGVHD, the concentration of IL-6 was doubled (compared to control), while in animals with Th1-dependent variant, this figure was even lower than in controls [6]. Since this cytokine is one of the main stimulators of platelet formation in the bone marrow [9], it may be the most important factor in the onset of thrombocytosis in our model.

It is assumed that an increase in the number of platelets in the blood of animals in our experimental model is a by-product of the main immunopathological processes that determine the development of cGVHD. However, it cannot be ruled out that changes in the number of these cells and their activity may also play a certain pathogenetic role in the formation of an SLE-like syndrome in this model. Many researchers point to the participation of platelets in immunopathological processes. Thus, in their work [2], performed on a large group of patients with SLE, the authors, on the basis of statistical processing of the obtained results, argue that the amount of platelet microparticles present in the blood of patients, which form immune complexes with IgG, correlates with the clinical manifestations of the disease and can be used to prediction of its course. A review of the literature on the role of platelets in the development of immune-mediated inflammatory diseases such as SLE or rheumatoid arthritis describes a variety of pathophysiological mechanisms that link platelet activity with cells of the immune system and with immune reactions [10].

Another recent work [8] describes the release by platelets from patients with SLE of microparticles containing extracellular mitochondrial DNA, which, according to the authors of the article, can play the role of an autoantigen in the development of this disease. Similar phenomena can be expected in the SLE model we studied, since, as we showed earlier, the concentration of extracellular DNA in the blood of mice with Th2-dependent variant of chronic GVHD noticeably increases [3].

Conclusion

Thus, the observed increase in the number of platelets in the blood of mice with cGVHD can be a starting point for a deeper study of the pathogenesis of immune system dysfunctions in this experimental model of SLE.

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