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РЕЦЕПТОР ЖИРНЫХ КИСЛОТ GPR84 И Th1/Th2-БАЛАНС В ЭКСПЕРИМЕНТАЛЬНОЙ СИСТЕМЕ *IN VIVO*

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Резюме. В ходе многочисленных исследований, в том числе в нашей лаборатории, было обнаружено, что баланс Th1/Th2 играет важную роль в регуляции реакций, определяющих исход иммунопатологических процессов как в моделях хронической РТПХ, так и в моделях острой РТПХ. Однако до сих пор открыт вопрос: от активности какого именно элемента регуляторного процесса в момент индукции РТПХ (например рецептора или фермента) зависит соотношение этого баланса. Сделано предположение, что степень активации рецептора GPR84 в момент индукции РТПХ может существенно влиять на Th1/Th2-баланс в организме и благодаря этому определяет направление развития и интенсивность патологического процесса. Целью настоящей работы являлось изучить влияние лигандов рецептора среднецепочечных жирных кислот GPR84 на баланс Th1/Th2 в экспериментальной модели *in vivo*.

В экспериментах были использованы самки мышей линий DBA/2 и гибридов (C57Bl/6 × DBA/2)F. В качестве исследуемых лигандов GPR84 использовали каприновую и лауриновую кислоты, а также синтетический лиганд 6-OAU. Хроническую РТПХ в полуаллогенной системе индуцировали вводя спленоциты мышей DBA/2 мышам-гибридам $B6D2F_1$: по 60-70 × 10^6клеток в/в двукратно с интервалом в 6 дней. Первое введение лигандов GPR84 производили через час после переноса донорских клеток и затем раз в сутки в течение двух недель. Эффект воздействия исследуемых препаратов на течение хронической РТПХ оценивали через три месяца после начала эксперимента.

Было показано, что введение животным лигандов к GPR84 в ходе индукции хронической РТПХ влияет на активность рецептора и на Th1/Th2-соотношение в организме. Введение синтетического лиганда 6-OAU увеличило количество животных, у которых иммунопатологический процесс развивался по Th1-зависимому варианту, почти в полтора раза по сравнению с группой без введения лиганда, что соответствовало литературным данным, полученным в системе *in vitro*. Эффект смеси каприновой и лауриновой кислот, видимо, опосредуется каким-то иным механизмом, отличным от активации GPR84. Следовательно, для осуществления перспективной возможности корректировать иммунные реакции путем включения в диету определенных жирных кислот требуются дальнейшие исследования.

Ключевые слова: системная красная волчанка, экспериментальная модель, лиганды жирных кислот, GPR84, Th1/Th2-баланс

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FATTY ACID RECEPTOR GPR84 AND Th1/Th2 BALANCE IN THE EXPERIMENTAL SYSTEM *IN VIVO*

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Abstract. While conducting numerous studies, including researchers in our laboratory, it was found that Th1/Th2 balance plays an essential role in the regulation of reactions that determine the outcomes of immunopathological processes in both chronic and acute GVHD models. However, the question about activity of which element in the regulatory process during GVHD induction (for example, a receptor or an enzyme) affects the ratio of this balance depends remains open. It has been suggested that the degree of activation of the GPR84 receptor during GVHD induction can significantly affect the host Th1/Th2 balance. And, by assessing this parameter, the direction of development and the intensity of the pathological process can be determined. The aim of this work was to investigate the effect of ligands such as medium-chain fatty acid receptor GPR84 on the Th1/Th2 balance in an experimental model in an *in vivo* system.

Female DBA/2 and hybrids (C57Bl/6 × DBA/2) F1 mouse strains were used in the experiments.The studied ligands of GPR84 were capric and lauric acids, as well as a synthetic ligand 6-OAU. Chronic GVHD in the semi-allogenic system was induced by injecting splenocytes from DBA/2 mice to $B6D2F_1$ hybrid mice: $60\text{-}70 \times 10^6$-cells iv twice with an interval of 6 days. The first administration of the GPR84 ligands was performed one hour after the donor cell transfer and then once a day for two weeks.The effect of the study drugs on the course of chronic GVHD was assessed three months after the onset of the experiment.

It was shown that the administration of GPR84 ligands to to animals during the induction of chronic GVHD affects the activity of the receptor and the host Th1/Th2 ratio. In the group with the injection of 6-OAU, the number of animals which the immunopathological process developed according to the Th1-dependent variant increased by more than 1.5-fold, compared with the control group. This fact is consistent with the literature data obtained in the *in vitro* system. Apparently, the effect of a mixture of capric and lauric acids is mediated by some other mechanism, differed from the GPR84 activation. Therefore, further research is required to realize the promising possibility of adjusting immune responses by including certain fatty acids in the diet.

Keywords: systemic lupus erythematosus, experimental model, fatty acid ligands, GPR84, Th1/Th2 balance

Introduction

In the organism of experimental animals, a balance between type 1 and type 2 T-helper cells plays a decisive role in developing the graft versus host reaction (GVHD) in the classical semi-allogenic system with adoptive transfer of lymphoid cells from one of the parental lines to hybrid mice of the first generation. Cell transfer from C57Bl/6 mice to hybrid (C57Bl/6 × DBA/2)F1 mice is accompanied by dominance of Th1-lymphocytes in immune responses and development of acute GVHD with pronounced tissue destruction and mass death of recipient animals. At the same time, if DBA/2 mice are used as donors, a different type of immune conflict develops in recipient mice: chronic GVHD with predominance of Th2-dependent reactions, intense polyclonal activation of B-lymphocytes, overproduction of antibodies and development of immunocomplex glomerulonephritis in some animals.

Moreover, over many years of studies in our laboratory it was found that Th1/Th2 balance plays an essential role in the regulation of reactions that determine the outcomes of immunopathological processes in both chronic and acute GVHD models. In particular, it was found that recipients develop two variants of this disease after the induction of chronic GVHD. Th2-dependent response, leading to the formation of glomerulonephritis and Th1-dependent – without nephritis, but with severe immunodeficiency [3]. Development of either of such scenarios is determined by the magnitude of Th1/Th2 ratio in individual animals at early stage of chronic GVHD development. It is possible to shift the balance of T-helper cells in either direction by using various immunomodulatory agents. It makes this model a convenient and sensitive test-system for identifying

agents that affect the Th1/Th2 balance. A similar division of recipients into Th1- and Th2-dependent variants of the developing disease was found in acute GVHD [10].

It has long been established that the inability of DBA mouse lymphocytes to induce acute GVHD after cell transfer to hybrid mice is accounted for by impaired recipient $CD8^+$T-cells [8]. In the critical phase of the immune conflict, such defective lymphocytes produce no sufficient IFN to sharply shift the balance towards Th1-cells and initiate massive generation of killer cells specific to the recipient antigens. Later, by backcrossing hybrids with the parental mouse strains C57Bl and DBA, it was also found that the defect inhibiting induction of acute GVHD by DBA mouse lymphocytes was genetically determined probably affecting the Th1/Th2 ratio [1]. However, the question remains as to which defective DBA mouse gene the observed differences are assigned to and how it is associated with development of opposite GVHD variants.

Based on the results of various studies carried out over the last twenty years, there is every reason to believe that the GPR84 gene is the most probable candidate for this role.

GPR84 was discovered in 2001 as an orphan receptor of the family of membrane G-protein-coupled proteins involved in cell signalling. However, several years later it was found that GPR84 may physiologically ligate medium-chain fatty acids (from C9 to C14), among which the most active were capric, undecanoic, and lauric acids as well as their hydroxylated metabolites [11]. Among the examined so far, the most important effects of GPR84 activation in cell cultures were demonstrated in activities which are directly related to the data described in this study.

Firstly, during the development of inflammatory response (after LPS injection) it was found that activation of GPR84 significantly upregulated expression of the IL-12 p40 subunit [2, 11], a cytokine that shifts the balance of helper cells towards Th1 pathway and plays a decisive role in developing acute GVHD. Secondly, the inhibitory effect of GPR84 activation on generation of Th2-lymphocytes and their production of IL-4 was established [7]. These data are in good agreement with the effect of this receptor on IL-12 expression. An increased IL-4 production by cells in mice knocked out for GPR84 has also been shown [9].

The results obtained in the study of the GPR84 gene deletion in the genome of various strains of laboratory mice are also of great importance [5]. The authors found that DBA/2 is among several strains bearing such gene deletion. Thus, the defects in $CD8^+$T-cells from such mice and the corresponding shift in Th1/Th2 balance towards Th2 can be accounted for by the decreased expression of IL-12 upon GVHD induction. These data convincingly support the assumption that the degree of GPR84 receptor activation can marketdly affect the Th1/Th2 ratio *in vivo*. Therefore, it might determine modality of development and intensity of the pathological process upon induction of GVHD.

The aim of current study was to test this assumption in an *in vivo* model by administering GPR84 ligands to recipient mice upon induction of chronic GVHD.

Materials and methods

In the experiments, we used 45 female mice of the C57Bl/6, DBA/2 strains and (C57Bl/6 × DBA/2)F_1 hybrids obtained from the SPF vivarium of the Institute of Cytology and Genetics SB RAS (Novosibirsk).

Capric (C10, Acros Organics, Germany) and lauric (C12, Acros Organics, Germany) acids, as well as the synthetic ligand 6-(octylamino)-2,4(1H,3H)-pyrimidinedione (6-OAU; Abcr GmbH, Germany), were used as the GPR84 ligands.

Chronic GVHD in the semi-allogenic system was induced according to the standard regimen by inoculating splenocytes from DBA/2 mice to B6D2F_1 hybrid mice: 60-70 × 10^6 cells iv twice with an interval of 6 days [3]. The outcome of the immunopathological process in individual animals was determined 3 months after the induction of GVHD 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 GVHD, 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.

Statistical analysis of the data obtained was carried out by using the software package Statistica 6.0.

Results and discussion

The response of C57Bl/6 and DBA/2 mice to an exogenous inflammatory stimulus was explored in a preliminary experiment. The animals were injected with LPS (sc, at a dose of 1 μg/mouse). Next, at various timepoints the intensity of the response was assessed after drug administration by measuring the neutrophil/lymphocyte ratio in peripheral blood as well as concentration of free (extracellular) DNA. It was found that magniude of changes in such parameters after administration of LPS in DBA/2 mice was significantly lower than in C57Bl/6 mice (data not shown). This is in agreement with literature evidence regarding attenuated proinflammatory responses in DBA/2 mice under various experimental settings.

TABLE 1. INDUCTION OF CHRONIC GVHD IN AN EXPERIMENTAL MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) UNDER THE INFLUENCE OF THE SYNTHETIC LIGAND GPR84 AND A MIXTURE OF FATTY ACIDS

Groups	Th1-dependent variant of cGVHD	Th2-dependent variant of cGVHD	Th1/Th2 %	Proteinuria*
1. cGVHD (control)	4	10	29/71	5.17
2. cGVHD + 6-OAU	5	6	46/54	5.63
3. cGVHD + (C10+C12)	0	12	0/100	5.63

Note. * mean concentration of protein in the urine of mice with developed glomerulonephritis (mg/ml).

During experiments with inducing chronic GVHD, animals were divided into three groups after donor cell transfer:

Group 1 – control (n = 14) – ip injection of 0.2 ml of PBS (saline);

Group 2 – experiment (n = 11) – ip injection of 0.4 mg 6-OAU in 0.2 ml of PBS;

Group 3 – experiment (n = 12) – injection of a mixture of fatty acids (0.35 mg C10 + 0.4 mg C12 in the form of a suspension in 0.5 ml of PBS), per os (using a gastric tube).

The first injection of GPR84 ligands to all animals was performed one hour after donor cell transfer and then once a day for two weeks.

The effect of the drugs on chronic GVHD was assessed three months after the onset of experiment. By determining the number of mice with developed immunocomplex glomerulonephritis in each of the indicated groups. The results of this experiment are presented in the Table 1.

These data show that even in the in vivo test-system activation of GPR84 by a synthetic ligand also leads to marked shift in the Th1/Th2 ratio towards Th1 and increased production of proinflammatory cytokines similar to what was previously shown in experiments with cell cultures. In the group with inoculated 6-OAU, the number of animals with immunopathological process developed along Th1-dependent variant increased by more than 1.5-fold, compared with the control group. Although donor cells initiating an immune conflict could not directly respond to inoculated 6-OAU (due to the ablated GPR84 gene). Nevertheless, the reactions of such cells, being actively involved in inducing a certain Th1/Th2 balance in recipient host, were significantly modified by the injected drug. Such data may be easily explained by assuming that the effect of the GPR84 activating agent is mediated by its effects on host cells, which genome contains a normally functioning GPR84 gene inherited from the parental C57Bl/6 mice. Injection of 6-OAU into recipients activating GPR84 increases the amount of IL-12 produced by the host cells. In turn, this cytokine can also affect donor cells affecting their activity and thereby shifting the Th1/Th2 balance to the pro-inflammatory side as well as ultimately reducing the number of nephritis cases in experimental group.

A more difficult obstacle is interpretation of intriguing data obtained in mice inoculated with a mixture of fatty acids. While planning the experiments, it was assumed that a mixture of capric and lauric acids, effectively binding to the GPR84 protein and cause its activation, should trigger an effect similar to that of a synthetic ligand in our test system. However, a sharp shift in the balance of T-helper cells to the dominant Th2-cells was unexpected. At first glance, this result contradicted our initial assumption and the effect of 6-OAU. Apparently, in this case, the effect of fatty acids on the Th1/Th2 ratio is mediated in another way, differed from the activation of GPR84 by these acids. As it turned out, one of the possible explanations may be related to the activation of the hydroxycarboxylic acid receptor 3 (HCA3) by capric acid, which is included in the mixture used in our experiments. Peters A. et al. found that the hydroxylated metabolite of this acid (3-OHC10) was able to activate not only GPR84 (with the same efficiency as the native C10), but also the HCA3 receptor [6]. Similar to the medium-chain fatty acid receptor, HCA3 is expressed on immune cells. However, unlike GPR84, activation of HCA3 may also suppress the production of proinflammatory cytokines in response to LPS [4]. Thus, we can assume that capric acid, acting through cognate receptor, is capable of shifting the Th1/Th2 ratio towards the prevalence of Th2-lymphocytes. However, further research is required to find out whether a similar process underlies the discovered phenomenon or results from action of some other factors.

Conclusion

The results of this study confirm that the GPR84 ligands are able to robustly affect the Th1/Th2 ratio *in vivo*, and also indicate a promise to assess a potrential for correcting immune responses by including certain fatty acids in the diet.

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