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ХАРАКТЕРИСТИКА CD4$^+$ ЦЕНТРАЛЬНЫХ И ЭФФЕКТОРНЫХ КЛЕТОК ПАМЯТИ ПРИ ПСОРИАЗЕ

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Резюме. Псориаз – хроническое аутоиммунное заболевание, при котором в патологический процесс вовлечены кожа и суставы. Установлено, что рецидив высыпаний при данном заболевании происходит за счет резидентных клеток памяти кожи. При этом количество CD4$^+$CCR3$^+$ эффекторных клеток памяти периферической крови коррелирует с тяжестью заболевания. Поэтому целью нашей работы является изучение фенотипа клеток памяти периферической крови пациентов с псориазом.

В исследование включили 6 здоровых доноров: средний возраст – 45,4 (min – 29, max – 55), женщины – 3, мужчины – 3; 10 пациентов с псориазом: женщины – 4 , мужчины – 6, средний возраст – 37,3 (min – 23, max – 57), из них 5 пациентов с PASI более 10 и 5 пациентов с PASI менее 10. Критериями исключения были наличие аутоиммунных, онкологических и гематологических заболеваний, системная терапия иммуносупрессивными препаратами в течение 1 месяца. Пациенты подписали добровольное информированное согласие на участие в исследовании. Выделение мононуклеарных клеток периферической крови проводили в градиенте плотности фиколл-урографина (р = 1,082 г/л) (BioClot GmbH, Германия). Полученные клетки окашивали конъюгированными с флуорохромами моноклональными антителами к поверхностным маркерам CD4$^+$ центральных (Tcm) и эффекторных (Tem) клеток памяти (CD4, CD45RO, CD197), α-цепи рецептора IL-7 (CD127) и γ-цепи рецептора IL-7 (CD132). Фенотипирование клеточных популяций проводили на клеточном анализаторе FACS CantoII. Статистический анализ полученных данных проводили с использованием пакета прикладных программ Statistica 6.0 (Statsoft, США).

Доля Tcm в периферической крови доноров составляла 33,4% (min – 18,2; max – 43,7), Tem – 28,7% (min – 13.6, max – 38,9), у пациентов с псориазом: Tcm – 28,65% (min – 13,3; max – 59,6), Tem – 21,5% (min – 9.3, max – 38.6). Среди центральных CD4$^+$-клеток памяти доля CD127$^+$CD132$^-$-клеток составляет 26,00%, CD127$^+$CD132$^+$ – 1,69%, CD127$^+$CD132$^-$ – 69,00%, CD127$^-$CD132$^+$ – 1,94%. Среди эффекторных CD4$^+$-клеток памяти доля CD127$^+$CD132$^-$-клеток составляет 23,58%, CD127$^+$CD132$^+$ – 1,18%, CD127$^+$CD132$^-$ – 69,84%, CD127$^-$CD132$^+$ – 0,70%. Обнаружена прямая корреляционная связь между количеством CD127$^-$CD132$^+$ центральных клеток памяти и значением PASI (r = 0,639, p < 0,05).

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У пациентов с псориазом доля центральных клеток памяти выше, чем у здоровых доноров, при этом количество эффекторных клеток памяти – ниже. Обнаружена прямая корреляционная связь между количеством центральных клеток памяти, экспрессирующих γ-цепь рецептора IL-7, и степенью тяжести заболевания. Высокой экспрессией CD132 характеризуются активированные клетки памяти. Можно предположить, что данная популяция клеток памяти играет роль в поддержании аутоиммунного воспаления у пациентов с данным заболеванием, а также участвует в репопуляции резидентных клеток памяти кожи.

Ключевые слова: $CD4^+$-клетки памяти, псориаз, аутоиммунные заболевания кожи, дерматология, резидентные клетки памяти, индекс PASI

CHARACTERIZATION OF CENTRAL AND EFFECTOR $CD4^+$ MEMORY CELLS IN PSORIASIS

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Abstract. Psoriasis is a chronic autoimmune disease in which the skin and joints are involved in the pathological process. It was found that the recurrence of rashes in this disease occurs due to the resident memory cells of the skin. The number of $CD4^+CCR3^+$ effector memory cells in peripheral blood correlates with the severity of the disease. Therefore, the aim of our work is to study the phenotype of peripheral blood memory cells in patients with psoriasis.

The study included 6 healthy donors: average age – 45.4 (min – 29, max – 55), women – 3, men – 3; 10 patients with psoriasis: women – 4, men – 6, average age – 37.3 (min – 23, max – 57), of which 5 patients with PASI > 10 and 5 patients with PASI < 10. The exclusion criteria for the study were the presence of autoimmune, oncological and hematological diseases, systemic therapy with immunosuppressive drugs for 1 month. Patients signed informed consent to participate in the study. Isolation of peripheral blood mononuclear cells was performed in a density gradient of ficoll-urographin (p = 1.082 g/L). Then cells were stained with fluorochrome-conjugated monoclonal antibodies to surface markers of central (Tcm) and effector (Tem) $CD4^+$ memory cells (CD4, CD45RO, CD197), the α-chain of the IL-7 receptor (CD127), and the γ-chain of the IL-7 receptor (CD132). Statistical analysis of the data obtained was performed using the Statistica 6.0 software package.

The percent of Tcm in the peripheral blood of donors was 33.4% (in – 18.2, max – 43.7), Tem – 28.7% (min – 13.6, max – 38.9), in patients with psoriasis: Tcm – 28.65% (min – 13.3, max – 59.6), Tem – 21.5% (min – 9.3, max – 38.6). In the peripheral blood of patients with psoriasis, among the central $CD4^+$ memory cells, the proportion of $CD127^+CD132^-$-cells is 26.00%, $CD127^+CD132^+$ – 1.69%, $CD127^+CD132^-$ – 69.00%, $CD127^-CD132^+$ – 1.94%. Among effector $CD4^+$ memory cells, the proportion of $CD127^+CD132^-$-cells is 23.58%, $CD127^+CD132^+$ – 1.18%, $CD127^+CD132^-$ – 69.84%, $CD127^-CD132^+$ – 0.70%. A direct correlation was found between the number of $CD127^-CD132^+$ central memory cells and the PASI value ($r = 0.639$, $p < 0.05$).

In patients with psoriasis, the proportion of central memory cells is higher than in healthy donors, while the number of effector memory cells is lower. A direct correlation was found between the number of central cells expressing the γ-chain of the IL-7 receptor and the severity of the disease. Activated memory cells are characterized by high expression of CD132. It can be assumed that this population of memory cells plays a role in maintaining autoimmune inflammation in patients with this disease, and also participates in the repopulation of skin resident memory cells.

Keywords: $CD4^+$ memory cells, psoriasis, autoimmune skin diseases, dermatology, resident memory cells, PASI index

Introduction

The skin is normally populated by a large number of T-lymphocytes. Contact with the antigen promotes the differentiation of naive T-cells into effector cells capable of performing protective functions. Most of these cells are characterized by a short lifespan, while a small subset of them transition to the pool of long-lived memory cells [11]. Psoriatic lesions usually recur in the same locations as those at the disease onset, which indicates the possibility of the existence of immune memory. It was found that the transplantation of clinically healthy skin from psoriasis mice led to the formation of psoriatic plaques in mice with immunodeficiency [1].

There is a population of resident memory T-cells ensuring the first line of adaptive cellular defense in the skin. These cells can express both CD8 and CD4 markers being also characterized by high expression of CD103, CD69, CD49, and CD44 [5]. $CD8^+CD69^+$ resident T-lymphocytes persist in the skin of patients with psoriasis for several months after effective methotrexate therapy [13]. Secukinumab therapy led to decreased number of resident memory effector T-cells ($CD8^+CD103^+CD49a^-CCR7^-$) and IL-17A-producing resident memory cells. At the same time, the number of resident memory cells significantly increased in clinically healthy skin after treatment, which was accounted for by high resistance of this population to biological therapy [7]. It was also found that the number of IL-22-producing memory $CD4^+$T-cells does not correlate with the duration of treatment, which indicates that the effector functions are preserved during therapy for a long time [4].

In addition to resident memory cells, there are central (Tcm) and effector (Tem) memory cells. Central $CD4^+$ memory cells (Tcm) are characterized by the ability to migrate from secondary lymphoid organs to the bloodstream, and featured with high expression of homing receptors CD62L and CCR7 as well as high proliferative potential. Memory effector cells (Tem) are characterized by the ability to migrate between secondary lymphoid organs and non-lymphoid tissues, as well as the absence or low expression of CD62L and CCR7, while adhesion molecules are highly expressed [2]. The use of alefacept led to apoptosis of effector $CD4^+$ memory cells, while the level of cell reduction correlated with the magnitude of the PASI index indicating the role of $CD4^+$ memory cells in the development of the disease [6]. Gaide et al. showed the common clonal origin of central and resident memory cells, which suggested that Tcm are capable of repopulating the pool of resident memory cells [8]. $CD4^+$ and $CD8^+$ circulating memory cells of patients with psoriasis are characterized by high expression of CCR4 (a chemokine receptor responsible for the migration of lymphocytes into the skin), and their count correlates with the severity of the disease [12]. It was found that central $CD8^+$ memory *vs* effector cells are characterized by a higher CCR4 expression. At the same time, the central $CD8^+CCR4^+$ and $CD4^+CCR4^+$-lymphocytes had the peak expression of the cutaneous lymphocyte antigen (CLA) necessary for skin tropism [3].

IL-7 plays a fundamental role in the development and maintenance of memory cells. It promotes cell survival due to its antiapoptotic action [10]. Therefore, **the aim of our work** was to study the phenotype of memory cells (expression of α- and γ-chains of the IL-7 receptor) in the peripheral blood of patients with psoriasis.

Materials and methods

There were enrolled 6 healthy volunteers (males and females), aged 28 to 58 years (mean age 48±2.7 years), and 10 patients with psoriasis aged 27 to 58 years, of which 5 patients had PASI more than 10 and 5 patients with PASI less than 10. The exclusion criteria were concomitant autoimmune, oncological and hematological diseases, systemic therapy with immunosuppressive drugs. Both groups were assigned after signing informed consent.

PBMCs were isolated by centrifugation in a Ficoll-Urographin (p = 1.082 g/L) density gradient . Next, cells were stained with fluorochrome-conjugated monoclonal antibodies to surface markers of central and effector $CD4^+$ memory cells, the IL-7 receptor α-chain , and the common γ-chain. The following antibody panel was used: CD4-PE (Sorbent LLC), CD45RO-PE/Cy7, CD197 (CCR7)-APC/Cy7, $CD127^-$PerCP/Cy5.5, CD132-APC (BioLegend, USA). Staining was performed with a combination of antibodies according to the manufacturer's recommended protocol. Phenotyping of cell populations and the study of proliferative activity were performed on a FACS CantoII cellular analyzer (BD, USA) by using the FACS Diva 6.1 software.

Statistical data analysis was carried out by using the Statistica 6.0 software package. The Mann–Whitney test was used to evaluate unrelated variables. The revealed differences were considered significant in case of $p < 0.05$.

Results and discussion

Patients with psoriasis were found to have significantly lowere count of $CD123^-CD132^+$-lymphocytes, as well as double positive $CD127^+CD132^+$-cells both in central and effector memory cells (Table 1). It can be assumed that, in this disease, cells expressing IL7R replenish the population of resident skin memory cells involved in the maintenance of local inflammation, which accounts for low number of $CD123^+CD132^+$ Tcm and Tem

TABLE 1. PHENOTYPE OF CD4+ CENTRAL AND EFFECTOR MEMORY CELLS OF HEALTHY DONORS AND PATIENTS WITH PSORIASIS

	Control	Psoriasis	
Tcm, %	33.4±10.14	40.80±11.07	p > 0.05
$CD127^+CD132^-$, %	29.40±14.96	26.90±17.08	p > 0.05
$CD127^+CD132^+$, %	10.55±1.49	1.68±1.94	p < 0.05
$CD127^-CD132^-$, %	50.31±9.63	69.46±18.01	p > 0.05
$CD127^-CD132^+$, %	9.73±6.78	1.94±2.26	p < 0.05
Tem, %	28.70±9.51	23.83±10.10	p > 0.05
$CD127^+CD132^-$, %	27.45±12.05	23.57±16,67	p > 0.05
$CD127^+CD132^+$, %	12.26±5.83	1.17±2.05	p < 0.05
$CD127^-CD132^-$, %	48.87±10.07	69.84±26.61	p < 0.05
$CD127^-CD132^+$, %	11.14±4.86	0.70±1.00	p < 0.05

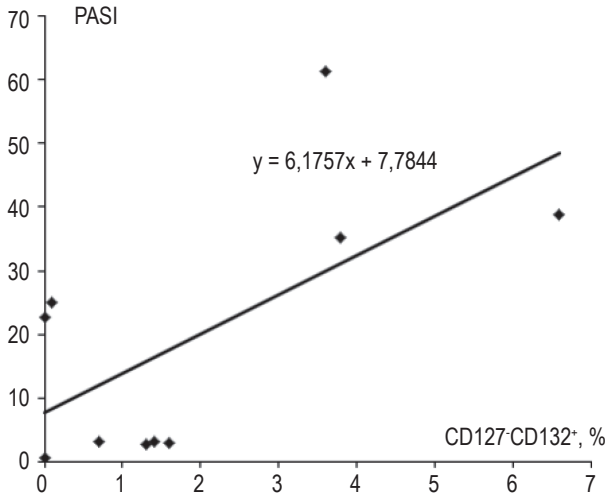

Figure 1. Direct correlation between the percentage of $CD127^-CD132^+$ central memory cells and the PASI value (r = 0.639, p < 0.05).

compared to apparently healthy donors. Expression of the IL-7 receptor γ-chain is characteristic of activated memory cells, which decreased number in the peripheral blood may also be due to the migration to skin foci of the disease. In view of the aforementioned, cell migration, percentage of $CD123^-CD132^+$ effector $CD4^+$ memory cells naturally increases. In the Tcm population, the number of double negative cells tends to decrease compared to the control group.

An inverse correlation was found between the proportion of $CD4^+$ central memory cells expressing the IL-7 receptor γ-chain ($CD123^-CD132^+$) and the PASI value ($r = 0.639$, $p < 0.05$) (Figure 1). We assume that this population of memory cells plays a role in maintaining autoimmune inflammation in patients with this disease, and also participates in the repopulation of skin resident memory cells.

Based on the study results , we conclude that $CD4^+$ central memory cells comprise one of the factors in developing severe psoriasis, which is indicated by a direct correlation between the percentage of activated Tcm expressing the IL-7 receptor γ-chain and magnitude of the PASI index. Also, this cell population is characterized by the peak expression of homing skin receptors [11].

According to the linear model of the immunological memory development [1], the central memory cells represent early stage. It is assumed that at this stage, the cells are transferred to the pool of skin resident memory cells [10]. Our data provide another argument in favor of this hypothesis. The dependence of the disease severity on the number of peripheral blood central $CD4^+CD123^-CD132^+$ memory cells, despite the fact that the proportion of this cell population is significantly lower in patients with psoriasis compared *vs* apparently healthy donors, indicates that $CD132^+$Tcm are involved in maintaining inflammation during psoriasis, and migrate from the bloodstream to peripheral tissues (presumably the skin).

Memory effector cells are characterized by minimal expression of CCR4 and CLA, but at the same time they express a higher level of CXCR3 and CCR5 molecules as compared to central memory cells [11, 13]. It is assumed that the expression of the above markers emphasizes the difference between the migration potential between central and effector memory cells: to the skin and to the areas of inflammation, respectively [13]. This accounts for significantly decreased number of activated Tcm and Tem in the peripheral blood of patients with psoriasis *vs* control subjects.

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