

СООТНОШЕНИЕ НАИВНЫХ CD45RA⁺ И CD31⁺Т-КЛЕТОК У ПАЦИЕНТОВ С РЕВМАТОИДНЫМ АРТРИТОМ ПРИ СТИМУЛЯЦИИ ГОМЕОСТАТИЧЕСКИМИ ФАКТОРАМИ *IN VITRO*

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Резюме. Большое внимание исследователей направлено на изучение роли факторов гомеостатической пролиферации в патогенезе аутоиммунных заболеваний, однако до сих пор недостаточно изучено влияние IL-7 и IL-15 на фенотип наивных Т-клеток при патологии. Целью данного исследования стало изучение соотношения CD45RA⁺ и CD31⁺ наивных клеток среди CD4⁺ и CD8⁺-лимфоцитов здоровых индивидуумов и пациентов с ревматоидным артритом (РА) при стимуляции IL-7, IL-15 *in vitro*. В периферической крови мы не обнаружили отличий по числу CD45RA⁺ и CD31⁺-клеток между группами доноров и пациентов. Стимуляция комбинацией IL-7 с IL-15 способствовала увеличению числа CD4⁺CD45RA⁺ и CD8⁺CD45RA⁺ у доноров относительно их уровня в периферической крови. Тогда как у пациентов с РА число CD8⁺CD45RA⁺-клеток снижалось под действием IL-15, комбинации IL-15 с IL-7 по сравнению с контролем без стимуляции, и достоверно отличалось, как и число CD4⁺CD45RA⁺-клеток, от содержания данных клеток у доноров в тех же условиях. Достоверных отличий по содержанию CD31⁺-клеток и числу CD31⁺-клеток, отвечающих пролиферацией на цитокины, как между группами доноров и пациентов с РА, так и различными вариантами культивирования не было выявлено. Таким образом, можно говорить о том, что под влиянием факторов гомеостатической пролиферации происходит пропорциональное увеличение CD31⁺Т-клеток как у доноров, так и пациентов с РА. При этом наивные Т-клетки доноров при культивировании сохраняют экспрессию CD45RA, а наивные Т-клетки пациентов частично ее утрачивают. Полученные данные говорят о том, что под действием факторов гомеостатической пролиферации при РА происходит конверсия фенотипа наивных Т-клеток в фенотип Т-клеток памяти.

Ключевые слова: наивные Т-клетки, CD31, гомеостатическая пролиферация, IL-7, IL-15, ревматоидный артрит

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PROPORTION OF NAIVE CD45RA⁺ AND CD31⁺T-CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS UNDER STIMULATION WITH HOMEOSTATIC FACTORS *IN VITRO*

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Abstract. Much attention of researchers is directed to study the role of factors of homeostatic proliferation in the pathogenesis of autoimmune diseases, however, the effect of IL-7 and IL-15 on the phenotype of naive T-cells has not yet been sufficiently investigated in the pathology. The aim of this study was to investigate the proportion of CD45RA⁺ and CD31⁺ naive cells among CD4⁺ and CD8⁺-lymphocytes from healthy individuals and patients with rheumatoid arthritis (RA) upon stimulation with IL-7, IL-15 *in vitro*. In peripheral blood, we did not find any differences in the number of CD45RA⁺ and CD31⁺-cells between the group of donors and group of RA patients. In donors, stimulation by a combination of IL-7 with IL-15 promoted an increase in the proportion of CD4⁺CD45RA⁺ and CD8⁺CD45RA⁺ relative to their level in the peripheral blood. Whereas in RA patients the number of CD8⁺CD45RA⁺-cells decreased under IL-15 and the combination of IL-15 with IL-7 compared to the control without stimulation, and it, as a proportion of CD4⁺CD45RA⁺-cells, significantly differed from the content of these cells under the same conditions in donors. There were no significant differences in the content of CD31⁺-cells and the number of CD31⁺-cells proliferating to cytokines, both between the groups of donors and patients with RA, and between different culture conditions. Thus, we can say that under the factors of homeostatic proliferation, there is a proportional increase in CD31⁺T-cells number, both in donors and in patients with RA. At the same time, naive T-cells from donors retain the expression of CD45RA during cultivation, while naive T-cells from patients partially lose it. The data obtained indicate that in RA, under factors of homeostatic proliferation the phenotype of naive T-cells is converted into the phenotype of memory T-cells.

Keywords: naive T-cells, CD31, homeostatic proliferation, IL-7, IL-15, rheumatoid arthritis

Introduction

Homeostasis of T-cells is contributed by two mechanisms: the generation of naive T-cells in the thymus and homeostatic proliferation (HP) on the periphery. The process of HP is based on signalling through the T-cell receptor and the receptors for IL-7, IL-15 [13, 14]. Markers of recent thymic emigrants (RTE) are presented by the surface molecule CD31 and the amount of T-cell excision circles (TREC) formed during T-cell receptor (TCR) gene rearrangement in the thymus [9]. At the periphery, RTEs undergo maturation and become naive T-cells. Despite the thymus involution and decline in migrating RTEs, the number of naive T-cells in the periphery does not undergo significant age-related changes suggesting about a more intensive HP of naive T-cells [6]. Also, the intensity of HP increases after lymphopenia induced by various factors, while the degree of HP intensity is directly related to the filling of lymphatic niches [6, 11, 12].

As a result of lymphopenia-induced proliferation, T-cell clones bearing TCRs recognizing self peptides expand, that can lead to the accumulation of potentially autoreactive cells [9]. The negative conse-

quences of HP also include conversion of naive T-cell phenotype into surrogate memory cells, that suggests the involvement of HP in developing autoimmune diseases, including rheumatoid arthritis (RA) [7, 13].

Over the past decade, more data have been accumulated confirming the active role for IL-7 and IL-15 in the pathogenesis of autoimmune diseases. Elevated levels of IL-7, IL-15 are detected in the blood serum and bone marrow of patients with RA. TNF-induced synoviocytes produce IL-15, which, in turn, promotes production of TNF by macrophages, as well as indirectly contributes to the production of IFN γ , IL-1 β , IL-17 by T-cells during inflammation [15]. It has been shown that under the influence of IL-7, T-cells predominantly produce cytokines related with Th1 and Th17-cells [10]. IL-7 mediates T-dependent activation of macrophages, dendritic cells and B-cells, which is accompanied by increased expression of differentiation factors, chemokines, adhesive and costimulatory molecules, catabolic enzymes [2].

Based on the data presented above, we examined *in vitro* effect of homeostatic factors IL-7 and IL-15 on percentage of CD45RA⁺ and CD31⁺ naive cells

among CD4⁺ and CD8⁺-lymphocytes from healthy individuals and patients with RA.

Materials and methods

Object of investigation

There were enrolled patients with rheumatoid arthritis ($n = 13.48 \pm 2.4$ years) with moderate and high disease activity, healthy individuals ($n = 13.45 \pm 2.1$ years), examined at the Department of Rheumatology, Clinic of Immunopathology of RIFCI (Novosibirsk) with the participation of rheumatologists. Written informed consent was obtained from all participants included in the study prior to collection of peripheral blood samples.

T-cell phenotyping by flow cytometry

Phenotyping of naive T-cells among PBMCs, isolated on the Ficoll-Urographin density gradient from the peripheral blood of donors and patients with RA, and in cell cultures was performed by staining with antibody panels. Peripheral blood cells were stained with fluorochrome-conjugated monoclonal antibodies to antigens: CD3-FITC (Sorbent, Russia), CD4-PE (Sorbent, Russia), CD45RA-PE/Cy7 (Beckman Coulter, USA), CD31-APC (BioLegend, USA). For staining culturing cells the following panel was used: CD3-PerCP/Cy5.5 (BioLegend, USA), CD4-PE (Sorbent, Russia), CD45RA-PE/Cy7 (Beckman Coulter, USA), CD31-APC (BioLegend, USA). Analysis of T-cell phenotype was performed on a flow cytometer FACS CantoII (BD, USA) using the FACS Diva software (BD, USA).

Cell culturing

PBMCs isolated from the peripheral blood of donors and patients with RA were cultured in the 24-well plates (TPP, Switzerland) in the culture medium RPMI-1640 (HyClone, USA) supplemented with 10% FCS (Gibco, USA) and antibiotics 50 µg/ml gentamicin (Dalchimpharm, Russia), 25 µg/ml thienam (Merck Sharp & Dohme Corp., USA), with/without IL-7 (50 ng/ml, PeproTech, USA) and IL-15 (50 ng/ml, PeproTech, USA), in humid atmosphere, with 5% CO₂, at 37 °C for 7 days. Before culturing, cells were stained with the vital dye CFSE (4 µM, Molecular Probes, USA) to assess the proliferative response of T-cells to cytokines. Non-proliferating cells have the highest intensity of fluorescent by CFSE; during cell division, the intensity of fluorescent dye decreases proportionally to the number of completed cell divisions.

Statistical analysis

Statistical data processing was carried out by using the software "Statistica 6.0" (StatSoft, USA) and "GraphPadPrism 9.0" (GraphPad, USA), using nonparametric statistics methods (Mann-Whitney test). For multiple comparisons within the group of donors and patients with RA, the Friedman method with posterior pairwise comparison was used to identify differences between the culture conditions.

Differences were considered statistically significant at $p < 0.05$.

Results and discussion

It was shown that no significant differences in percentage of naive CD45RA⁺, CD31⁺T-cells in the peripheral blood between the groups of donors and patients were observed. Treatment with IL-15 or IL-7 combined with IL-15 (IL-7+15) revealed that percentage of CD4⁺CD45RA⁺ and CD8⁺CD45RA⁺ in the group of donors was significantly increased compared to group of patients (Figure 1, M-U test, $p < 0.05$). In addition, the stimulation with IL-15, IL-7+15 in the group of donors resulted in increased number of CD4⁺CD45RA⁺-cells compared to that in the peripheral blood (Figure 1A). In donors, an increase in percentage of CD8⁺CD45RA⁺-cells was observed under stimulation with IL-7 combined with IL-15 (Figure 1C). In RA patients, no significant changes in the number of CD45RA⁺-cells cultured with the homeostatic factors were found (Figure 1B), excepting CD8⁺45RA⁺-cells, which decreased after exposure to IL-15 and IL-7+15 vs control intact T-cells (Figure 1D).

No significant differences in the CD31 expression on T-cells were found between the groups of donors and patients, as well as under the different culture conditions (Table 1). We estimated the number of proliferated CD31⁺-cells in the cultures (Table 2), and found no significant differences between the groups of donors and patients with RA. However, the number of CD4⁺CD31⁺-cells proliferating in response to IL-15 in the group of patients with RA compared with the group of donors tended to increase ($p = 0.07$). The peak response of naive T-cells to stimulation was observed by combining both cytokines IL-7, IL-15.

Thus, it may be concluded that under the influence of HP factors, percentage of CD31⁺T-cells, both in donors and in patients with RA was increased. However, donor cells during culturing retain CD45RA expression, the percentage of such cells increased under the influence of IL-15 as well as IL-15 combined with IL-7. In RA patients, the percentage of CD45RA-expressing cells is reduced under the same conditions. The data obtained suggested that in RA, under the influence of HP factors resulted in conversion of naive-to-memory T-cell phenotype.

Our data about the effect of HP factors on the number of CD31⁺T-cells, proliferation with sustained phenotype are consistent with the published data. It was shown that *in vitro* IL-7-induced proliferation of CD4⁺CD31⁺ naive T-cells does not affect the stable surface CD31 expression [1]. Loss of the CD31 molecule can occur after receiving a signal through the T-cell receptor [8]. CD31⁺T-cells from RA patients showed a proliferation level comparable to that in donor cells in response to IL-7, IL-15. Typically, peripheral blood T-cells showed low responsiveness

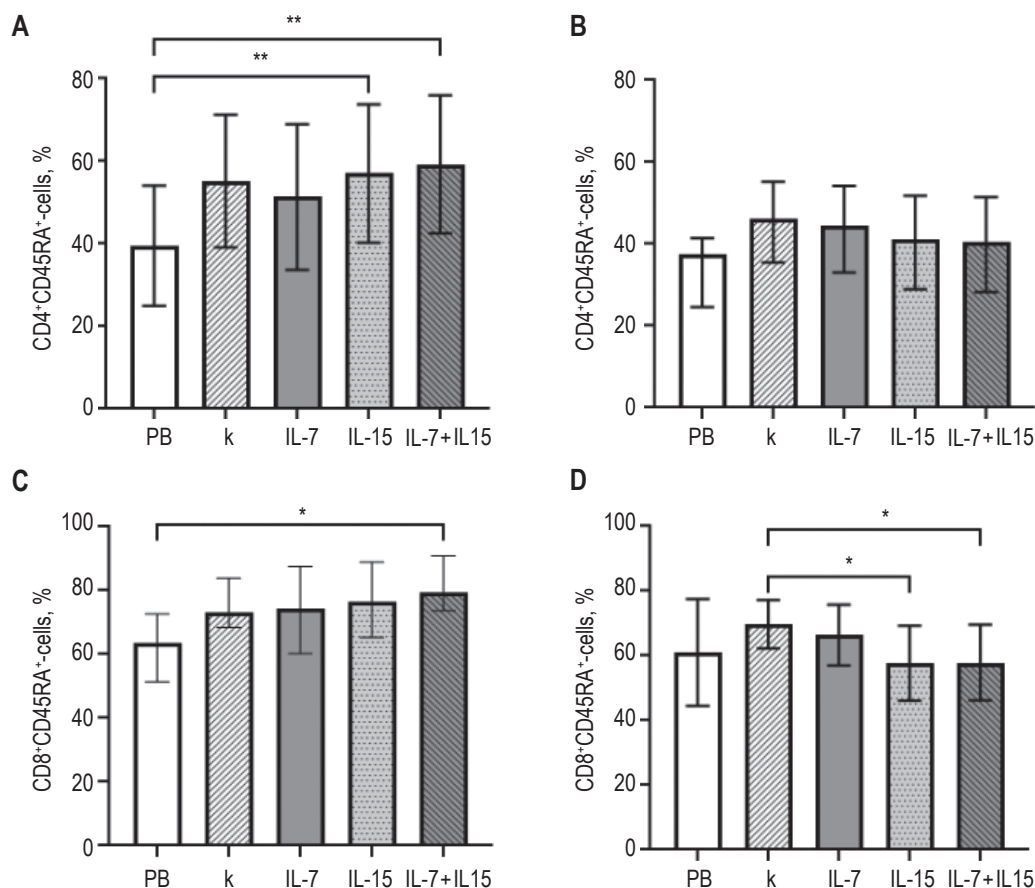


Figure 1. Proportion of CD4⁺CD45RA⁺ and CD8⁺45RA⁺-cells in cultures *in vitro* and in the peripheral blood of healthy volunteers and patients with rheumatoid arthritis

Note. A, percentage of CD4⁺CD45RA⁺-cells from donors. B, percentage of CD4⁺CD45RA⁺-cells from patients with RA. C, percentage of CD8⁺45RA⁺-cells from donors. D, percentage of CD8⁺45RA⁺-cells from patients with RA. Data are present as median with interquartile range (25th and 75th percentiles); PB, peripheral blood; k, culture without stimulation. *, significant differences, $p < 0.05$; **, significant differences, $p < 0.01$

TABLE 1. CONTENT OF CD31⁺ CELLS AMONG CD4⁺ AND CD8⁺-LYMPHOCYTES IN THE PERIPHERAL BLOOD AND *IN VITRO* CULTURES IN HEALTHY INDIVIDUALS AND PATIENTS WITH RA, Me (Q_{0.25}-Q_{0.75})

	CD4 ⁺ CD31 ⁺ , %	CD8 ⁺ CD31 ⁺ , %
PB_donors	32.9 (20.1-44.0)	75.5 (64.1-83.1)
PB_RA patients	26.4 (22.9-32.4)	74.7 (72.9-78.9)
k_donors	32.5 (19.3-45.0)	76.8 (65.5-82.2)
k_RA patients	26.0 (19.1-28.0)	75.6 (61.0-76.7)
IL-7_donors	30.0 (22.6-45.8)	78.6 (71.8-80.3)
IL-7_RA patients	23.6 (22.9-37.4)	77.9 (72.0-79.1)
IL-15_donors	29.7 (18.3-44.2)	75.4 (65.0-81.8)
IL-15_RA patients	22.1 (20.2-30.8)	77.6 (72.1-80.4)
IL-7+15_donors	37.3 (24.0-45.7)	77.8 (67.7-82.9)
IL-7+15_RA patients	28.0 (22.9-37.3)	77.1 (74.6-82.3)

Note. PB, peripheral blood; k, culture without stimulation.

TABLE 2. RESPONSIVENESS OF CD4⁺CD31⁺ AND CD8⁺CD31⁺-CELLS FROM HEALTHY INDIVIDUALS AND PATIENTS WITH RA TO HOMEOSTATIC CYTOKINES *IN VITRO*, Me (Q_{0.25}-Q_{0.75})

Cell subset	Proliferated cells, %			
	k	IL-7	IL-15	IL-7 + IL-15
Donors CD4 ⁺ CD31 ⁺	1.8 (1.2-4.8)	12.5 (6.8-39.5)*	8.7 (5.5-18.7)*	27.5 (13.4-59.2)*
RA patients CD4 ⁺ CD31 ⁺	1.7 (0.8-2.2)	14.4 (5.4-27.7)*	18.2 (5.8-36.6)*	38.9 (11.0-43.4)*
Доноры CD8 ⁺ CD31 ⁺	1.4 (0.6-2.9)	30.9 (21.4-54.7)*	54.2 (37.8-76.6)*	68.4 (58.3-88.4)*
RA patients CD8 ⁺ CD31 ⁺	1.4 (0.8-2.8)	27.8 (16.3-48.5)*	70.6 (44.7-77.6)*	75.2 (39.7-78.5)*

Note. *, significant differences compared to cells without stimulation (k), p < 0.05.

to mitogenic stimulation, while synovial CD4⁺-lymphocytes are hyper-reactive *in vitro*, including the high response to IL-7 stimulation [14]. In addition, IL-7 is able to enhance the response of T-cells to phytohemagglutinin in patients with RA under the clinical remission, confirming its involvement in the disease pathogenesis [5].

In RA, the decrease in the percentage of CD45RA⁺-cells was revealed in cultures added with IL-15 or IL-15 combined with IL-7, suggesting that under the influence of HP factors, an increased percentage of CD45RA⁺CD31⁺-cells may occur. It has been shown that, under the action of proinflammatory cytokines, IL-7 promotes accumulation of cultured central memory T-cells *in vitro*, which can be differentiated from naive T-lymphocytes under inflammatory

conditions [3]. IL-15 itself can act as a pro-inflammatory cytokine, promoting the production of TNF by macrophages and other pro-inflammatory cytokines by T-cells [15].

Conclusion

Thus, this study demonstrated that HP factors, individually or in combination, promote the proliferation of CD31⁺-positive cells and sustained their percentage both in health and RA. However, taking into account the lower number of cells expressing CD45RA in patients with RA, the proliferation of CD4⁺31⁺ and CD8⁺CD31⁺-cells treated with IL-15 and IL-15 combined with IL-7 occurs along with losing phenotype of naive T-cells in autoimmune pathology.

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