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СРАВНЕНИЕ ФЕНОТИПИЧЕСКИХ СВОЙСТВ ВРОЖДЕННЫХ ЛИМФОИДНЫХ КЛЕТОК НА РАЗНЫХ СТАДИЯХ РЕВМАТОИДНОГО АРТРИТА

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Резюме. Аутоиммунные заболевания на сегодняшний день занимают лидирующее место по частоте встречаемости в популяции, среди которых один процент занимает ревматоидный артрит (РА). Ремиссия при данном виде заболевания достигается крайне редко и требует постоянного использования фармакотерапии. В связи с этим необходимо подробно изучать патогенез РА для поиска новых мишеней лекарственных препаратов. Известно, что в развитии РА принимают участие Т-хелперы (Th)1 и Th17. Однако некоторые исследователи предполагают, что в развитии РА играют роль ILC. ILC являются «врожденными аналогами» Th, ввиду того, что данная субпопуляция синтезирует такие же цитокины. ILC1 является врожденными аналогами Th1, ILC2-Th2, ILC3-Th17. ILC представляют собой резидентные в тканях врожденные лимфоидные клетки, которые имеют функциональное разнообразие и регулируют направленность иммунного ответа с помощью продукции цитокинов.

В качестве материала мы использовали мононуклерные клетки периферической крови (МНК ПК) от пациентов (n = 19) и условно-здоровых доноров (n = 10). Группа пациентов была разделена в зависимости от терапии: ГИБП и МТХ, а также в зависимости от стадии РА (ранний и очень ранний артрит, развернутый и поздний). МНК ПК были окрашены моноклональными антителами и определялись как Lin^-CD127^+, в общей популяции оценивали количество $CD294^+$ILC (ILC2), $CD117^-CD294^-$ ILC были идентифицированы как ILC1, а $CD117^+CD294^-$ILC были определены как ILC3.

Мы получили следующие результаты: количество ILC1 было достоверно снижено у пациентов, получавших МТ по сравнению с пациентами, находящимися на ГИБП и условно здоровыми донорами. Однако пациенты на МТХ с поздней стадией РА имели низкие уровни ILC2 и ILC3 по сравнению с

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пациентами на ГИБП. Доля ILC2 достоверно возрастала у пациентов на ранних стадиях РА по сравнению с пациентами с поздней стадией РА. Однако ILC1 были значительно снижены у пациентов, получавших МТХ, а ILC3 значительно увеличились у пациентов, получавших МТХ по сравнению с ГИБП.

Экспрессия PD1 на ILC1 была повышена по сравнению с пациентами, получавшими ГИБП. Однако ILC3 пациентов с поздними стадиями на МТХ имели повышенную экспрессию PD1 по сравнению с пациентами, принимавшими ГИБП. ILC3 доноров был значительно повышен по сравнению с пациентами на ГИБП.

Ключевые слова: врожденные лимфоидные клетки, ревматоидный артрит, контрольные точки иммунного ответа, проточная цитометрия, цитокины

COMPARISON OF PHENOTYPIC PROPERTIES OF INNATE LYMPHOID CELLS AT VARIOUS STAGES OF RHEUMATOID ARTHRITIS

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Abstract. Autoimmune diseases currently take a leading place in terms of frequency of occurrence in the population, among which 1 percent is occupied by rheumatoid arthritis (RA). Remission in this type of disease is extremely rare and requires constant use of pharmacotherapy. Studying the pathogenesis of RA is necessary to study to search for new drug targets. It is known that T helpers 1 (Th) and Th17 are involved in the development of RA. However, some researchers suggest that ILCs play a role in the development of RA. ILCs are "innate analogues" of Th, due to the fact that this subpopulation synthesizes the same cytokines. ILC1 is innate analogs of Th1, ILC2-Th2, ILC3-Th17. ILCs are tissue-resident innate lymphoid cells that have functional diversity and regulate the direction of the immune response through the production of cytokines.

We used peripheral blood mononuclear cells (PBMCs) from patients (n = 19) and conditionally healthy donors (n = 10) as material. The group of patients was divided biologic disease-modifying anti-rheumatic drugs (bDMARDs) and Metotrexate (MTX) and of stage of RA (early and very early arthritis, advanced and late). PBMCs were stained with monoclonal antibodies. ILCs were identified as Lin-CD127$^+$, CD294$^+$ILCs (ILC2) were measured in the general population, CD117-CD294-ILCs were identified as ILC1, and CD117$^+$CD294$^-$ ILCs were identified as ILC3.

We obtained the following results: ILC1 was significantly reduced in patients treated with MTX comparison with patients on bDMARDs and healthy donors. However, patients on MTX with advanced RA had low levels of ILC2 and ILC3 compared to patients on bDMARDs. ILC2 significantly increased in patients with early stages of RA comparison with patients with advanced RA. However, ILC1 was significantly reduced in patients treated with MTX, and ILC3 increased significantly in patients treated with MTX comparison with bDMARDs. Expression of PD1 on ILC1 was increased compared to patients treated with bDMARDs. However, ILC3 patients with advanced stages on MTX had increased expression of PD1 comparison with patients taking bDMARDs. The ILC3 of donors was significantly increased comparison with patients on bDMARDs.

Keywords: ILC, rheumatoid arthritis, immune checkpoint molecules, flow cytometry, cytokines

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Introduction

Today rheumatoid arthritis (RA) is one of the most common diseases among autoimmune diseases [8, 9]. RA is associated with progressive disability, systemic damage of organs and tissues, as well as with economic costs for society [6]. Until today, research

on rheumatoid arthritis is important and a significant problem due to low efficiency of medicines and the risk of developing unwanted effects. Therefore, it is necessary to study in detail the pathogenesis of RA and to search for new drug targets. Remission of RA is achieved extremely rarely and requires permanent use of pharmacotherapy [6]. It is interesting to research the role of innate lymphoid cells (ILC) in the development of autoimmune inflammation in RA, especially the role of plasticity of these cells. ILCs are tissue-resident innate lymphoid cells which have a functional diversity similar to T cells. In addition, ILCs regulate the direction of the immune response through the production of cytokines. Accordingly, understanding of these processes will allow the development of new therapeutic strategies aimed at reducing inflammation or enhancing antitumor immunity and based on the possible reprogramming of T cell populations towards one or another phenotype [1, 2].

ILCs are same as Th1 because they respond to intracellular pathogens, secrete IFNγ, and they depend on T-bet for their differentiation. ILC2s are like Th2 cells which produce high levels of interleukin (IL)-4, IL-5 and IL-13 in response to IL-33, IL-25 and thymic stromal lymphopoietin (TSLP). ILC-2s express high levels of the transcription factors GATA3 and RORα. ILC-3s are innate analogues of Th17 cells. It depends on RORγt [1, 2, 7, 11].

Materials and methods

The study included 10 volunteers and 19 patients with RA divided into 3 groups according to the disease stage and treatment: (1) late and advanced stages 10 patients were treated with biologic disease-modifying anti-rheumatic drugs (bDMARDs); (2) late and advanced stages 5 patients were treated with methotrexate (MTX); (3) early and very early stages 4 patients were treated with MTX 9 ml of blood was collected from donors and patients. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood in a ficoll-urografin density gradient (1.077 g/ml). Isolated PBMCs were stained with fluorochrome-conjugated monoclonal antibodies: anti-Lineage (CD3/14/16/19/20/56) and anti-FceR1 alpha-FITC, anti-CD294-APC/Cy7, anti-CD127-PerCP/Cy5.5, anti-CD336-PE, anti-CD117-APC.C3. ILCs were defined as Lin^-CD127^+, $CD294^+$ILCs were estimated as ILC2, $CD117^-CD294^-$ILCs were defined as ILC1, and $CD117^+CD294^-$ILCs were identified as ILC3. The cell phenotype was analyzed on a FACS Canto II flow cytometer (BD Biosciences, USA). Statistical processing of the obtained data was carried out using the GraphPad Prism 9.0.0 application package with one way ANOVA. Differences were considered significant at $p < 0.05$.

Results and discussion

We found that ILC 1 is significantly reduced in patients treated with MTX in comparison with volunteers (D) and patients on bDMARDs in all stages of RA. However, we found that in patients with advanced and late stages of RA treated with MTX had significantly reduced ILC2 and ILC3 compared to volunteers and patients in bDMARDs (Figure 1). ILCs2 were significantly increased in patients in early and very early stages of RA in comparison with patients on late stage of RA on MTX and bDMARDs. However ILCs1 were significantly reduced in patients which were treated with MTX, but ILCs3 were significantly increased in patients on MTX comparison with patients in bDMARDs (Figure 2).

ILC1 of patients who were treated with MTX had significantly increased expression of PD1 in comparison with patients on bDMARDs. However we noted relative to ILC3 that expression of PD 1 is significantly increased in patients who were treated with MXT (late stages of RA) in comparison with patients with use bDMARDs. Also we found that ILC3 of donors are significantly increased in comparison with patients were treated with bDMARDs, however were compared to ILC1 and ILC2 donors and patients on bDMARDs we found trend. In the proportion of ILC2 $cKit^+$ we didn't obtain significant results (Figure 3).

It has been shown that various cells play a role in the development of RA, such as DCs, T and B cells and other cells. ILC have been seems have been involved in the pathogenesis of development RA too [2, 10, 11, 13].

This study demonstrates that numbers of ILCs depend on stage of RA as well as therapy. Some research reports that numbers of ILC1 and ILC3 is increased in the peripheral blood, synovial fluid, and lymph node of patients with RA, however numbers of ILC2 were low [5]. Apparently, the balance of ILC changes in patients with RA. In early stage of RA we observed ILC1 was low, but ILC2 is increased in the peripheral blood. These results are consistent with previous studies [12]. While the number of ILC2 in the synovial fluid (SF) of patients was decreased in active phase of RA, it was increased in remission [3, 12]. In later stage of RA we observed that numbers of ILC1 is significant increased. ILC 2 has been suggested to play a protective role synthesizing IL-4 and IL-5 activated Th2 to downregulate the inflammatory processes in RA [12]. ILCs2 secrete IL-10 and TBF b to reduce inflammation in gut by suppressing both ILC1 and ILC3 [2, 11, 13].

As known, patients with RA have an overactive immune response. These patients have a biased immune response, T helpers (Th)1 and Th17 which are significantly increased in comparison with Th2. ILCs are "innate analogues Th". ILCs locate on barrier

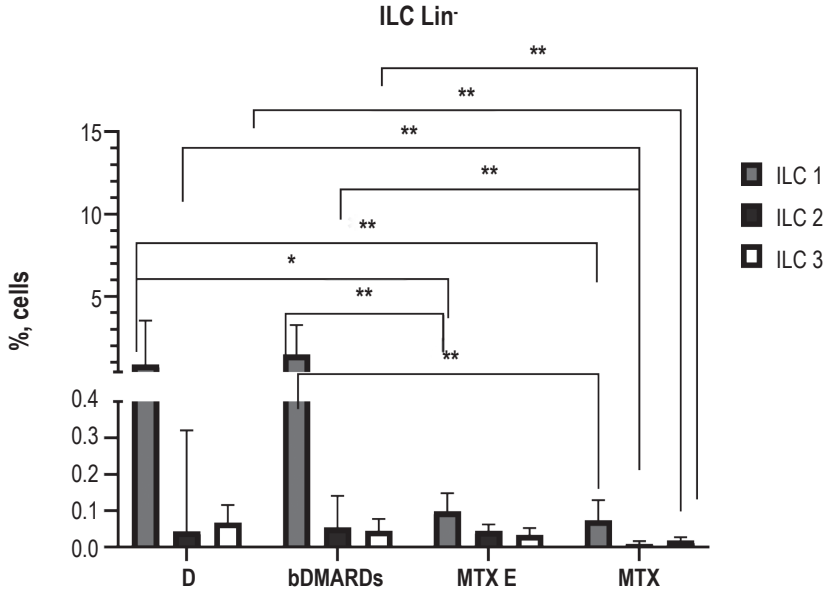

Figure 1. Number of ILC Lin-. D, healthy donors, bDMARDs (biologic disease-modifying anti-rheumatic drugs), MTX E (patients who were treated MTX with early stage RA), MTX (patients who were treated MTX with advance stage RA)

Note. Data are presented as median ± interquartile range with n = 10 (D), n = 12 (bDMARDs), n = 4 (MTX E.), n = 5 (MTX).*, significant differences are $p < 0.05$ by employing one-way ANOVA.

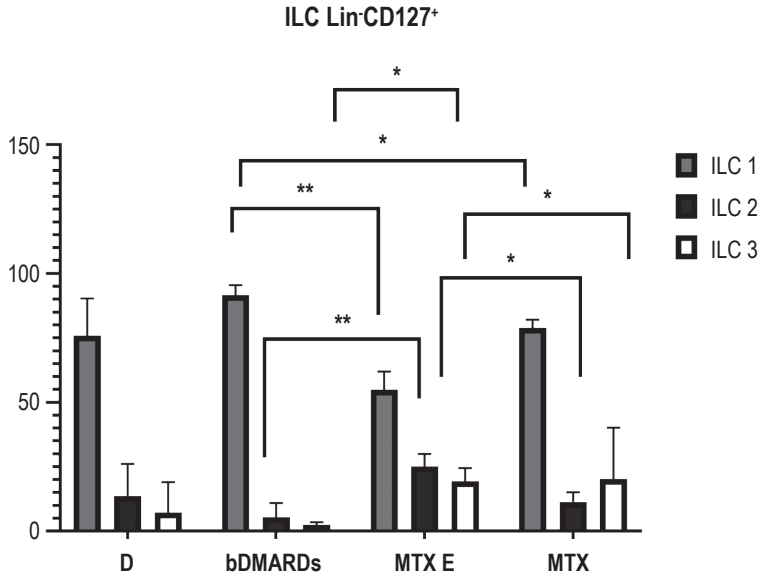

Figure 2. Number of ILC Lin-CD127+. D, healthy donors, bDMARDs (biologic disease-modifying anti-rheumatic drugs), MTX E (patients who were treated MTX with early stage RA), MTX (patients who were treated MTX with advance stage RA)

Note. Data are presented as median ± interquartile range with n = 8. *, significant differences are $p < 0.05$ by employing one-way ANOVA.

tissues and first respond on various stress signals by synthesizing cytokines to affect to TH. So we conclude that balance Th depends on ILCs and in that way anti-rheumatic therapy can balance the population of T helpers. [3]. Herman et al. [4] reported that MTX significantly reduces Th1 cells and modulates the immune status towards Th2 dominance. We obtained that balance of ILC depends on therapy as well. Patients which were treated with MTX have higher numbers of ILC2 and ILC3. However, Tamimoto et al. [10] reported that bDMARDs (rituximab) directs the immune response to Th 1. We obtained similar results that bDMARDs significantly increase ILC1. Thus numbers of ILC depends on stage and therapy of RA.

PD1 is an important negative regulator of differentiation that plays similar role on ILC as on other cells. The numbers of ILCs 2 depends on expression of PD 1 [11]. ILC synthesize high level of Th2 cytokines such as IL-5, IL-13, IL-9 in the absence PD1 or

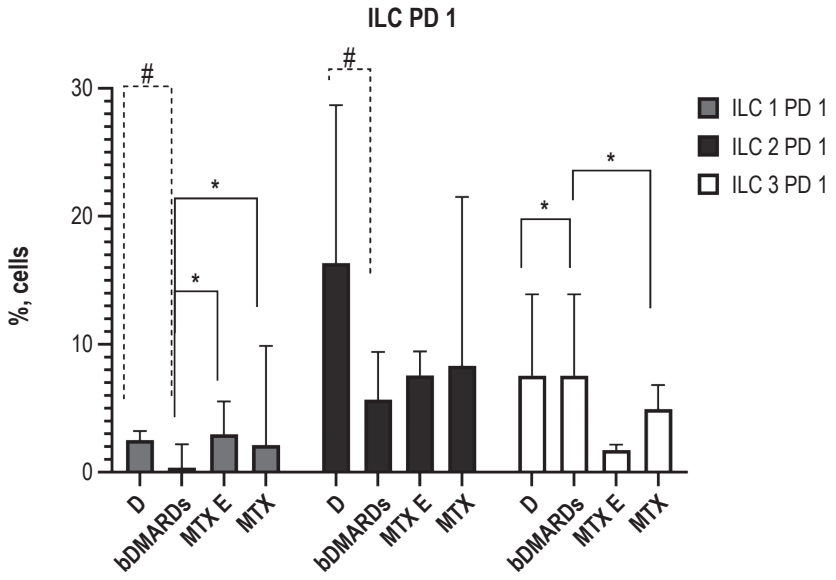

Figure 3. Expressions of PD-1 and PDL-1 immune checkpoint molecules on ILC. D, healthy donors, bDMARDs (biologic disease-modifying anti-rheumatic drugs), MTX E (patients who were treated MTX with early stage RA), MTX (patients who were treated MTX with advance stage RA)

PD-1-knockout (KO) mice, so that high lever PD1 can to inhibit of ILC [11]. We found that expression PD1 of ILC1 with patients on bDMARDs significantly reduced which can explain high level of numbers of ILC. Thus PD1 plays an important role to maintain the number of ILC.

Conclusion

We found that the balance of ILC depends on therapy and stage of RA. However, further research is needed to confirm the relationship between the balance of ILC and antirheumatic drugs to improve the effectiveness of treatment.

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