ИДЕНТИФИКАЦИЯ Th1-ПОЛЯРИЗОВАННЫХ КЛЕТОК Th17: РЕШЕНИЕ ПРОБЛЕМЫ

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Резюме. Т-хелперы, продуцирующие IL-17 (Th17), обладают высокой пластичностью: рестимуляция лимфоцитов в воспалительном окружении способна индуцировать их трансформацию в клетки с другим фенотипом, и наиболее частым является сдвиг в направлении Th1. Результатом такой трансформации является появление клеток, экспрессирующих наряду с классическими маркерами клеток Th17 ключевые Th1-ассоциированные молекулы. В наиболее общей форме такая популяция представлена $CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^+IFN\gamma^+$Т-клетками, и в современной литературе она чаще всего обозначается как Th17.1. Часть клеток Th17.1 может полностью утрачивать продукцию IL-17, сохраняя при этом экспрессию других Th17-ассоциированных молекул, – это так называемые клетки ex-Th17 ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$Т-клетки). Как следствие, популяция Th1-поляризованных Th17 включает клетки Th17.1, ex-Th17 и ряд дополнительных переходных форм. Она имеет уникальные функциональные свойства – повышенный провоспалительный потенциал и способность преодолевать гистогематические барьеры. Именно этим клеткам в настоящее время отводится ключевая роль в патогенезе многих аутоиммунных заболеваний, а процесс редифференцировки Th17 в Th1 рассматривается как перспективная терапевтическая мишень. Однако развитие этого направления осложняет слабая сопоставимость данных о размерах такой популяции. Проведенный в рамках настоящей работы анализ методов определения Th1-поляризованных Th17 *in vivo* и *in vitro* позволил разрешить эти противоречия и разработать оптимальные подходы к идентификации данной популяции. В большинстве работ, особенно клинических, ее идентифицируют по коэкспрессии ключевых цитокинов (IL-17/IFNγ) или хемокиновых рецепторов (CCR6/CXCR3), редко – по их комбинации. При таком подходе коэкспрессия CCR6/CXCR3 маркирует общую популяцию Th1-подобных Th17, включающую и Th17.1, и ex-Th17, тогда как коэкспрессия цитокинов IL-17/IFNγ идентифицирует клетки Th17.1, а субпопуляцию ex-Th17 в этом случае ошибочно классифицируют как классические Th1. Такая «недооценка» субпопуляции ex-Th17 существенно занижает результаты, поскольку именно на долю ex-Th17 приходится основная часть Th1-подобных Th17. И только одновременная оценка коэкспрессии цитокинов и Th17-ассоциированных мембранных молекул позволяет идентифицировать клетки Th17.1 и ex-Th17 отдельно, что важно учитывать при интерпретации данных по проблеме и при планировании клинических исследований.

Ключевые слова: Th17, редифференцировка, Th1-поляризованные Th17, IFNγ-продуцирующие Th17, Th17.1, ex-Th17

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IDENTIFICATION OF Th1-POLARIZED Th17 CELLS: SOLVING THE PROBLEM

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Abstract. Helper T cells producing IL-17 (Th17) have high plasticity: restimulation of lymphocytes in an inflammatory environment can induce their transformation into cells with another phenotype, and a shift towards Th1 is the most common. The result of this transformation is the appearance of cells expressing along with the classical markers of Th17 cells key Th1-associated molecules. In its most general form, this population is represented by $CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^+IFN\gamma^+$T cells, and in the current literature it is most often referred to as Th17.1. Some Th17.1 cells can completely lose the production of IL-17, while maintaining the expression of other Th17-associated molecules; these are the so-called ex-Th17 cells ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$T cells). Consequently, the population of Th1-polarized Th17 includes Th17.1, ex-Th17 cells and a number of additional transitional forms. It has unique functional properties – an increased pro-inflammatory potential and the ability to overcome histohematic barriers. It is these cells that are currently assigned a key role in the pathogenesis of many autoimmune diseases, and the process of Th17 redifferentiation into Th1 is considered as a promising therapeutic target. However, the development of this direction is complicated by the weak comparability of data on the size of such a population. The analysis of methods for determining Th1-polarized Th17 *in vivo* and *in vitro*, carried out in this work, made it possible to resolve these contradictions and develop optimal approaches to identifying this population. In most studies, especially clinical ones, it is identified by co-expression of key cytokines (IL-17/IFNγ) or chemokine receptors (CCR6/CXCR3), rarely by their combination. In this approach, co-expression of CCR6/CXCR3 marks the total population of Th1-like Th17, including both Th17.1 and ex-Th17, while co-expression of IL-17/IFNγ cytokines identifies only Th17.1 cells, and the subpopulation of ex-Th17 is misclassified as classic Th1 in this case. Such "underestimation" of the ex-Th17 subpopulation significantly marks down the results, since it is ex-Th17 that accounts for the bulk of Th1-like Th17. And only a simultaneous assessment of the co-expression of cytokines and Th17-associated membrane molecules allows identification Th17.1 and ex-Th17 cells separately, which is important to consider when interpreting data on the problem and when planning clinical trials.

Keywords: Th17, redifferentiation, Th1-polarized Th17, IFNγ-producing Th17, Th17.1, ex-Th17

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Introduction

Differentiation of T helper cells producing IL-17 (Th17) is usually initiated upon activation of naive $CD4^+$ T cells in the presence of cytokine combinations IL-6/TGF-β or IL-6/IL-1β, and later IL-23 enters the process, providing cell expansion and supporting their functioning, in particular, the synthesis of IL-17 [11]. Classical Th17 cells express the key transcription factor RORC, carry specific membrane markers such as the lectin-like killer cell receptor CD161 and the chemokine receptor CCR6, and are capable of producing their characteristic cytokines IL-17A, IL-17F, and IL-22 [9]. However, differentiated Th17 lymphocytes are unstable and can transform into cells of a different phenotype upon restimulation in a local cytokine environment. The most common variant of this transformation is a shift towards Th1, which is accompanied by the formation of cells that co-express along with traditional Th17-associated molecules Th1 cell markers – transcription factors STAT4/T-bet, the chemokine receptor CXCR3, and the key Th1 cytokine IFNγ [8, 10, 12].

Normally, the population of IFNγ-producing Th17 is present in trace amounts in peripheral blood, but its content in infiltrates of inflamed tissues reaches 60% [15], and it is these cells that are currently assigned a key role in the pathogenesis of many diseases, including autoimmune ones. Thus, the presence of IFNγ-producing Th17 was found in the sites of inflammation in sarcoidosis [15], in the CNS in multiple sclerosis [3, 6, 13], in the synovial tissue of patients with rheumatoid arthritis [2, 12], in inflamed tissues of the gastrointestinal tract of patients with Crohn's disease [1, 7], and for many pathologies a direct association with this non-classical T helper population has been convincingly shown. As a result, the number and activity of Th1-polarized Th17 cells are currently considered as promising diagnostic and/or prognostic markers. However, this is hampered

by inconsistencies in how this population is defined and by incomparability of data between studies.

The purpose of this work is to analyze methods for assessing Th1-polarized Th17 *in vivo* and *in vitro*, and to develop optimal approaches to identify this population.

Materials and methods

A literature search was performed in the PubMed database using appropriate keywords, without language and publication date limitations. The subject of interest was generally not a biological effects, but rather the ways of identification the non-classic T cell population and comparing its size between studies.

Results and discussion

Contradictions in the data on IFNγ-producing Th17 are primarily associated, apparently, with a large number of potential markers of these cells, and the markers are diverse. As noted earlier, this population combines the phenotypic and functional characteristics of both lines, Th17 and Th1, including the expression of membrane molecules, cytokines, and key transcription factors, although the latter are not usually used for identification. In the most general form, this population is presented in the works as $CD^+CD161^+CCR6^+CXCR3^+IL\text{-}17^+IFN\gamma^+T$ cells [10, 12]. It is called differently in the current literature; in this paper we will use its most common name, Th17.1 [2, 14, 15]. However, Th17.1 cells are not the only variant of Th1-polarized Th17 lymphocytes: some of these cells lack the production of IL-17, but demonstrate the expression of the Th17-associated transcription factor RORC and CD161/CCR6 membrane molecules ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+T$ cells) [1, 12]. In the literature, they are usually referred to as ex-Th17. Most of the available evidence suggests that Th17.1 and ex-Th17 cells are not separate subpopulations, but rather different stages of Th17 redifferentiation into Th1, during which classical Th17 first acquire the expression of Th1-associated molecules, and at the next stage lose IL-17 synthesis, producing only IFNγ, but retaining other "attributes" of the original population.

A more detailed analysis of data on the Th17.1 subpopulation shows that in a number of cases, when these cells are identified only with membrane molecules, two more chemokine receptors, CCR4 and CCR10, are added to the line of markers in order to separate the populations of classical Th17 and Th22, which upon identification using these membrane markers have the same phenotype ($CCR6^+CXCR3^{-/low}$), but can be differentiated by the combination of CCR4/CCR10: CCR4 is present in both populations, while CCR10 is highly expressed on Th22 cells but absent in Th17 [14]. As a result, the subpopulation of cells that co-produce IL-17/IFNγ will have the $CD4^+CD161^+CCR6^+CCR4^{-/low}CCR10^-CXCR3^+$ phenotype, in contrast to the classical Th17 with the $CD4^+CD161^+CCR6^+CCR4^+CCR10^-CXCR3^-$ phenotype [14]. In addition, in *ex vivo* studies, Th17.1 and exTh17 cells are usually isolated from pre-fractionated memory T cells, either central ($CCR7^+CD45RA^-$) or effector ($CCR7^-CD45RA^-/CD45R0^+$).

Of course, this is not the limit of detail; there are studies in which, along with membrane molecules, a wide range of intracellular molecules are evaluated, both at the mRNA and protein levels, however, in most studies, especially clinical ones, this subpopulation is identified by key cytokines (IL-17/IFNγ), chemokine receptors (CCR6/CXCR3) or, very rarely, combinations thereof. Obviously, when using only CCR6/CXCR3 chemokine receptors as markers, a general population of Th1-like Th17 is identified, including both Th17.1 and ex-Th17 [2, 3, 13], whereas in the case of detection of Th1-like Th17 cells by co-expression of IL-17/IFNγ cytokines, we are talking about the Th17.1 subpopulation [1, 4, 12]. It is important to emphasize that the ex-Th17 subpopulation ($IL\text{-}17^-IFN\gamma^+T$ cells) is in this case improperly classified as classic Th1. And only a simultaneous assessment of the expression of cytokines (IL-17/IFNγ) and Th17-associated membrane molecules (CD161 or CCR6) allows us to identify both the Th17.1 subpopulation ($CD4^+CD161^+IL\text{-}17^+IFN\gamma^+T$ lymphocytes) and the second the key subpopulation of Th1-like Th17 – ex-Th17 ($CD4^+CD161^+IL\text{-}17^-IFN\gamma^+T$ cells), differentiating it using CD161 from classical Th1 lymphocytes lacking this marker ($CD4^+CD161^-IL\text{-}17^-IFN\gamma^+T$ cells) [10].

Conclusion

The use of CCR6/CXCR3 membrane markers makes it possible to identify both major subpopulations of Th1-like Th17 lymphocytes, Th17.1 and ex-Th17, but does not allow them to be differentiated from each other. Evaluation of co-expression of IL-17/IFNγ cytokines makes it possible to detect Th17.1 cells, but not ex-Th17 cells; in this case, they are improperly attributed to the population of classical IFNγ-producing Th1. Moreover, such an "underestimation" of the ex-Th17 subpopulation significantly reduces the informativeness of the results, since it accounts for the bulk of T lymphocytes co-expressing Th17/Th1-associated membrane markers CCR6/CXCR3 [5, 15]. Not surprisingly, the size of the study population varies greatly from study to study. Therefore, when interpreting and comparing data on Th1-like Th17-lymphocytes, it is fundamentally important to take into account the method of their identification, and when planning work, to give preference to a combined approach, with simultaneous assessment of cytokines (IL-17/IFNγ) and Th17-associated membrane molecules (CD161 or CCR6), which allows to determine separately the subpopulations of Th17.1 and ex-Th17.

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