BIOCHEMISTRY

MARKERS OF Th1 POLARIZED Th17 CELLS (LITERATURE REVIEW)

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ABSTRACT

T helpers (Th) producing IL-17 (Th17) have high plasticity and under the influence of external conditions are able to redifferentiate into cells with a different phenotype, primarily in Th1-lymphocytes, forming a population that combines the characteristics of both Th17 and Th1 and has a high pro-inflammatory potential, as well as a unique ability to overcome histohematic barriers. These cells are currently assigned a key role in the pathogenesis of many inflammatory diseases, including autoimmune ones: they account for up to half of the lymphocytes present in infiltrates of inflamed tissues. The paper discusses the reasons for the increased plasticity of Th17 cells in comparison with the main T helper populations (Th1 and Th2) and considers in detail the mechanisms of formation of IFNγ producing Th17, taking into account not only the redifferentiation of mature Th17, but also possible alternative pathways, in particular, Th1 cell redifferentiation or naive $CD4^+$ T lymphocytes direct differentiation into cells with an intermediate Th1/Th17 phenotype. The main inducers of differentiation of IFNγ producing Th17 cells and the reversibility of this process are also discussed. Particular attention is paid to the methods for identifying Th1 polarized Th17 cells: this population is heterogeneous, and its size significantly depends on the type of markers used to characterize these cells – Th1/Th17-associated transcription factors, key cytokines, as well as chemokine receptors and other membrane molecules. As a result, the data in the works on this problem are poorly comparable with each other. The unification of approaches to identifying a population of Th1 like Th17 cells will solve this problem and make it possible to use an assessment of the size and activity of such a population as diagnostic or prognostic markers.

Key words: *Th17, Th1, plasticity, redifferentiation, Th17.1, ex-Th17*

Received: 07.12.2022
Accepted: 29.05.2023
Published: 11.07.2023

For citation: Kuklina E.M., Glebezdina N.S. Markers of Th1 polarized Th17 cells (literature review). *Acta biomedica scientifica*. 2023; 8(3): 55-62. doi: 10.29413/ABS.2023-8.3.5

МАРКЕРЫ Th1-ПОЛЯРИЗОВАННЫХ КЛЕТОК Th17 (ОБЗОР ЛИТЕРАТУРЫ)

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РЕЗЮМЕ

Т-хелперы (Th, T helpers), продуцирующие IL-17 (Th17), обладают высокой пластичностью и под влиянием внешних условий способны редифференцироваться в клетки с другим фенотипом, прежде всего в Th1-лимфоциты, формируя популяцию, сочетающую в себе характеристики как Th17, так и Th1 и обладающую высоким провоспалительным потенциалом, а также уникальной способностью преодолевать гистогематические барьеры. Именно этим клеткам в настоящее время отводится ключевая роль в патогенезе многих воспалительных заболеваний, включая и аутоиммунные: в инфильтратах воспалённых тканей на их долю приходится до половины присутствующих там лимфоцитов. В работе обсуждаются причины повышенной пластичности клеток Th17 в сравнении с основными Т-хелперными популяциями (Th1 и Th2) и подробно рассматриваются механизмы формирования IFNγ-продуцирующих Th17 с учётом не только редифференцировки зрелых Th17, но и возможных альтернативных путей, в частности редифференцировки клеток Th1 или непосредственной дифференцировки наивных $CD4^+$Т-лимфоцитов в клетки с промежуточным Th1/Th17-фенотипом. Также обсуждаются основные индукторы дифференцировки IFNγ-продуцирующих клеток Th17 и обратимость этого процесса. Особое внимание в обзоре уделено способам идентификации Th1-поляризованных клеток Th17: эта популяция неоднородна, и её размер существенно зависит от типа маркеров, используемых для характеристики данных клеток – Th1/Th17-ассоциированных транскрипционных факторов, ключевых цитокинов, а также хемокиновых рецепторов и других мембранных молекул. Как следствие, данные в работах по этой проблеме плохо сопоставимы друг с другом. Унификация подходов к выявлению популяции Th1-подобных Th17 позволит решить эту проблему и даст возможность использовать оценку размера и активности такой популяции в качестве диагностических или прогностических маркеров.

Ключевые слова: *Th17, Th1, пластичность, редифференцировка, Th17.1, ex-Th17*

Статья поступила: 07.12.2022
Статья принята: 29.05.2023
Статья опубликована: 11.07.2023

Для цитирования: Куклина Е.М., Глебездина Н.С. Маркеры Th1-поляризованных клеток Th17 (обзор литературы). *Acta biomedica scientifica*. 2023; 8(3): 55-62. doi: 10.29413/ABS.2023-8.3.5

The population of T-helpers producing interleukin (IL) 17 (Th17) shows considerable heterogeneity and plasticity. The cytokine milieu is capable not only of adjusting the program of differentiation of naive $CD4^+$ T lymphocytes into Th17, but also of inducing redifferentiation of mature Th17, and first of all, we are talking about the acquisition by these cells of a Th1-like phenotype associated with high production of interferon (IFN) γ [1–4]. The nomenclature of such Th1 polarized Th17s is not uniform, although it reflects their transformed state: in different papers these cells are designated as Th17/Th1 [5, 6], Th1/Th17 [7, 8], Th1/17 [9], Th17/1 [10], Th17-1 [11], Th17.1 [12–14], as well as Th1* [15], "non-classical Th1s" [6] or simply as "IFNγ-producing Th17". However, it is not only a matter of nomenclature – the analysis of these works shows that the population of Th1-like Th17 is heterogeneous, and the above-mentioned works often refer to phenotypically and functionally different subpopulations.

Despite the small size of IFNγ-producing Th17 subpopulation in peripheral blood, its content in infiltrates of inflamed tissues reaches 60 % [13], and it is these cells that are currently assigned a key role in the pathogenesis of many inflammatory diseases, including autoimmune ones [5, 7, 8, 10, 13, 14]. Unification of data on non-classical Th17 cell variants is therefore of high relevance and is the subject of this review.

CHARACTERIZATION OF Th1-LIKE Th17 CELLS

The fact that the Th17 population includes a fraction of lymphocytes producing, along with IL-17, the Th1-associated cytokine IFNγ was noted in one of the first studies of Th17 cells in humans [16]. At that time, membrane markers to differentiate such a non-classical population from traditional Th17 were also identified – chemokine receptors CCR6, CCR4 and CXCR3 [16]. In particular, it has been shown that peripheral blood memory T cells expressing IL-17A in response to *ex vivo* stimulation carry the CCR6 receptor on the membrane [5, 16] and are divided into two subpopulations with different cytokine profiles depending on the co-expression of CCR4 and CXCR3 molecules: CCR6/CCR4 co-expression on the membrane marks memory T cells that selectively produce IL-17A but not IFNγ, whereas $CCR6^+CXCR3^+$ T cells produce IL-17A and IFNγ or IFNγ alone [16]. Although the concept of IFNγ-producing Th17 cells was later corrected, the combination of the above-mentioned chemokine receptors ($CCR6^+CCR4^-CXCR3^+$) in combination with markers of memory T cells is still widely used to identify this T-helper subpopulation, especially in clinical studies that do not involve prolonged cultivation and evaluation of intracellular factor expression [7, 12, 13, 15].

Mechanisms of formation of T lymphocytes co-expressing Th17 and Th1 markers

Traditionally, Th17 cell differentiation is initiated in the presence of IL-6, transforming growth factor β (TGFβ), IL-1β and IL-23, sequentially activating transcription factors STAT3 and RORC (in mice, RORγt) [17]. The primary inducers of differentiation are IL-6/TGFβ or IL-6/IL-1β, and IL-23 is included in the process later, when the IL-23R receptor appears on the membrane of activated T lymphocytes: signaling through this receptor stimulates cell expansion and is necessary to maintain cell function, in particular, to synthesize IL-17 [18]. Classical Th17 express the key transcription factor RORC, carry specific markers such as the lectin-like killer cell receptor CD161 and the chemokine receptor CCR6 on the membrane, and are able to produce the characteristic cytokines IL-17A, IL-17F, and IL-22 [17, 19].

However, the Th17 population is unstable – differentiated Th17 lymphocytes are capable of transforming into cells of a different phenotype in the local cytokine milieu upon restimulation, and the Th17 shift towards Th1 is most easily realized. An effective inducer of this shift is expectedly IL-12, a major cytokine in Th1 differentiation. Elevated levels of IL-12 induce the development of a subpopulation of Th1-like Th17 cells, in which the expression of Th1-associated transcription factors T-bet/STAT4 and the chemokine receptor CXCR3 is initiated, as well as the synthesis of the key Th1 cytokine IFNγ [3, 10]. As a consequence, the newly formed subpopulation has phenotypic features common to Th17 and Th1 lineages ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^+IFN\gamma^+$ T cells) [6, 10]. It is variously identified and labeled in the current literature – in this paper we will use the most common name Th17.1 [12–14]. A part of Th17.1 cells ($IL\text{-}17^+IFN\gamma^+$Th17 cells) may completely lose IL-17 production and differentiate into so-called "ex-Th17" cells, which produce only IFNγ, but retain the expression of Th17-associated transcription factor RORC, membrane molecules CD161 and CCR6 ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$T cells) [5, 10, 20, 21], as well as the ability to effectively respond to IL-23 [10]. In addition, some authors have recorded additional transitional forms, for example, with different variants of expression of membrane molecules CD161 and CCR6: $CD4^+CD161^+CCR6^-CXCR3^+IL\text{-}17^-IFN\gamma^+$ T lymphocytes or $CD4^+CD161^-CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$ T cells [22]. Normally, the content of Th1-like Th17 in the peripheral blood of healthy donors is extremely low, but these cells account for the bulk of $CD4^+IL\text{-}17^+$ T lymphocytes in the site of inflammation, including autoimmune one [10], so another informal name for them is "pathogenic Th17", as opposed to classical Th17, which are not pathogenic in various models of autoimmunity and produce substantial amounts of the anti-inflammatory cytokine IL-10 [23].

It should be noted that IL-12 is not the only inducer of Th17 redifferentiation into Th1: it has been shown that IL-23, a typical for the Th17 lineage cytokine, can also induce the appearance of cells with a Th1-like phenotype upon restimulation [23–25]; therefore, it is currently attributed not only to Th17 expansion, but also to their pathogenicity [3, 23]. Moreover, the high level of IL-23R expression by memory T cells is restricted to non-classical Th17 cells co-expressing IFNγ and CXCR3 [12].

An important factor regulating the Th17-Th1 transition is TGFβ, a cytokine that, in combination with IL-6, is consi-

dered a classic stimulator of the primary differentiation of naive CD4$^+$T lymphocytes into Th17. Although TGFβ has now been shown to be dispensable for primary stimulation, it plays an important role in the fate of Th17 by inhibiting the expression of the Th1-associated transcription factor T-bet [10, 23]. This stabilizes the phenotype of classical Th17 and prevents the formation of pathogenic Th1-like variants of this population [10].

Another important point is related to the origin of T lymphocytes co-expressing Th17 and Th1 markers – all the above considerations assume that these cells are formed from mature differentiated Th17, and this has indeed been demonstrated in many studies [3, 10, 26], although at least two other mechanisms of their origin are theoretically possible: first, they may develop directly from naive CD4$^+$T lymphocytes during primary differentiation; second, they may transform from classical Th1. There are no data on the first option yet, but its probability is low, since one of the main inducers of Th1-like Th17 development – IL-23 – has no receptor on naive T lymphocytes [24], and the second inducer – IL-12 – will initiate the development of classical Th1 in this case. Regarding the origin of IFNγ-producing Th17 from Th1, this option has been evaluated in many studies and has not been confirmed to date. In contrast, it has been shown that fractionated Th1 is unable to transform into Th17 or Th17.1 in response to the Th17-polarizing cytokines IL-1β, IL-23, IL-6, and IL-21 *in vitro* [10], and the clonal structure of T cell receptors (TCR) in the Th17.1 population is closer to that of Th17 than Th1 [10]. Therefore, polarized Th17 cells are considered as a major source of IFNγ-producing Th17 cells. The reversibility of Th17 redifferentiation into Th1 is controversial: some authors report the impossibility of "non-classical Th1" (ex-Th17) returning to Th17 due to epigenetic mechanisms [27], while others have shown *ex vivo* transdifferentiation of "non-classical Th1" into Th17 and Th1/Th17 under Th17-polarizing conditions [22].

Causes of Th17 cell plasticity toward Th1 cells

As for the plasticity of the Th17 population, it is important to note that regardless of the direction of redifferentiation, it is generally less stable than the main T-helper populations, Th1 and Th2: unlike the latter, Th17 cells do not form a positive feedback loop during differentiation, the so-called autoactivation, in which the produced cytokine (IFNγ for Th1 or IL-4 for Th2), acting as an auto- or paracrine factor, binds to receptors on the cell membrane and enhances its own production, which stabilizes the corresponding phenotype. In addition, the second important component of the phenotype stabilization process – alternative T-helper lineage differentiation suppression – does not work in Th17 cells, whereas in Th1 and Th2 cells this mechanism is well established, including in relation to Th17. Thus, the Th1-associated regulator T-bet binds to the transcription factor RUNX1 and blocks its interaction with RORγt, eventually suppressing Th17 differentiation [28]. The major Th2 response inducer GATA3 [29], the cytokines IL-2 (via STAT5) [30], IFNγ (via STAT1) and IL-12 (via STAT4) also have inhibitory effects against RORγt. Moreover, unlike the key regulators of Th1/Th2 lineages, T-bet and GATA3, RORγt regulates the transcription of a significantly smaller number of loci in Th17 cells [31], which, in the absence of reliable mechanisms to stabilize its expression, does not allow us to consider this factor as a full-fledged "master regulator" capable of providing an effective program of Th17 differentiation, which determines the high instability of the population.

Regarding the preferential plasticity of Th17 towards Th1, several reasons are noted. One is that a key cytokine in Th17 cell differentiation, IL-23, shares a common subunit with IL-12, a major Th1 inducer, and the IL-23R receptor also shares one of the two subunits with IL-12R [32]. As a consequence, the signals initiated in Th17 upon IL-23 binding to the corresponding receptor activate not only STAT3 (a key transcription factor in Th17 differentiation) but also STAT4 (a differentiation factor for Th1), albeit to a significantly lesser extent [33]. That is, the stabilizing IL-23-dependent signal for the Th17 lineage, implemented through STAT3, simultaneously promotes IFNγ co-expression and a shift towards the Th1 phenotype through STAT4 activation [34]. At the molecular level, Th17-Th1 plasticity is associated with permissive epigenetic modifications in Th1-associated loci of Th17 cells [35], in particular in the *IFNG* locus: while in Th2 cells the *IFNG* locus has no noticeable traces of remodeling, in Th17 cells the chromatin structure at this locus bears high similarity to that in Th1 cells [20]. In the work of A. Mazzoni et al. the transformation of *in vitro* generated Th17 into "non-classical" Th1 (ex-Th17) was accompanied by DNA demethylation at *TBX21* (encodes T-bet) and *IFNG* loci with simultaneous DNA methylation at *IL-17A/RORC2* [36], although in an earlier study using memory Th17 clones under similar conditions no epigenetic modifications were detected at the *IFNG* locus [37]. Whether epigenetic priming of Th1-associated loci of Th17 cells is associated with the above-described cross-activation of the transcription factor STAT4 in response to IL-23/IL23R-dependent signaling remains an open question.

MARKERS OF Th1-LIKE Th17 CELLS

To date, two major variants have been identified in the Th1-polarized Th17 cell population. The first is Th17-lymphocytes, producing along with classical IL-17 the major Th1 cytokine IFNγ, as well as co-expressing the transcription factor T-bet, the chemokine receptor CXCR3 and a number of other Th1-associated molecules [3, 10]. This subpopulation combines phenotypic and functional characteristics of both Th17 and Th1 lineages and is most generally represented in the literature as CD4$^+$CD161$^+$CCR6$^+$CXCR3$^+$IL-17$^+$IFNγ$^+$T cells [6, 10]. In some cases, when these cells are identified using membrane molecules only, without assessment of cytokine synthesis, two more chemokine receptors, CCR4 and CCR10, are added to the line of markers to separate the populations of classical Th17 and Th22, which, when identified using these membrane markers, have the same phenotype (CCR6$^+$CXCR3$^{-/low}$), but can be dif-

ferentiated by the CCR4/CCR10 combination: CCR4 is represented in both populations, whereas CCR10 is highly expressed on Th22 cells but absent in Th17 cells [12]. As a result, the interested subpopulation of Th17 cells co-producing IL-17/IFNγ will have a $CD4^+CD161^+CCR6^+CCR4^{-/low}$ $CCR10^-CXCR3^+$ phenotype, in contrast to classical Th17 with a $CD4^+CD161^+CCR6^+CCR4^+$ $CCR10^-CXCR3^-$ phenotype [12]. In addition, in *ex vivo* studies, Th17.1 and ex-Th17 cells are typically isolated from pre-fractionated memory T cells, either central ($CCR7^+CD45RA^-$) or effector ($CCR7^-$ $CD45RA^-/CD45R0^+$). Obviously, 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, but in most studies, especially clinical ones, this subpopulation is identified by key cytokines (IL-17/IFNγ), chemokine receptors (CCR6/CXCR3) or a combination of both. These cells do not have a single name, they have many designations in the literature (to be discussed below), in this paper we use the most common one – Th17.1 [12–14].

The second variant of Th1-polarized Th17 cells is actually the result of further redifferentiation of Th17.1 towards Th1, in which the cells lose IL-17 synthesis, producing only IFNγ, but retain the other "attributes" of the initial population – expression of Th17-associated transcription factor RORC and membrane molecules CD161/CCR6 ($CD4^+CD161^+CCR6^+CXCR3^+IL\text{-}17^-IFN\gamma^+$T cells) [2, 5, 10, 20]. These are the so-called "ex" Th17 (ex-Th17), aka "non-classical Th1" [6] or Th1* [15]. It should be noted that the ex-Th17 subpopulation cannot be identified only by cytokine production or only by the expression of membrane markers: in the first case it overlaps with classical Th1 and in the second case with Th17.1 cells. This is why they are often mistakenly "underestimated" or "overestimated".

However, identification problems apply not only to ex-Th17 cells but also to the Th1-polarized Th17 population as a whole. As noted above, the nomenclature of such cells is not uniform; different studies have labeled these cells as Th17/Th1, Th1/Th17, Th1/17, Th17/1, Th17-1, Th17.1, and so on. Although it is clear from the name that these are products of Th17 to Th1 transformation, careful analysis of such papers shows that they often refer to different subpopulations.

Let's look at a few examples. The work of R. Ramesh et al. [12] is one of the few in which a subpopulation of Th17-lymphocytes co-producing IL-17/IFNγ has been identified as accurately as possible to date: in it, memory T cells – central ($CD4^+CCR7^+CD45RO^+$) and effector ($CD4^+CCR7^-CD45RO^+$) – were sequentially isolated from human peripheral blood cells, which were further fractionated based on the expression of CCR6/CCR4/CXCR3 chemokine receptors and IL-17/IFNγ intracellular cytokines ($CD4^+CCR6^+CCR4^{lo}CXCR3^{hi}IL\text{-}17^+IFN\gamma^+$T lymphocytes). The subpopulation was first designated in the paper as Th17.1, which is the most common variant of its name to date.

However, other works using the same designation, Th17.1 [13, 14] do not refer to the same population – or rather, not to it alone. So, in a study by J. Ramstein et al. [13], Th17.1 cells were identified in peripheral blood and bronchoalveolar lavage of sarcoidosis patients by co-expression of CCR6/CXCR3 ($CD4^+CCR6^+CCR4^-$ $CXCR3^+$T lymphocytes). Similarly, the Th17.1 subpopulation in blood memory cells of healthy donors and rheumatoid arthritis patients ($CD4^+CD45R0^+CCR6^+CCR4^-CXCR3^+$T lymphocytes) was determined by co-expression of CCR6/CXCR3 in the study of W. Dankers et al. [14]. Clearly, when only CCR6/CXCR3 chemokine receptors are used as markers, a general Th1-like Th17 population including Th17.1 and ex-Th17 is identified. The subpopulation denoted in the literature as Th1/Th17 [7, 8], was also identified by co-expression of CCR6/CXCR3 chemokine receptors – in circulating $CD70^+$ T lymphocytes of multiple sclerosis patients ($CD4^+CD70^+CCR6^+CXCR3^+$T lymphocytes) [8] and in central memory cells from peripheral blood of such patients CCR6/CXCR3 ($CD4^+CCR7^+CD45RA^-$ $CCR6^+CXCR3^+$T lymphocytes) [7]. There were no cytokines determined here, so we are talking about a general Th1-like Th17 population including both Th17.1 and ex-Th17. This also applies to the subpopulation of circulating Th1* cells in the study by N. Nishihara et al. ($CD4^+CD45RO^+CXCR3^+CCR4^-CCR6^+$T lymphocytes) [15] or to Th1/17 cells from the blood of healthy donors in the study by T. Duhen et al. ($CD4^+CD45RO^+CD25^-$ $CD127^+CCR6^+CXCR3^+$T lymphocytes) [9]. Remarkably, in a number of studies described above, subsequent evaluation of cytokines in T lymphocytes co-expressing CCR6/CXCR3 showed that only a small fraction of these cells co-produce IL-17/IFNγ, whereas the major part (in bronchoalveolar lavage – up to 60 %) – IFNγ alone [9, 13].

On the other hand, there are many studies in the literature, in which Th1-like Th17 cells are identified by co-expression of IL-17/IFNγ cytokines, such as the Th17-1 subpopulation identified in the blood of healthy donors ($CD4^+IL\text{-}17^+IFN\gamma^+$T-lymphocytes) [11], Th17/Th1 cells from the blood and intestinal mucosa of patients with Crohn's disease ($CD4^+IL\text{-}17^+IFN\gamma^+$T lymphocytes) [5], and Th17/Th1 cells identified in the blood and synovial fluid of patients with juvenile idiopathic arthritis ($CD4^+IL\text{-}17^+IFN\gamma^+$T lymphocytes) [10]. All these papers obviously refer to a subpopulation, which is named Th17.1 in our review. In a study by L. Maggi et al. [6], the Th17-specific membrane molecule CD161 was used as markers along with IL-17/IFNγ cytokines to identify Th17/Th1 cells in blood: its use allowed to identify not only the subpopulation of IL-17/IFNγ-co-producing Th17, i. e. Th17.1 ($CD4^+CD161^+IL\text{-}17^+IFN\gamma^+$T-lymphocytes), but also the second major subpopulation of Th1-like Th17 – ex-Th17, or "non-classical" Th1 ($CD4^+CD161^+IL\text{-}17^-$ $IFN\gamma^+$ cells), differentiating it with CD161 from classical Th1 ($CD4^+CD161^-IL\text{-}17^-IFN\gamma^+$T cells) [6].

To summarize: the use of membrane markers CCR6/CXCR3 allows the identification of both major subpopulations of Th1-like Th17, Th17.1 and ex-Th17, but does not differentiate them from each other. Evaluation of IL-17/IFNγ cytokine co-expression only detects Th17.1 but not ex-Th17. Only a combined approach using membrane molecules as markers along with cytokines makes it possible to separate these subpopulations.

Transcription factors deserve special attention as potential markers in such studies: although the evaluation of T-bet and RORC expression is a standard in defining classical Th1 and Th17 populations, their use to identify subpopulations of Th17.1 and ex-Th17 seem to be inefficient: few studies show that if RORC expression in Th17.1 and ex-Th17 subpopulations is similar and comparable to that in classical Th17, T-bet has low expression in Th17.1, although its level in ex-Th17 is comparable to that in classical Th1 [13].

CONCLUSION

Despite the fact that Th1-polarized Th17 are also detected in healthy donors, the interest in this population is primarily due to the presence (and significant prevalence) of these cells in foci of inflammation in multiple sclerosis [7, 8, 24], sarcoidosis [13], rheumatoid arthritis [10, 14], and inflammatory bowel disease [5, 38], and their contribution to the development of these diseases has been proven. Moreover, the process of Th17 redifferentiation into Th1 is currently considered as a promising target for therapy. In this regard, the issue of method unification for isolation of these cells is very relevant, especially considering that the population of Th1-like Th17 cells is heterogeneous and includes at least two variants – Th17.1 and ex-Th17 – apparently reflecting different stages of Th17 transformation. The role of each subpopulation in pathogenesis and their unique properties are still poorly understood: there are only a few studies in which Th17.1 and ex-Th17 cells have been isolated and evaluated individually. Most researchers use one of two methods to identify Th1-like Th17 cells – by co-expression of CCR6/CXCR3 chemokine receptors or IL-17/IFNγ cytokines – and obtain results that are not always comparable with each other. The reason for such contradictions seems to be that the ex-Th17 subpopulation, overlooked in the determination of IL-17/IFNγ-co-producing cells, varies greatly depending on localization and milieu: while in peripheral blood its size is small (~5 %), as in short-term culture during Th17-to-Th1 transformation *in vitro*, in sites of inflammation the share of ex-Th17 reaches 60 % [9, 13]. In this regard, the use of CCR6/CXCR3 chemokine receptors as markers provides a more accurate representation of the size of the Th1-like Th17 population than the assessment of cytokine synthesis, but the preferred strategy for the identification of these cells seems to be the simultaneous assessment of the cell expression of IL-17/IFNγ cytokines and Th17-associated membrane markers (CCR6 and/or CD161): it allows not only to identify both subpopulations, Th17.1 and ex-Th17, but also to separate them and to differentiate ex-Th17 from the classical Th1 population.

Financing

The study was financially supported by the Russian Science Foundation and the Perm Territory under research project No. 22-25-20121.

Conflict of interest

There are no conflicts of interest, financial or otherwise.

Compliance with Ethical Standards

This article does not describe studies performed by the authors involving humans or using animals as subjects.

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