УДК 612.112.94: 57.016.4: 612.017.1
https://doi.org: 10.20538/1682-0363-2020-3-144-155

Multiple subsets of regulatory T-cells

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ABSTRACT

Regulatory T-lymphocytes play a central role in the immunological tolerance system. To date, existence of many different subpopulations of regulatory T-cells have been described. However, a number of questions related to the function, differentiation, and homeostasis of these subpopulations in a body remain unclear. Interactions between the previously discovered pairs of helper and regulatory T-lymphocytes require further study. The main topic is identification and establishment of the functions of regulatory memory cells. Interstitial migration of activated regulatory T-lymphocytes is also a promising direction. In this review, we summarized the main findings in multiple subsets of regulatory T-lymphocytes, discussed unclear data that will require further studies, and showed an application for regulatory T-lymphocytes in medicine.

Key words: regulatory T-lymphocytes, immunological tolerance, memory Treg, effector Treg, central Treg, tissue-specific Treg.

Conflict of interest. The authors declare no obvious and potential conflicts of interest related to the publication of this article.

Source of financing. The authors claim that there is no funding for the study.

For citation: Kupriyanov S.V., Sinitsky A.I., Dolgushin I.I. Multiple subsets of regulatory T-cells. *Bulletin of Siberian Medicine.* 2020; 19 (3): 144–155. https://doi.org: 10.20538/1682-0363-2020-3-144-155.

Разнообразие субпопуляций регуляторных Т-клеток

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РЕЗЮМЕ

Регуляторные Т-лимфоциты являются центральными клетками системы иммунологической толерантности. В настоящее время описано существование множества различных субпопуляций регуляторных Т-клеток (T-reg), однако большое количество вопросов, касающихся функционального назначения, путей дифференцировки и гомеостаза этих субпопуляций в организме, остаются неизученными. Продемонстрированные ранее пары хелперов и соответствующих им регуляторных Т-лимфоцитов требуют дальнейшего изучения их взаимодействий друг с другом. Актуальной темой является идентификация и установление функций клеток регуляторной памяти. Тканевая миграция активированных регуляторных Т-лимфоцитов также является перспективным направлением. В этом обзоре собраны и систематизированы данные о различных субпопуляциях регуляторных Т-лимфоцитов, выделены актуальные вопросы данной

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Ключевые слова: регуляторные Т-лимфоциты, иммунологическая толерантность, Treg памяти, эффекторные Treg, тканеспецифичные Treg.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования.

Для цитирования: Куприянов С.В., Синицкий А.И., Долгушин И.И. Разнообразие субпопуляций регуляторных Т-клеток. *Бюллетень сибирской медицины.* 2020; 19 (3): 144–155. https://doi.org: 10.20538/1682-0363-2020-3-144-155.

INTRODUCTION

The immune system is a complex and diverse structure with the task to maintain homeostasis. The main roles of the immune system are eliminating infectious agents, killing tumor cells, and immunoregulation. The system of immunological tolerance, which protects the body's own tissues from being attacked by immune cells, includes many different cells, such as tolerogenic antigen-presenting cells [1], regulatory T-cells, and B-cells [2].

Previously, it was believed that the population of regulatory T-cells (Tregs) was homogeneous, but over time the accumulated data contradicted this idea. Currently, the existence of various Treg subpopulations is accepted [3]. However, despite numerous studies, this area of immunology remains underdeveloped. There is little information on the differentiation of Tregs, the formation of their various subpopulations, and their interactions with other cells. The main mechanisms of tolerogenic action have been described (contact suppression due to suppressive molecules such as CTLA-4 [4] and PD-1 [5]; secretion of anti-inflammatory cytokines such as TGF-b [2], IL-10 and IL-35 [6]; sequestration of growth factors such as IL-2, necessary for activation of effector cells [7]; and metabolic activity, for example, the conversion of ATP to adenosine, which limits the pro-inflammatory effect of immune cells [8]). However, it is unclear how this happens *in vivo* and in what situations these mechanisms are implemented.

Unresolved issues in this area need to be studied, since their practical application can make a great contribution to solving many clinical problems. Currently, the treatment of autoimmune pathology is imperfect, and in some cases it cannot lead to compensation in patients. It is also associated with severe side effects, like infection and the risk of developing cancer [9]. Establishing the role of individual Treg subpopulations in the control of autoimmune processes can provide important information for new targets of therapeutic intervention and the creation of new effective and safe treatments for autoimmune diseases.

MULTIPLE SUBSETS OF REGULATORY T-CELLS

Treg cells can be classified as naive and activated, the latter of which have passed antigen recognition and proliferation processes in peripheral lymphoid organs. Naive Tregs can be designated as cells derived from the thymus (tTreg) [10] and these cells did not undergo TCR activation. They can be recognized by their expression of the CD45RA isoform, whereas previously activated cells have the CD45R0 isoform [11]. Treg cells that differentiate from Th0 (naive T-lymphocytes, but not Tregs) are referred to as peripheral (pTreg) in the literature, since they differentiate in peripheral lymphoid organs after TCR activation; these cells are also activated Treg cells [10].

The separation of tTreg and pTreg was based on the expression of the transcription factor Helios [12], which is expressed by Tregs of thymic origin. It has been shown that the level of Helios in tTreg was increased with activation; this makes possible to identify both naive (CD45RA+) and activated cells (CD45R0+) in the group of regulatory T-cells of thymic origin [12]. Naive thymic cells express CCR7 and CD62L, which allows them to migrate to the lymph nodes. Therefore, this population has been designated central Tregs (cTreg) in the literature [3]. These cells contain a large amount of CD25 (high affinity IL-2 receptor

alpha chains); this may allow them to deprive the surrounding T-cells of IL-2, limiting their proliferation [3]. Activated cells (CD45R0+) have other functional characteristics like the expression of suppressive molecules such as IL-10, CTLA-4, ICOS, TGIT, CD39 [13-15] and chemokine receptors which mediate their migration into various tissues [3]. Besides, in vitro induced Tregs (iTreg) [10], which are obtained by applying cultivation of T-lymphocytes outside the body, currently constitute a separate group; these cells may differ significantly from Treg cells in vivo. Among activated Tregs, differences were found between activated Treg cells derived from tTreg and pTreg cells. It was demonstrated that activated Helios+ Tregs (tTreg) and Helios$^-$ Tregs (pTreg) can differ in the cytokine profile [16] (described in more detail below in the section "Th-Treg pairs").

Based on tropism, Treg cells can be divided into tropic to lymphoid formations (peripheral lymph nodes) and tropic to non-lymphoid tissues [3]. Currently, there is data that allows determination of resident tissue-specific Tregs (section "Tissue-specific Tregs"). These cells are tropic to the microenvironment of certain tissues; however, they are not circulating (migrating) or recirculating cells. Therefore, Treg population may be further subdivided into circulating (recirculating) and resident cells.

It is difficult to identify which cells are recirculated (i.e. exit from one tissue and move through the bloodstream to the other tissue). It is also impossible to confidently determine which resident cells cannot leave tissues and recirculate under any circumstances. Treg cells studied in peripheral blood (i.e., circulating populations) also cannot be precisely defined as migrating in one direction or recirculating from one tissue to another.

Likely, these processes are quite dynamic and cells with the same origin and functional status can become both tissue-resident and recirculating populations depending on the context. Nevertheless, study in this area will provide much more understanding to the functioning of the tolerogenic system, since circulating and resident cells have different properties (described in more detail in the following sections). Cell tropism can be determined by the presence of appropriate tissue-specific chemokine receptors. The central (tropic to lymphoid formations) populations include naive thymic Tregs. However, there has recently appeared data to expand this group. According to the study by Wei X. et al. [6], there are two subsets of activated Treg in mice: IL-10+Bcl-6+ Tregs and IL-35+Ebi-35+ Tregs. IL-35+ Tregs demonstrate tropism for secondary lymphoid organs (these cells are localized in the peripheral lymph nodes / white pulp of the spleen and express CCR7 and CD62L), which identifies this subpopulation as central. According to the authors' assumptions, this subpopulation differentiates from thymic Tregs [6], which remain tropic to lymphoid organs after activation. There are some similarities between the functions of thymic naive Tregs and IL-35+ Tregs. These two subpopulations are located in the lymph nodes and are able to suppress initiation of the immune response. IL-35 promotes the differentiation of pTregs from naive T-lymphocytes [17]. Moreover, it was shown that differentiated cells are able to synthesize IL-35 themselves [17], which suggests that maintaining the constancy of IL-35+ Tregs can also occur due to the conversion of naive cells into IL-35 producing ones.

Activated Treg cells are referred to as effector Tregs in the literature [3]. These cells are a CD45RO+CD45RA-FOXP3^{high} subset and tropic to non-lymphoid tissues. IL-35+ Tregs are an exception to this condition. In addition, there is currently strong evidence for the existence of memory Tregs (Memory Tregs section), which also applies to activated cells. Activated non-regulatory T-lymphocytes are currently divided into various groups which include central memory T-cells, effector memory T-cells, and terminally differentiated T-cells (TEMRA) [18].

Based on these data, it is rational to distinguish between the group of effector Treg cells and Treg memory cells based on a number of features (for more details, see the Memory Tregs section). In addition, activated cells can be divided into tissue-specific cells and specific to subpopulations of helper T-lymphocytes. Thus, activated Tregs can be divided into tissue-specific, helper-specific Tregs and such separate groups as the effector Treg cells and Treg memory cells can also be identified.

TISSUE-SPECIFIC TREGS

The Treg tissue group is a subset of regulatory cells that suppress local tissue inflammation and provide homeostasis in peripheral tissues. The functions of these cells differ depending on the type of tissue. Thus, Treg cells of muscle tissue affect the repair of muscle fibers, accumulating in the tissue upon damage [19]. In adipose tissue, Tregs suppress

local inflammation, which is manifested by impaired glucose tolerance [20]. Intestinal Tregs regulate tolerance to antigens of food and commensal microflora of the gastrointestinal tract [21]. Therefore, different groups of tissue Treg cells perform various functions of maintaining homeostasis in the tissue, and not only monitor the activity of immune cells.

It should be noted that in some tissues, such as muscles or the central nervous system, the presence of resident Treg cells is limited; their accumulation is observed only when damage occurs [3]. However, for such tissues, Treg cells can play a significant role in regional lymph nodes, as they rapidly divide and migrate into the tissue if it is damaged. It can be assumed that resident Tregs exist for the lymph nodes of such tissues. It was shown that part of the memory T-cells is present in the peripheral lymph nodes [22].

TH-TREG PAIRS (HELPER-SPECIFIC TREGS)

There are subgroups of Tregs specific to certain groups of helper T-lymphocytes [16]. These cells specialize in suppressing a specific population of Th cells. The existence of Tregs specific for Th1, Th2, Th17 [16] and Tfh [23] was identified. Helper-specific Tregs are characterized by a specific set of chemokines and transcription factors. For example, Treg cells that suppress Th1: these cells express T-bet transcription factor (which is also expressed in Th1) and are dependent on cytokines associated with Th1: gamma interferon (IFNγ) and IL-27 [3, 16]. For the development of Th2-specific Treg cells, expression of the transcription factor of Th2 GATA3 cells is necessary [24]. Meanwhile, in order to suppress responses of the lymph node germinal center, which are provided by Tfh, Treg cells called T-follicular regulators (Tfr) are needed [23].

This cell population expresses Blimp-1, in contrast to Tfh cells, whose development is inhibited by Blimp-1 [25]. Further study of helper-specific subpopulations of Treg cells and their transcriptional program is necessary. It should also be noted that these cells have functional features similar to helper cells; they produce suppressive cytokines together with pro-inflammatory cytokines which are characteristic of their effector analogues (IL-17 for Th17, IL-4 for Th2, IFNγ for Th1) [16]. This circumstance can potentially disrupt the action of tolerogenic mechanisms of helper-specific regulatory T-cells due to the pro-inflammatory effects of these cytokines. Transition of Tregs to Th17 under the influence of various stimuli has been shown [26], which may indicate functional instability of these cells. An increase in such subpopulations has also been shown in autoimmune pathologies such as multiple sclerosis [27] and type 1 diabetes mellitus [28]. However, as it has been rightly noted in the work by T. Duhen et al. [16], the production of pro-inflammatory cytokines in Tregs may differ significantly from their effector analogues.

Thus, IL-22 was often co-produced with IL-17 in Th17 cells, which was not observed in the Th17-like Treg population [16]. All of these cytokines are produced together with IL-10 in Th17-like Tregs. It was also found that IFNγ and IL-17, under certain conditions, can have an immunoregulatory effect [29, 30]. It can be assumed that such Treg subpopulations are characterized by certain programs for establishing tolerance due to a combination of pro- and anti-inflammatory cytokines; these mechanisms need further study. In this regard, it is possible that an increase in the number of such Tregs in multiple sclerosis may be a compensatory body reaction to autoimmune inflammation [16].

It should be noted that, at the moment, it is impossible to accurately determine that the helper-specific Tregs existing in the norm and similar cells that increase in amount during autoimmune pathologies are identical. Helper-specific Tregs are heterogeneous in origin. Being activated (CD45R0+) cells, most of these Tregs expressed Helios [16], which may indicate their origin from the group of thymic Tregs. However, among the $CD25^{hi}$ $CD127^{lo}$ Th1- and Th17-like Tregs, a group of $Helios^{-}$ cells, probably pTregs, were also found [16]. These cells produced IL-10 in larger quantities than Helios+ cells [16]. Thus, helper-specific Treg cells of different origin could increase in amount during autoimmune diseases. It is possible that the expression of Helios factor is suppressed, and this is interconnected with a change in the cell functioning. A deeper analysis and comparison of different groups of helper-specific Tregs in normal and pathological conditions will help to better explain the role of these cells in the immune system.

MEMORY TREGS

Currently, cells of regulatory memory, memory Tregs, are being determined [22]. These cells can remain viable in the absence of stimulation by the

antigen (autoantigen) for a long time, and also effectively suppress the immune response when activated. Memory Tregs are characterized by an increased ability to suppress effector cells [22]. The existence of memory Treg cells was quite controversial because it is difficult to prove that memory Tregs are preserved in the absence of constant stimulation by the antigen, provided that normally autoantigens are constantly presented in the body [22]. The biological meaning of the existence of such cells can consist of the following points:

With age, there is a decrease in the thymus function and the production of naive T-lymphocytes [2], which suggests the existence of long-lived memory cells that support the lymphoid population in the absence of a constantly updated pool of naive T-lymphocytes.

In the absence of a pathological process, the presentation of autoantigens is performed by immature dendritic cells or specialized tolerogenic cells that contain a small number of costimulatory molecules and do not produce pro-inflammatory cytokines in sufficient quantities [1]. Such antigen presentation leads to anergy, apoptosis, or the formation of a regulatory phenotype in T-lymphocytes. When autoantigens with costimulatory molecules and pro-inflammatory cytokines are presented, activation of autoreactive T-cells occurs, which can lead to autoimmunity [2].

In this situation, the existence of Treg memory cells, which would be activated in parallel with autoreactive cells, would contribute to autoimmunity control. Thus, it is possible that the autoantigen presentation under normal conditions is not sufficient to activate memory Tregs, and these cells can only be activated under pro-inflammatory conditions when the autoantigen presentation can lead to the expansion of autoreactive cells.

Access of immune cells to tissues separated from the immune system by histological barriers (immunoprivileged tissues) is limited [2]. In case of damage to the barriers due to trauma or inflammation, autoantigens from these tissues become available for recognition by the immune system, and this situation can lead to the development of an autoimmune process [31]. To prevent this, Treg cells persisting in the absence of autoantigen presentation for a long time (for example, in regional lymph nodes) may exist; these cells, upon a repeated episode of damage, can migrate with effector cells into the tissue to prevent the autoimmune process.

Regulatory cells can be specific not only to autoantigens, but also to foreign antigens that are not expressed in the body. In a model of acute influenza infection, the number of virus-specific Treg cells was shown to increase 50-fold during the initial response [32]. Subsequently, like in the case of effector T-cell populations, the number of virus-specific Treg cells decreased after resolution of the primary infection.

However, a fraction of these Treg cells persisted for more than 50 days after infection. Upon re-infection, the pool of such Tregs underwent a 10-fold expansion, which is similar to an increase in the population of effector memory T-cells. In addition, Treg memory cells significantly inhibited the clonal expansion of the effector T-cell population and cytokine production. They also reduced tissue damage without impairing clearance of the virus [32]. These results were reproduced by another group using a similar infection model [33]. The mechanisms that allow Tregs to improve elimination of pathogens are currently unknown, but these experiments demonstrate the need for the interaction of various parts of the immune system for an adequate immune response, as well as the existence of not only suppressive, but also a regulatory function of Tregs.

Not all antigens can be constantly expressed in tissues; expression of some molecules may be activated during inflammation [34]. Tregs specific to such markers can also be memory cells.

A number of studies have been carried out proving the existence of Treg memory cells [32, 33, 35]. An experimental model has been created to suppress or activate the expression of a specific antigen in the skin [35]. Meanwhile, the expression of this antigen in the thymus was not suppressed. Upon presentation of this antigen, a group of regulatory T-cells that suppressed the immune response to the antigen developed in the skin. When its expression was turned off, the existence of regulatory T-lymphocytes specific to this antigen was detected, which remained for a long time in the skin in the absence of antigen presentation and, when its expression was re-activated, suppressed inflammatory reactions more efficiently than primary Treg cells [35].

The determination of memory Tregs in humans is somewhat more complicated. Human T-cells express the CD45RO isoform in the thymus and turn into CD45RA+ after migration to the peripheral lymph nodes [36]. After recognition of the antigen

at the periphery, these cells switch back to the form of CD45RO. Almost all *in vitro* CD45RA+CD4+ T-cells lose their expression of CD45RA and switch to the CD45RO+ phenotype after 4 days of TCR stimulation [37].

At this point, human memory Tregs are designated as T-cells expressing the CD45RO marker, indicating a previous activation. However, CD45RO expression alone does not define a T-cell as a true memory cell [22]. This marker does not distinguish between cells that persist in the absence of antigens and cells that constantly recognize antigens. However, CD45 isoforms are now widely used to distinguish between naive Tregs and cells activated by antigen recognition (among which memory cells are also represented). In the study by M. Miyara et al., based on the expression of CD25, CD45RA, and FOXP3, the peripheral blood T cells of healthy people were divided into two subsets of CD45RA+FOXP3^{low} and CD45RA-FOXP3^{hi}, which were called "resting" and "activated" Tregs [11]. It was demonstrated that, after antigen stimulation, resting Tregs proliferate and differentiate into activated Tregs [11]. It was shown that the number of CD45RA+ Treg cells in peripheral blood decreases with age, which is accompanied by an increase in the population of CD45RO+ Treg [38].

These results confirm the hypothesis that CD45RA+ Treg cells are a resting population that turns into activated CD45RO+ (among which there may be memory Treg cells) under the influence of antigen activation [22]. In turn, CD45RO+ Tregs can be divided into subpopulations in accordance with the expression of HLA-DR [39]. These groups differ in functional characteristics: suppressive ability and cytokine secretion. HLA-DR+ Tregs expressed higher levels of activation markers (CTLA-4, ICOS) and had a more pronounced suppressive effect *in vitro* but produced lower levels of cytokines. Perhaps, this group is Treg memory cells due to their more differentiated phenotype [22].

HLA-DR- cells can be considered recently activated, but not fully differentiated Treg cells. However, it has been shown that HLA-DR is expressed on recently activated conventional T-cells in humans [40]. In this regard, it is possible that CD45RA-HLA-DR+ Tregs are newly activated "effector" Treg cells, and not Treg memory cells [22]. It is also worth noting that most memory Tregs may be located in peripheral tissues [41], and in blood they can appear only when moving between tissues or between tissues and lymph nodes.

It was shown that almost all Treg cells in adult skin express CD45RO, while a significant part of the regulatory skin cells of the fetus were attributed to a subpopulation of CD45RA [42]. Tregs in adult skin also express high levels of other markers associated with memory T-cells, including CD27 and BCL2 [42]. It is important to note that, in comparison with effector T-cells, memory Tregs from human skin expressed unique tissue-specific TCR sequences, did not express CCR7, and could not migrate from skin [42]. All of this data shows that differentiated Treg memory cells may be located in tissues and do not appear in peripheral blood.

At present, there is no unified approach to the determination of regulatory memory T-cells, however, many distinctive features such as expression of activation molecules (CD45RO, HLA-DR), chemokine profile, and metabolic profile can help in solving this problem [22]. It is also important to note that memory Tregs may use other homeostatic factors. It was shown that memory Tregs are less dependent on IL-2 (which is necessary for the survival of naive and activated cells), but more sensitive to IL-7 (memory Tregs in the skin showed increased expression of IL-7R, i.e. CD127, which is usually low or not expressed at all on Treg cells in peripheral blood) [43]. This fact demonstrates different biology of ordinary Tregs and memory Tregs and can serve as one of the markers of regulatory memory T-cells.

RESULTS INTEGRATION

Based on the study of IL-35+ and IL-10+ Tregs, two main directions can be formulated: preventive, i.e. maintaining homeostasis by reducing the activation of non-regulatory T-lymphocytes or exposure to other cells (for example, antigen-presenting), and suppressive, aimed at limiting the already existing focus of inflammation. These two actions were divided between lymphoid IL-35+ Tregs (preventive) and non-lymphoid IL-10+ Tregs (suppressive) [6]. Such a separation is justified since an immune response is initiated in the lymph nodes and an inflammatory reaction occurs in tissues. However, in this review, using the preventive and suppressive effect of regulatory T-cells as a basis, a model is proposed, not of the anatomical distribution of these effects, but of a functional one, i.e. by the predominant type of cells that have these effects (Fig. 1).

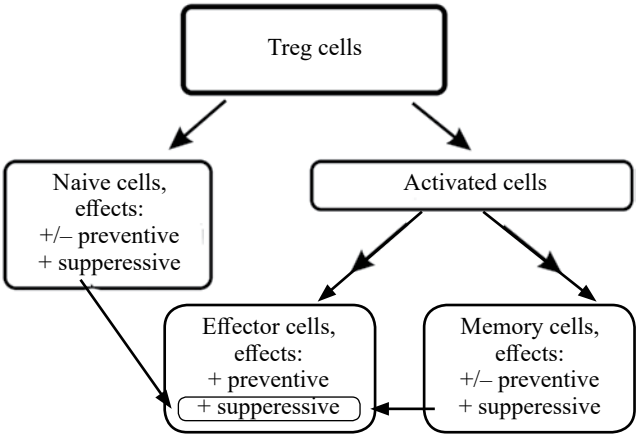

Fig. 1. Types of regulatory T-cells

By activation status, regulatory T-cells are divided into naive and activated. The activated cells include effector cells and memory cells. Each group has preventive and suppressive effects to various extents. Effector cells exhibit an active preventive effect or a suppressive effect depending on their number. Naive and memory cells have a passive preventive effect due to the competition for resources with other T-cells; upon activation, these populations divide and differentiate into effector cells and the suppressive effect is manifested due to their increased number.

Both lymphoid and non-lymphoid populations have both effects. Moreover, probably all populations (naive, effector, memory cells) have both effects, but they are realized to varying degrees. This is indirectly indicated by the results of the experiment [6], when one of the two populations (lymphoid or non-lymphoid) was removed, the development of autoimmune pathologies did not occur, which indicates a partial overlap in their function.

Naive Tregs have some preventive effect due to the competition with Th0 for IL-2 (a passive effect, since they do not synthesize anything) [7]. At the same time, these cells have a pronounced suppressive effect upon TCR activation due to proliferation and differentiation into Treg CD45R0 + effector cells [11]. Memory Treg cells may have similar properties like competing for IL-7 with other memory T-cells and providing proliferation and differentiation of effector Tregs upon re-activation. The effector Tregs, which constantly recognize antigens and are in an active functional state (produce suppressive cytokines and contact suppression molecules), have both preventive (active) and suppressive effects, which depends on the number of cells.

An increase in the number of effector Tregs during the proliferation of naive and memory cells translates the preventive effect into a suppressive one. It should be noted that due to the high expression of contact suppression molecules [22], memory cells may also have a preventive effect while maintaining a state of functional rest. However, this requires the presence of a sufficient number of these cells and this method can potentially lose the paracrine effects of suppressive cytokines to maintain a preventive effect. On the other hand, as a suppressive effect, this method may be more successful. As mentioned earlier, HLA-DR+ Tregs showed higher levels of contact suppression molecules CTLA-4 and had a more pronounced suppressive effect *in vitro*, but produced lower levels of cytokines [39].

The lymph nodes contain (at least in mice) naive Treg cells and activated IL-35+ Treg cells. IL-35+ cells exhibit effector features with functional activity (this is evidenced by gene expression profiles) [6]. Thus, lymphoid tissues contain groups of cells exhibiting both effects. Moreover, the existence of Treg memory cells in lymphoid tissue is not ruled out.

Various types of cells exhibiting both preventive and suppressive properties are also present in non-lymphoid tissues. Adipose tissue resident Treg cells are shown as functionally active cells that recognize local tissue antigens and, therefore, persist in adipose tissue and control homeostasis [20]. This description is suitable for effector regulatory T-cells that have a preventive effect. It was also demonstrated that with an increase in IL-33, which acts as an alarmin in tissue damage, these resident Tregs proliferate intensively, which demonstrates their suppressive effect [20]. It is not clear whether this population is homogeneous, and all of these effector cells have proliferative potential or there are resting memory cells among them.

Given the existence of several types of memory cells with different functional statuses in T-lymphocytes (Tcm, Tem) [18], such a division can also exist among Tregs. It may turn out that among adipose tissue resident Treg cells there are active effector cells with sufficient proliferative potential. However, an important feature of these resident cells is that adipose tissue Treg cells proliferate in response to exogenous administration of IL-33, while Treg cells in the lymph nodes did not show such proliferation [20]. This fact may mean that there are specific stim-

uli for each specific Treg group that induced their proliferation. Therefore, a suppressive effect can be observed under certain conditions. Treg IL-35 cells were practically not proliferated compared to IL-10 Tregs upon activation by monoclonal antibodies to CD3 [6]. This population may be effector with low proliferative potential or there may be a specific stimulus (stimuli) to which this population can respond by proliferation. This issue requires further study.

Despite the fact that in both lymphoid and non-lymphoid tissues there are cell populations responsible for preventive and suppressive effects, these tissues and Treg cells that they include should be considered as a single functional system for maintaining immunological tolerance. Some changes, for example, an increase in the level of pro-inflammatory IFNγ+ T-cells, were observed in the absence of IL-10+ Tregs alone [6], which indicates incomplete functional overlap between IL-10+ and IL-35+ Tregs. Autoimmune pathology did not occur in these mice, however, a background predisposing to this could be created (it has not been investigated). This can be considered as one of the links in the pathogenesis of autoimmune diseases.

The absence of such changes in mice with deletion of IL-35+ Tregs can be explained by the presence of additional functional reserves of lymphoid tissues in the form of naive populations of Tregs (also having both effects) or the effect of IL-10+ Tregs, which despite being directed to peripheral tissues (chemokine profile), were detected in the lymph nodes (perhaps, they were in the process of exiting the nodes) [6]. The chemokine profile of IL-35+ Tregs showed the orientation of these cells to lymphoid tissues, therefore, in the absence of IL-10+ Tregs, these cells could not migrate to peripheral tissues. This circumstance may explain the increase in the number of effector IFNγ+ T-cells. In the absence of lymphoid effector Tregs, non-lymphoid Tregs compensated for their function. The absence of non-lymphoid populations led to an increase in the level of pro-inflammatory cells (partial compensation), but not to the development of the disease. Spontaneous autoimmune colitis developed only in the absence of both populations [6], which indicates the existence of connections between tolerogenic systems of the lymph nodes and peripheral tissues.

To achieve immunological tolerance and control immunity, lymphoid (regional lymph nodes) and non-lymphoid tissues act in concert, partially compensating and complementing each other. The following scheme is proposed to describe the operation of this system (Fig. 2).

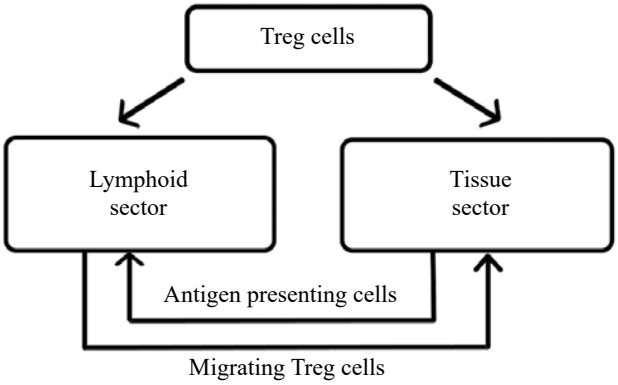

Figure 2. A unified system of immunological control

Lymphoid and tissue populations of regulatory T-cells act as a unified system of immunological control. Tissue sector affects lymphoid sector due to antigen-presenting cells. The lymphoid sector is a source of cells for suppressing tissue inflammation (the suppressive effect) and renewal of the tissue sector (transition of migrating populations to tissue resident ones after performing suppressive functions or outside of performing suppressive functions). Migrating cells compete with tissue resident cells; more adapted cells win the competition, which leads to dynamic immunoregulation under changing conditions.

An interaction takes place between the lymph nodes and peripheral tissues: antigen-presenting cells migrate from the tissues [2] and activated cells migrate from the lymph nodes to the tissues where they can become progenitors of tissue resident cells [22]. Adipose tissue resident Treg cells were capable of proliferation, i.e. self-renewal of the population [20].

This fact calls into question the need for migration of precursors from the lymph nodes. Nevertheless, in conditions of inflammation, due to the increase in the number of pro-inflammatory cells, the participation of Treg cells migrating into the tissue may be necessary to realize the suppressive effect. This may be especially important in relation to helper-specific populations that migrate to the places of accumulation of their helper analogues. Some of the migrating cells may remain after suppression of the immune response in tissues [22] to maintain homeostasis

(preventive effect). Due to the presence of competition for resources (cytokines and p-MHC complexes) in lymphocytes [44], these populations will compete with resident cells. As a result, dynamic regulation of the system of immunological tolerance can occur (more functional cells adapt to provide a preventive effect).

Thus, peripheral tissues affect the lymphatic population of Treg cells due to antigen-presenting cells stimulating both the preventive (presentation of antigen in small quantities to maintain the persistence of effector populations) and suppressive effects (presentation of antigen in significant quantities together with inflammatory signals to activate naive cells and memory cells). The lymph nodes provide a suppressive effect for tissues through the production of effector Tregs and regulate the preventive effect due to tissue resident progenitor cells that compete with local Treg cells for resources. As a result, cells more adapted to tissue stimuli will persist in the tissues, providing local homeostasis and immunological tolerance.

It is also important to emphasize the specificity of the preventive action. Its focus of reducing activation and preventing the development of the immune response is non-specific. However, the methods by which this effect is achieved may be different for each Treg group. This is indicated by the fact that activated Helois+ and Helios⁻ Tregs, being effector cells, differed in the amount of IL-10 produced [16]. Additionally, in the study by X. Wei et al. [6], the authors note that activated cells producing IL-10 act on this type of cytokine to affect many types of cells. This is due to the broad expression of IL-10R, while IL-35 acts mainly on T-cells. Thus, it can be assumed that due to differences in functional modes (cytokine spectrum), the preventive effect of a certain Treg group will be selective for different cell populations; it makes the regulation of immunological tolerance more adaptive to changing conditions.

APPLICATION PROSPECTS

The separation of different populations of regulatory T-lymphocytes entail many significant consequences. Different populations are able to act as diagnostic and prognostic markers. For example, insufficiency of specific populations may be a prognostic marker for the development of pathologies associated with this group of cells (insufficiency of Treg subpopulations that suppress Th2 may be a risk factor for the development of allergic diseases).

Therapy of autoimmune diseases is currently an urgent problem, but the currently existing methods based solely on suppressing the immune response are imperfect because they do not always allow disease control and at the same time have a large number of serious side effects [9]. Thus, it is necessary to create new methods for the treatment of autoimmune diseases based not on full immunosuppression, but on correction of the immune response. One of the varieties of new techniques is tolerogenic cell vaccines [45].

These methods are based on the introduction of autologous tolerogenic cells specific to a causally significant autoantigen (i.e., autoantigen to which the development of an autoimmune reaction is expected) [45]. In autoimmune diseases, there are impaired regulations of the immune response including impaired control of immunological tolerance [2]. The regulatory system, as shown above, includes the joint work of regulatory cells of lymphoid and non-lymphoid tissues. Accordingly, the impact on both levels of the immunological tolerance system should be more effective than on any one level. This approach should be true for the use of tolerogenic cell vaccines. The impact on both levels of immunological regulation, either using two types of tolerogenic populations or using agents that affect both types of populations, can significantly improve the methodology of tolerogenic cell vaccines.

To implement this strategy for the treatment of autoimmunity, further study of various populations and levels of immunological tolerance, as well as their disorders and shifts in autoimmune pathologies is necessary. The result of such studies may be the emergence of new immunotherapy techniques that can restore the system of immunological tolerance and are devoid of the disadvantages of immunosuppressive therapy.

CONCLUSION

Currently, new groups of regulatory T-cells, their influence on the processes of immunological tolerance, the immune response, and the role of these cells in pathological conditions are being investigated. However, at the moment there is no clear structuring of various subpopulations and their roles in the implementation of immunological control. In this review, attempts were made to theoretically system-

atize data on Treg subpopulations. Further study and systematization of various Treg groups may open up many new practical directions in the diagnosis and treatment of various diseases, especially autoimmune ones.

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