Treg CELLS IN ISCHEMIC STROKE: A SMALL KEY TO A GREAT ORCHESTRION

O.A. Zhukova[1], D.A. Chudakova[2], V.V. Belopasov[3], E.V. Shirshova[1], V.P. Baklaushev[1, 2, 4], G.M. Yusubalieva[1, 2, 4]

[1] Federal Scientific and Clinical Center for Specialized Medical Assistance and Medical Technologies of the Federal Medical Biological Agency, Moscow, Russian Federation

[2] Federal Center of Brain Research and Neurotechnologies, Moscow, Russian Federation

[3] Astrakhan State Medical University, Astrakhan, Russian Federation

[4] Pulmonology Scientific Research Institute under Federal Medical and Biological Agency of Russsian Federation, Moscow, Russian Federation

ABSTRACT

Ischemic stroke is a global medical problem and one of the leading causes of death or disability worldwide. The main approach of ischemic stroke therapy in the most acute period, which can prevent or minimize the development of a neurological deficit, is the restoration of the blood flow in the ischemic brain tissue using enzymatic thrombolysis or endovascular thromboextraction. When the therapeutic window is missed, the modulation of the acute inflammatory response may play an important role in determining the fate of neurons in the penumbra. The key players in this process are T-regulatory cells (Tregs) — an immunosuppressive population of CD4+ T-cells with the CD4+, CD25+ CD127^{low}, FoxP3+ phenotype. Despite the existing reports that Tregs (or certain Treg subpopulations) can exacerbate microcirculatory disorders in the ischemic tissue, many stadies convincingly suggest the positive role of Tregs in ischemic stroke. Resident CD69+ Tregs found in the normal mammalian brain have neuroprotective activity, produce IL-10 and other anti-inflammatory cytokines, control astrogliosis, and downregulate cytotoxic subpopulations of T cells and microglia. Systemic administration of Treg in stroke is accompained by a decrease in the volume of cerebral infarction and decreased levels of secondary neuronal death. Thus, the methods allowing Treg activation and expansion ex vivo open up several new avenues for the immunocorrection not only in systemic and autoimmune diseases, but, potentially, in the neuroprotective therapy for ischemic stroke. The relationship between Treg, inflammation, and cerebrovascular pathology is of particular interest in the case of ischemic stroke and COVID-19 as a comorbidity. It has been demonstrated that systemic inflammation caused by SARS-CoV-2 infection leads to a significant suppression of Treg, which is accompanied by an increased risk for the development of ischemic stroke and other neurological complications. Overall, the information summarized herein about the possible therapeutic potential of Treg in cerebrovascular pathology may be of practical interest not only for researchers, but also for clinicians.

Keywords: *Treg; regulatory T cells; ischemic stroke; COVID-19; biomarkers.*

For citation:

Zhukova OA, Chudakova DA, Belopasov VV, Shirshova EV, Baklaushev VP, Yusubalieva GM. Treg Cells in Ischemic Stroke: A Small Key to a Great Orchestrion. *Journal of Clinical Practice*. 2023;14(3):36–49. doi: https://doi.org/10.17816/clinpract568210

Submitted 10.08.2023 Revised 15.09.2023 Published 29.09.2023

INTRODUCTION

Statistical forecasts show an annual increase in the number of patients with ischemic stroke until 2030. Ischemic stroke is a leading cause of patient disability and the second most common cause of mortality worldwide, following ischemic heart disease [1, 2].

Currently, the main international and Russian therapeutic recommendations for treating patients in the first hours of an ischemic stroke focus on rapidly restoring blood flow and preventing ischemic brain tissue necrosis. These include neuroprotection, thrombolysis, mechanical thromboextraction, and anticoagulant therapy [3]. Difficulties arise when the therapeutic window to restore cerebral blood flow is missed. In this case, the therapeutic options are limited to protecting brain cells from oxidative stress [3] by preventing ferroptosis [4] and providing symptomatic therapy. Several biomolecules (TIM-3, DOR, PD-1, ROCK, ADAMTS-13, S1PR, etc.) that may play a role in stroke pathogenesis [1, 5–7] are being investigated as potential targets for stroke

journal of clinical practice 2023 Vol 14 №3

РЕГУЛЯТОРНЫЕ Т-КЛЕТКИ ПРИ ИШЕМИЧЕСКОМ ИНСУЛЬТЕ: МАЛЕНЬКИЙ КЛЮЧ ОТ БОЛЬШОЙ «МУЗЫКАЛЬНОЙ ШКАТУЛКИ»

О.А. Жукова[1], Д.А. Чудакова[2], В.В. Белопасов[3], Е.В. Ширшова[1], В.П. Баклаушев[1, 2, 4], Г.М. Юсубалиева[1, 2, 4]

[1] Федеральный научно-клинический центр специализированных видов медицинской помощи и медицинских технологий, Москва, Российская Федерация

[2] Федеральный центр мозга и нейротехнологий, Москва, Российская Федерация

[3] Астраханский государственный медицинский университет, Астрахань, Российская Федерация

[4] Научно-исследовательский институт пульмонологии, Москва, Российская Федерация

АННОТАЦИЯ

Ишемический инсульт — глобальная медицинская проблема и одна из основных причин смертности и инвалидности во всём мире. Основным направлением терапии ишемического инсульта в острейшем периоде, способным предотвратить или минимизировать развитие неврологического дефицита, является восстановление кровотока в ишемизированной ткани мозга с помощью ферментативного тромболизиса или эндоваскулярной тромбоэкстракции. В случаях, когда терапевтическое окно упущено, важное значение в судьбе ишемизированных нейронов в зоне пенумбры может иметь модуляция иммунного ответа с целью подавления системной воспалительной реакции. Ключевую роль в этом процессе играют Т-регуляторные клетки — иммуносупрессивная популяция CD4+ Т-клеток, имеющая фенотип CD4+, CD25+ CD127^{low}, FoxP3+. Несмотря на отдельные сообщения о том, что Treg (или их определённые субпопуляции) могут усугублять микроциркуляторные нарушения в ишемизированной ткани, большинство исследователей убеждены в позитивной роли Treg при ишемическом инсульте. Резидентные CD69+ Treg, обнаруженные в нормальном мозге млекопитающих, обладают нейропротективной активностью, вырабатывают IL-10 и другие противоспалительные цитокины, контролируют астроглиоз и подавляют цитотоксические субпопуляции Т-клеток и микроглии. Системное введение Treg при инсульте сопровождается уменьшением объёма инфаркта мозга и упреждением вторичной гибели нейронов. Возможность активировать и наращивать Treg ex vivo открывает широкие перспективы по иммунокоррекции не только при системных и аутоиммунных заболеваниях: потенциально эта технология может быть применима в качестве нейропротективной терапии при ишемическом инсульте. Связь Treg, воспаления и цереброваскулярной патологии особенно показательна на примере развития ишемического инсульта на фоне COVID-19. Показано, что системное воспаление, обусловленное инфицированием SARS-CoV-2, приводит к значительному угнетению Treg, что сопровождается повышенным риском развития ишемического инсульта и других неврологических осложнений. Обобщённые сведения о возможном терапевтическом потенциале Treg при цереброваскулярной патологии могут представлять практический интерес не только для исследователей, но и для клиницистов.

Ключевые слова: *Treg; регуляторные Т-клетки; ишемический инсульт; COVID-19; биомаркеры.*

Для цитирования:
Жукова О.А., Чудакова Д.А., Белопасов В.В., Ширшова Е.В., Баклаушев В.П., Юсубалиева Г.М. Регуляторные Т-клетки при ишемическом инсульте: маленький ключ от большой «музыкальной шкатулки». *Клиническая практика.* 2023;14(3):36–49.
doi: https://doi.org/10.17816/clinpract568210

Поступила 10.08.2023 Принята 15.09.2023 Опубликована 29.09.2023

therapy; however, their therapeutic potential remains to be evaluated in randomized clinical trials.

Recent studies [2, 5–7] have highlighted the crucial role of inflammatory response within the ischemic penumbra and associated immune response in determining the course of ischemic perifocal tissue, focal organization, and neuronal plasticity during the recovery of lost function. The role of regulatory T cells (Tregs) in anti-inflammatory signaling cascade regulation and immune response suppression has been widely studied [2, 5, 8–10].

Studies on animals with experimental cerebral ischemia have accumulated considerable data on the potential translational significance of Tregs in stroke. Although some studies have noted a relationship between Tregs and cerebral microvascular dysfunction and the development of microthrombosis [11–13], several studies have reported a positive effect of Tregs in ischemic stroke. Ex vivo-engrafted Treg administration reduces the size of experimental brain infarction [9]. Tregs have been found to reduce the area of inflammatory tissue damage in ischemic stroke, block neuroinflammation, regulate neural tissue regeneration and angiogenesis, improve white matter recovery, inhibit astrogliosis, and reduce the cytotoxic activity of microglia, resulting in favorable effects on the recovery of neurological deficits [10, 13–16].

Pilot clinical trials have been conducted on the therapeutic potential of Tregs in neurology for multiple sclerosis, and the results have been favorable [8]. Additionally, preclinical studies on the safety and efficacy of Tregs in experimental stroke therapy show promise [9]. The next step is to evaluate the clinical relevance and feasibility of introducing Treg therapy for ischemic stroke into clinical practice. The urgency of the problem is highlighted by the lack of effective neuroprotective methods for fatal stroke.

Thus, we analytically reviewed over one hundred Russian and foreign publications that investigate the diagnostic, prognostic, and therapeutic applications of Tregs in stroke and that were published between 2018 and 2023.

IMMUNE RESPONSE TO ISCHEMIC STROKE: ROLE AND THERAPEUTIC POTENTIAL OF T-REGULATORY CELLS

Ischemic stroke can cause nerve tissue damage and increased permeability of the blood–brain barrier, leading to an intense inflammatory reaction and immune response. This response can be either innate or adaptive with a local autoimmune orientation. Maintaining a balanced immune response is crucial because an overexpressed or suppressed response can have harmful consequences to the patient. The course and outcome of ischemic stroke are determined by the features and orientation of the immune response [9, 18–20].

The brain is an immunoprivileged organ, with resident immune cells (microglia), borderline macrophages, and T cells present in the membranes' structures [21]. In stroke, blood–brain barrier damage allows immune cells to migrate to the zone of ischemic injury [17], accompanied by mitochondrial degradation, oxidative stress, lactoacidosis, metabolic changes, excitotoxicity, and neuroinflammation in the damaged brain area, which can cause different forms of cell death, including apoptotic or non-apoptotic (necroptosis, ferroptosis, cuproptosis, parthanatos, and pyroptosis) [22–25]. The release of alarmins, which are bioactive molecules that act as danger signals and include heat shock proteins, S100B, HMGB1, beta-amyloid, hyaluronan, etc., triggers innate immunity mechanisms. This, in turn, stimulates the pro-inflammatory polarization of microglia and peripheral immune cell migration to the ischemic penumbra. Subpopulations of T- and B-lymphocytes, neutrophils, monocytes, astrocytes, and microglia are involved in different stages of this cascade. In this case, Tregs control the immune response and autoimmune reactions by suppressing the proliferation and/or effector activity of T- and B-cells, natural killer cells (NK-cells), and antigen-presenting cells [14]. We will not discuss the similar immunosuppressive role of regulatory B-cells (Bregs) [28]. Local inflammation is a common occurrence in conditions such as multiple sclerosis, hemorrhagic stroke, brain injury, and demyelinating diseases of the central nervous system [8, 10, 26].

In rodent experiments, Tregs have been found to reduce the degree of damage to the blood–brain barrier in the first hours after stroke development. This effect is observed even when recombinant tissue plasminogen activator, a drug approved by the US Food and Drug Administration for thrombolytic therapy, is administered within the first hours after stroke [15]. In reducing CCL2 expression by vascular endothelial cells, Tregs have a positive effect on the permeability of the blood–brain barrier and survival of oligodendrocytes and their precursors [16] and inhibit or suppress reactive astrogliosis [29]. Simultaneously engaging stem cells in the subventricular niches, spleen, and intestine along the brain–intestine axis [30] determines the characteristics of cellular response in neuroinflammation in the brain in the poststroke period. Tregs play a role in reducing the degree of demyelination [31], such as in multiple sclerosis [8], and in reducing inflammation in different types of nervous system tissue lesions [10].

Tregs perform functions beyond their immunosuppressive role. They participate in the regulation of metabolism and cellular sensitivity to insulin. Additionally, they influence stem cells in various locations, such as the bone marrow or intestine, potentially affecting stem cells in subventricular and

https://doi.org/10.17816/clinpract568210

journal of clinical practice 2023 Vol 14 №3

subgranular niches [30]. This influence may further impact the cellular response to ischemic stroke.

A therapeutic approach aimed at modulating the immune response through Tregs is an attractive option [5]. Two possible strategies exist:

1) Pharmacological action on Tregs and immune mechanisms using low-molecular-weight drugs, microRNAs, monoclonal antibodies, and cytokines
2) Transplantation of autologous Tregs of a certain phenotype to the patient after ex vivo augmentation [8]

Experimental data showed that Tregs are a potential therapeutic solution for immunoprotection and neural tissue repair on day 1 after stroke development. Implementing Treg-targeted therapy for poststroke ischemia has become a subject of discussion in recent publications [32–34].

In mice with experimental stroke, exogenous Treg infusion has been shown to increase the number of Tregs from the first day to the second week and persists for a month. This reduces stroke volume and long-term functional recovery [16]. Therefore, it is critical to determine the molecular and cellular mechanisms of Treg-cell regulation of the immune response during the development of ischemic stroke [35]. The potential clinical application of autologous Treg transplantation as a therapeutic tool in ischemic stroke is promising, given its successful application in multiple sclerosis [8] and the observed reduction of Treg levels and dysfunction in stroke [36]. This gives hope that they will find clinical application in ischemic stroke [34].

Phenotypic and functional characteristics of Tregs

The human T-lymphocyte population includes $CD4^+$ T-helper cells (Th), $CD8^+$ cytotoxic T cells (CTL), γδ T cells, and Tregs, with several distinguishable subpopulations (Table 1) [37–43]. Tregs are a functionally distinct subpopulation of CD4+ T cells, with the FoxP3 transcription factor (forkhead box protein 3) considered as its obligatory marker [44]. The immunosuppressive activity of Tregs is closely related to the level of FoxP3 expression, and a decrease in FoxP3 content is associated with its reduction [45]. Autoimmune syndromes associated with the absence of functional Tregs develop because of mutations in the gene encoding FoxP3 [46]. The transcription factor FoxP3 is the determinant in the differentiation of T cells along the immunosuppressor pathway, producing Tregs from nonregulatory T cells (Tconv) [47].

In vivo, FoxP3 is induced by signaling pathways from the T-cell receptor in the thymus [48]. In humans, FoxP3 is represented by three functionally different isoforms [49], and the expressions of the different isoforms affect the development of some diseases. For instance, in allergic rhinitis, the expression of FOXP3Δ2 isoform leads to a decrease in the number and functional activity of Tregs [51]. Therefore, the presence of these isoforms should be considered when phenotypically characterizing Tregs.

The *FOXP3* gene, which encodes the FoxP3 protein in humans, is in the X chromosome in the centromeric region (region Xq11.3-q13.3). This

Table 1

Basic subpopulation of Treg cells

Subpopulation	Description/function	Basic markers	Source
nTregs	Naive “natural” Tregs formed in the thymus	CD4, FoxP3, Helios, $CD25^{high}$, CD39, CD73, $CD127^{low}$, CD152, CD357, LAG3	[10, 37]
Induced iTregs, also known as peripheral pTregs Subdivided into Th3, Tr1, and iTr35	Peripheral-induced effector Tregs formed from CD4+ T-helper cells (Th)	CD4, FoxP3, Helios-	[37, 38]
Activated Tregs	Activated Tregs versus Tconv	CD25, CD127, FOXP3, IKZF2, ITGA4, IKFZ, IKZF2, ITGA4, TRIM-	[39]
Cytotoxic Tregs	Tregs providing direct cytotoxicity	CD8 and FoxP3+ and FoxP3- subpopulations	[40]
Subpopulation with low CD25 levels	Treg subpopulation found in systemic autoimmune diseases	CD4, $CD25^{-/low}$, FoxP3	[41]
Resident brain Tregs	Resident Tregs of the neural tissue	FoxP3, CD69, PD1, KLRG1, 5-HT7, CD103, Neuropeptide Y, Osteopontin	[42]
mTregs	Memory Tregs, tissue-resident	CD4, CD25, FoxP3, CD45RA-, CD45RO+	[43]

location may contribute to the higher risk of stroke and lower survival rate after stroke in menopausal women compared with men of the same age group [52]. The immune response differs between men and women, which is partly due to the karyotype and influence of sex hormones. Many of these hormones affect Tregs. For instance, this study examined the variations in $CD4^+CD25^+$ $FoxP3^+$Treg levels during different stages of the menstrual cycle in healthy women. However, the effect of these changes on ischemic stroke requires further investigation [53]. Progesterone stimulates the formation of inducible regulatory T cells (iTregs) [54], whereas testosterone promotes the expansion of Tregs and affects chromatin modification in the region encoding FoxP3 [55]. In mice, the gene encoding FoxP3 is also in the X chromosome. An experiment was conducted on transgenic mice in which the SRY gene, necessary for the development of a male-type organism, was transferred to the autosome. The results showed that the difference in the course of stroke, regardless of the level of sex hormones, is primarily determined by the karyotype. Karyotype XX was associated with a larger infarct volume after experimental middle cerebral artery occlusion than XY [56].

There are two primary methods of Treg pool formation. The first involves the development of naive "natural" Tregs (nTreg) in the thymus, which are activated in peripheral tissues. These cells exhibit a phenotype that includes CD4+, $Helios^+$, TCR(CD3), $CD25^{high}$, CD39, CD73, $CD127^{low}$, CD152 (CTLA-4), CD357(GITR), and LAG3 [10, 57]. In the second pathway, CD4+ T-helper cells (Th) differentiate into Th1 cells (under the influence of cytokines IL-12, IFN-γ, IL-27), Th2 cells (under the influence of IL-4), Th17 cells (under the influence of IL-1β, IL-23, IL-6, TGF-β), or iTregs (in the presence of IL-2 and TGF-β) after leaving the thymus. CD4 and FoxP3 are the main markers of iTregs. These cells secrete TGF-β, either IL-10 or IL-35, and can be subdivided into Th3, Tr1, and iTr35 cells, respectively [10, 48]. The T-cell receptor repertoires of nTregs and Tregs, whose precursors are T-helper cells, differ [58]. Furthermore, they differ in the methylation profiles of certain CpG DNA in the enhancer region of the gene encoding FoxP3. In cells formed from T-helper cells, this region is methylated [59].

$CD8^+$ cells and $CD4^+$ Tregs have similar functions [40]. In patients with inflammatory diseases such as systemic lupus erythematosus, the number of $CD4^+$, $CD257^{low}$, and $FoxP3^+$ subpopulations increases [41]. Additionally, $CD4^+$, $CD25^+$, $CD127^{low}$, and $FoxP3^+$ cells can be divided into naive nTregs and memory mTregs, which have distinct functions (Table 1).

FoxP3 is detected in some nonregulatory T cells; however, this does not result in an immunosuppressive phenotype [60]. A detailed panel of markers, including $IKFZ^{++}$ and $ITGA4^+$, and the absence of the TRIM marker on Tregs, which is highly expressed in Tconv cells, distinguishes between these cell populations [10, 39].

Tregs differ significantly from other T cells regarding metabolism. In effector T cells, the main regulators of metabolism are mTor, HIF-1a, Myc, ROHA, ICOS, and BCL6. However, in Tregs, this role is played by mTor, Myc, and FoxP3 [61]. Recent studies have shown that FoxP3 controls cell metabolism, facilitating cell adaptation to low glucose and high lactate. This adaptation allows Tregs to function effectively in ischemic tissues [62].

The immunoregulatory functions of Tregs are carried out through the secretion of anti-inflammatory cytokines, such as IL-10 and TGF-b, and the expression of immunosuppressive molecules, including CD152, CD39, and PD-1. Additionally, the binding of extracellular IL-2 and increased expression levels of CD25 (IL-2 receptor) can reduce the intensity of the immune response. Further, regulatory molecules expressed on the cell membrane, such as cytotoxic T-lymphocyte glycoprotein 4 (CTLA4, CTLA-4, CD152), interleukin IL-10, and transforming growth factor TGF-b1, modulate the activity of antigen-presenting cells [63]. CD152 plays a role in downregulating the costimulatory molecules CD80 and CD86 on dendritic cells [64]. These molecules are crucial for stimulation to prevent the proliferation and apoptosis of activated T cells. Moreover, Tregs can directly eliminate T- and B-cells involved in inflammation [61] through perforin and granzyme secretion.

Modern methods of single-cell DNA sequencing have revealed a high degree of phenotypic heterogeneity in cell populations involved in the development of ischemic stroke [65]. A transcriptome analysis has shown that Tregs exhibit high phenotypic heterogeneity, and their ability to adapt to the target tissue is manifested differently in various tissues [66]. Depending on the tissue microenvironment, Tregs can express additional phenotypic markers, such as various integrins, including ITGA4 [39, 57]. RNA sequencing of single cells has clarified the phenotypic markers of various Treg subpopulations, including tissue-specific ones, in both mice and humans [66, 67].

The number of Tregs in the normal brain tissue of a healthy person is small [29], and they are classified as

https://doi.org/10.17816/clinpract568210

journal of clinical practice 2023 Vol 14 №3

resident cells [42]. They differ from peripheral Tregs by expressing *Blimp1, Ccr6, Ccr8, AREG, ST2, IL10, CD69, PD1, KLRG1, 5-HT7, CD103, Neuropeptide Y*, and *Osteopontin* genes [42]. $CD69^+$ resident Tregs found in the normal brain of mice and humans have specialized protective functions and are rapidly activated under neuroinflammation conditions. These cells can regulate astrogliosis levels by producing amphiregulin, polarize microglia into a neuroprotective state, and suppress tissue inflammation by producing IL-10 [42].

The idea that Tregs are a homogeneous cell population is outdated. Treg subpopulations differ both phenotypically and functionally. This heterogeneity emphasizes the importance of detailed phenotypic and functional characterization and makes us critical of earlier studies that treated Tregs as a single population. Recruiting immune cells into the brain parenchyma of patients with ischemic injury in the poststroke period for future clinical applications should consider the diametrically opposite local and systemic responses upon activation of different Treg subpopulations.

Treg level as a prognostic marker of stroke

Clinical and laboratory studies indicate a decrease in Tregs in peripheral blood on day 1 after an ischemic stroke. This is due to their active migration from the cerebral microcirculatory bed to the zone of ischemia and inflammation in the brain [36, 68]. This process is dynamic and reversible; therefore, a decrease in peripheral Tregs in ischemic stroke may not always be detected [69]. Hence, it is critical to determine whether the dynamics of peripheral Treg levels, either as a whole or as individual subpopulations, can provide additional prognostic data in cases of ischemic stroke.

The level of certain Treg subpopulations may be crucial for recovery from ischemic stroke. Research has demonstrated that certain Treg subpopulations can produce brain-derived neurotrophic factor (BDNF), a secretory protein that supports neuronal viability. A study has shown an increase in the number of Tregs producing BDNF ($BDNF^+$Tregs) in the peripheral blood of patients after ischemic stroke. In patients with a more favorable neurological outcome, the number of $BDNF^+$Tregs in peripheral blood after 6 months was higher than that in other patients [70].

Treg levels in peripheral blood may depend on neuroinflammation severity and infarction volume. In patients with an infarct volume of 28.6 ml, the Treg level in peripheral blood decreased insignificantly on day 1 after infarction. However, in patients with an infarct volume >28.6 ml, a significant decrease in Treg levels was observed on day 1, followed by an increase on days 3, 7, and 14 [71].

A recent study has found that patients with acute ischemic stroke had increased Treg frequency and mTreg frequency (with $CD45RA^+$ Tregs accounting for about 90% of circulating Tregs) and decreased nTreg frequency and nTreg/mTreg ratio. The nTreg/mTreg ratio on the day of hospitalization may independently predict an unfavorable 90-day outcome in acute ischemic stroke. In contrast, patients with high $CD4^+$ Tregs among lymphocytes upon hospital admission had a lower risk of adverse outcomes at discharge [74]. The number of circulating Tregs 48 and 72 h after infarction negatively correlates with infarct volume. That is, increased Treg levels decrease infarct volume [69, 75].

In summary, the level of peripheral Treg may serve as a biomarker for predicting stroke outcomes.

APPROACHES TO Treg LEVEL MODULATION FOR ISCHEMIC INSULT THERAPY

The immunomodulatory and reparative properties of Tregs make them an effective immunosuppressive therapy for the “graft versus host” reaction in some autoimmune diseases, including multiple sclerosis [8]. Isolating, ex vivo expanding, and subsequently transplanting $CD4^+CD25^+CD127^{low}Foxp3^+$ Tregs has allowed for long-term remission of the autoimmune process in such patients. The experimental data confirmed that Tregs induce a neuroprotective effect during the acute stage of ischemic stroke and promote brain recovery during the chronic stage. See below for further details. This effect is mediated by various mechanisms, including direct intercellular interactions and secretion of soluble factors [76].

Transplantation of ex vivo-generated Tregs requires licensing of the production site and registration of the cells themselves as a high-tech drug or obtaining permission to use Tregs as a hospital exception. The technologies of mediated modulation of the amount and functional activity of Tregs are much simpler from the viewpoint of regulatory legislation.

Retinoic acid is a Treg modulator that stimulates Tregs via the TGF-β/Smad3 signaling pathway [77]. It is produced *in vivo* by dendritic cells [78]. Moreover, chorionic gonadotropin, which was patented in 2023 by Russian researchers for autologous induction of Tregs ex vivo, is a known Treg modulator [79]. Additionally, the immunosuppressant rapamycin, which inhibits the mTOR kinase signaling pathway cascade, can stimulate the expansion of human

Tregs ex vivo along with retinoic acid [80]. The use of rapamycin as a pharmacological agent to trigger Treg expansion has been proposed. Research has shown that exposure to rapamycin leads to selective expansion of Tregs [81]. Further, signaling through the mTOR pathway has been found crucial for the activation and maintenance of the immunosuppressive activity of Tregs [82]. These findings indicate a need to modify current ideas about the mechanism of action of rapamycin in Treg expansion.

In mouse experiments, low doses of 5-aza-2'-deoxycytidine, an inhibitor of DNMT DNA methyltransferase that leads to DNA demethylation, resulted in increased Treg level. This effect is expected because of the impact of DNA methylation on FoxP3 expression and subsequent increase in FoxP3 levels in cells after treatment with 5-aza-2'-deoxycytidine. When combined with TGF-β exposure, this treatment made iTregs more similar in phenotype to nTregs.

In mice experiments, the administration of the IL-2 complex and an antibody recognizing IL-2 resulted in an increase in Tregs in the peripheral blood, spleen, and lymph nodes. Moreover, it stimulated CD39 and CD73 expression in these cells. Ultimately, this immunotherapy protected the brain from ischemic damage in transient middle cerebral artery occlusion [85].

In vitro and *in vivo* studies have shown that nerve tissue autoantigens trigger the conversion of Tconv to Tregs [86]. Additionally, exosomes containing TGF-β, Smad2, and Smad4, which are secreted by embryonic stem cells (ESC-sEVs), lead to a significant increase in Tregs after stroke. This is due to TGF-β/Smad signaling pathway activation, which induces Treg expansion. Therefore, ESC-sEVs may be considered candidates for immune modulation [87]. In mice, recombinant IL-33 administration after ischemic stroke increased IL-10-expressing Tregs in the brain. This decreased the infarct size, the amount of activated microglia, and brain-infiltrating cytotoxic T cells. A more than threefold increase was observed in the number of Tregs in the brain 3 days after stroke compared with controls [88].

An experimental model of cerebral occlusion showed that microglia induced the expression of sirtuin 2 (Sirtuin2) after a stroke. This, in turn, suppressed the anti-inflammatory activity of Tregs, resulting in a significant increase in the transcription factor HIF-1α in Tregs. Conversely, inhibition of HIF-1α blocked the increase in the level of sirtuin 2 [89]. Pharmacologic inhibition of HIF-1α in ischemic stroke may prevent Treg activity suppression by microglia. Inhibition of HIF-1α is considered as a possible approach to stroke therapy, regardless of its role in Treg regulation [90].

The relevance of this problem is evidenced by the number of published studies. Remote ischemic conditioning or remote ischemic postconditioning, a method that induces short reversible episodes of ischemia with reperfusion in certain tissues or organs to protect other distant tissues and organs from ischemic/reperfusion damage, was applied to mice, resulting in increased Treg levels and decreased affected area during stroke and, in turn, indicating that Tregs play a causative role in this process. The same effect was induced by nicotinamide adenine dinucleotide hydrate [91]. A study has discussed the function of the receptor for advanced glycation end products (RAGE) in regulating the metabolism of peripheral $CD4^+$ T cells. The study demonstrated that neutralizing RAGE action by adding circulating RAGE (sRAGE) to cells stimulates $CD4^+$ T-cell polarization toward the Treg phenotype and reduces stroke size [92]. Currently, active research on the therapeutic potential of microRNAs expressed in Tregs or activating their expansion is underway [93–95].

Notably, several biologically active substances used to treat ischemic stroke affect Tregs [96], including melatonin, estrogen, statins, and vitamin D [96]. Melatonin is particularly attractive owing to its well-known neuroprotective properties, such as in the treatment of cerebrovascular pathology [97]. Research has demonstrated that melatonin impacts T cells, including their activation and differentiation. However, the effect of melatonin is context-dependent. In cases of pathological processes characterized by inflammation, melatonin suppresses the immune response. Conversely, in conditions accompanied by immunosuppression, it stimulates the immune response [98]. For instance, melatonin has increased the number of Tregs in peripheral blood in patients with systemic lupus erythematosus [99]. Another drug candidate that affects Tregs in ischemic stroke is the multitarget drug metformin [100]. Studies have shown that the use of metformin, both along with the immunosuppressant tacrolimus and as monotherapy, leads to an increase in the number of Tregs [101].

Studies assessing the potential use of inhibitors of the sphingosine-1-phosphate receptor (S1P receptor) in stroke therapy have been ongoing for over a decade [102]. One such inhibitor, fingolimod, led to increased FoxP3+Tregs in the spleen and peripheral blood and in the brain after ischemia in a mouse model of ischemic

https://doi.org/10.17816/clinpract568210

journal of clinical practice 2023 Vol 14 №3

stroke [103]. In a small study cohort consisting of 5 healthy volunteers and 12 patients who had experienced acute ischemic stroke 72 h prior, treatment with the poly(ADP-ribose) polymerase 1 (PARP-1) inhibitor JPI-289 resulted in an increase in Tregs. The study showed a potential therapeutic benefit of JPI-289 for treating acute ischemic stroke. The pro-inflammatory cytokines (IFN-γ, TNF-α, and IL-17) decreased, whereas the anti-inflammatory cytokines (IL-4, IL-10, and TGF-β1) increased. Initially, the proportion of Tregs in peripheral blood was significantly higher in healthy subjects [104]. The effect of PARP-1 inhibition on Treg functions can be explained by the fact that PARP-1 performs poly-ADP ribosylation of FohP3 [105].

Modulation of the immune response, including ischemic stroke, by metabolites of commensal or pathogenic microflora of the gastrointestinal tract has recently gained attention. Published data show that metabolites of commensal microflora, such as butyrate, belong to histone deacetylase inhibitors (HDACi) and can stimulate the formation of peripheral Tregs by increasing the level of histone acetylation in the locus where the gene encoding FoxP3 is located [106]. This is particularly interesting given the general immunomodulatory and neuroprotective role of histone deacetylase inhibitors. HDACi increase CTLA-4 expression, stimulating the immunosuppressive function of human Tregs [107].

Recruitment of Tregs to the brain in ischemic stroke

Recruitment of immune cells to the brain parenchyma during ischemic injury begins with an increase in the permeability of the blood–brain barrier. This increase occurs early in ischemic stroke, within 10 min after reperfusion in rodents and within 2–6 h (average: 3.8 hours) from the onset of stroke in humans [108]. From approximately 24 h, there is a permanent pathological increase in the permeability of the blood–brain barrier, which continues for several weeks [109]. Simultaneously, mediators released in the inflammatory focus stimulate the production of chemokines, such as CXCL8 in humans and CXCL1 and CXCL2 in rodents. This enables peripheral monocytes, neutrophils, NK-cells, and lymphocytes to penetrate the blood–brain barrier and trigger the inflammatory cascade [110]. Tregs infiltrate the brain parenchyma later than other T cells [111]. Although immunophenotypically naive Tregs stay briefly, activated Tregs can migrate to the ischemic hemisphere of the brain and remain there for up to 30 days, particularly in mice [112]. Additionally, local and systemic immunologic mechanisms contribute to the pathogenesis of ischemic stroke, in which peripheral Tregs are likely involved [113].

COVID-19, Tregs, and stroke

The relationship between systemic inflammation, Tregs, and ischemic stroke has become prominent because of the COVID-19 pandemic. Immunopathological mechanisms triggered by SARS-CoV-2 infection, especially in long COVID-19, alter the Treg system, increasing the risk of ischemic stroke and other complications [114, 115]. Previously, we discussed the neurological consequences of COVID-19, including cerebral circulatory disorders [116], and the pathogenetic mechanisms responsible for the increased risk of stroke during and after COVID-19 [26]. In this section, we summarize the main immunopathological links between COVID-19 and increased stroke risk, specifically focusing on the role of Tregs.

Four key pathogenetic mechanisms of COVID-19 damage to the nervous system have been identified. First, SARS-CoV-2 is neurotropic and can directly infect the neuroepithelial cells of the olfactory analyzer, causing damage to the first pair of cranial nerves, allowing the virus to penetrate and spread in the brain. Second, SARS-CoV-2-induced systemic inflammation leads to hypercoagulability and an increased risk of thrombosis, including in cerebral vessels. Third, hypoxemia caused by partial lung damage inevitably affects the central nervous system, leading to a range of complications. The most severe manifestation of these complications is critical-state encephalopathy. SARS-CoV-2 infection can cause immune-mediated damage to nervous tissue. This is due to the production of pro-inflammatory cytokines by resident macrophages and $Th17^{+}$ cells and a shift in the immune balance toward systemic inflammation in response to central nervous system cell infection [114–116]. Tregs play a key role in this mechanism, which increases the risk of stroke.

A 2021 meta-analysis has found that COVID-19 survivors have at least a threefold increased risk of stroke compared to non-survivors [117]. Moreover, all COVID-19 patients experience decreased Treg levels and impaired function [118], which is a key mechanism of the immunopathologic manifestations of the disease [119, 120]. A decrease in Treg levels leads to a Treg/Th17 imbalance, promoting the development of systemic inflammation. This worsens both the prognosis of

COVID-19 and comorbid disease outcomes and coronavirus infection complications [120, 121]. The most significant and prolonged decrease in Treg levels was observed in long COVID-19 [121, 122].

Transcriptome analysis of over 100,000 viral antigen-recognizing CD4+ T cells from 40 COVID-19 patients revealed that hospitalized patients, as opposed to outpatients with mild forms of the disease, exhibit a significant increase in cytotoxic T-helper cells (CD4-CTL) compared with SARS-CoV-2-reactive Tregs [123]. These findings reveal a shift in the immune balance toward systemic inflammation, which is associated with the severity of coronavirus infection. Transcriptome analysis has revealed that the severity of COVID-19 is positively correlated with Treg functional impairment, as evidenced by decreased expressions of FoxP3 and immunosuppressive cytokines IL-10 and TGF-β [121]. Conversely, in COVID-19 reconvalescents, the pool of long-lived Tregs with high HLA-DRA expression levels is restored after ≥4 months, and a unique cluster of Tregs overexpressing TGF-β appear [124].

The present study examines the relationship between COVID-19, vaccination, and the risk of stroke. This retrospective study analyzed a cohort of 466 patients and found that those who received the SARS-CoV-2 vaccine had a more favorable course of ischemic stroke compared with the control group. The authors believe that vaccination, which affects the pathogen, may trigger a sanogenetic mechanism by increasing the Treg level in cerebral vessels that provide microcirculation in the area of cerebral ischemia or infarction [125].

CONCLUSIONS

The role of Tregs in the modulation of ischemic stroke is widely acknowledged. A comprehensive set of markers for all Treg subpopulations will enable a more accurate assessment of the functioning of individual Treg populations at different stages of ischemic stroke, from the initial acute phase to its long-term consequences. Available data indicate that Tregs have a protective role in ischemic stroke by affecting the size of the ischemic penumbra and limiting the size of brain infarction, promoting the recovery of neurological deficits. Therapeutic approaches aimed at increasing the number and activity of Tregs may be the optimal solution to protect ischemic neural tissue from immune inflammatory responses, prevent astrogliosis, provide systemic neuroprotection, and facilitate more efficient recovery of neurological impairment after a stroke.

ADDITIONAL INFORMATION

Funding source. This study was supported by the grant of Federal Medical and Biological Agency of Russia (The project “TILs-Glioblastoma”).

Competing interests. The authors declare that they have no competing interests.

Authors’ contribution. *O.A. Zhukova, D.A. Chudakova, V.V. Belopasov* — analysis of the literature, writing a draft version of the manuscript; *E.V. Shirshova, V.P. Baklaushev* — manuscript editing; *G.M. Yusubalieva* — conceptualization of the manuscript, literature review, manuscript editing. The authors made a substantial contribution to the conception of the work, acquisition, analysis of literature, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Источник финансирования. Работа выполнена в рамках государственного задания ФМБА России (НИР «TILs-Глиобластома»).

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

Вклад авторов. *О.А. Жукова, Д.А. Чудакова, В.В. Белопасов* — анализ литературы, написание чернового варианта рукописи; *Е.В. Ширшова, В.П. Баклаушев* — редактирование текста рукописи; *Г.М. Юсубалиева* — идея обзора, анализ литературы, редактирование рукописи. Авторы подтверждают соответствие своего авторства международным критериям ICMJE (все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией).

REFERENCES / ЛИТЕРАТУРА

1. Feigin VL, Brainin M, Norrving B, et al. World Stroke Organization (WSO): Global stroke fact sheet 2022. *Int J Stroke*. 2022;17(1):18–29. doi: 10.1177/17474930211065917
2. Sakai S, Shichita T. Inflammation and neural repair after ischemic brain injury. *Neurochem Int*. 2019;(130):104316. doi: 10.1016/j.neuint.2018.10.013
3. Fedin AI, Badalyan KR. Review of clinical recommendations for the treatment and prevention of ischemic stroke. *J Neurology Psychiatry named after S.S. Korsakov*. 2019;119(8-2):95–100. (In Russ). Федин А.И., Бадалян К.Р. Обзор клинических рекомендаций лечения и профилактики ишемического инсульта // *Журнал неврологии и психиатрии им. С.С. Корсакова*. 2019. Т. 119, № 8-2. С. 95–100. doi: 10.17116/jnevro201911908295
4. Alim I, Caulfield JT, Chen Y, et al. Selenium drives a transcriptional adaptive program to block ferroptosis and treat stroke. *Cell*. 2019;177(5):1262–1279. doi: 10.1016/j.cell.2019.03.032

https://doi.org/10.17816/clinpract568210

5. Zhang SR, Phan TG, Sobey CG. Targeting the immune system for ischemic stroke. *Trends Pharmacol Sci*. 2021;42(2):96–105. doi: 10.1016/j.tips.2020.11.010
6. Vorobyev SV, Yanishevsky SN, Kudryavtsev IV, et al. Involvement of the immune response in the pathogenesis of ischemic stroke. *Medical Advice*. 2023;(3):8–16. (In Russ). Воробьев С.В., Яншевский С.Н., Кудрявцев И.В., и др. Участие иммунного ответа в патогенезе ишемического инсульта // *Медицинский совет*. 2023. № 3. С. 8–16.
7. Thapa K, Shivam K, Khan H, et al. Emerging targets for modulation of immune response and inflammation in stroke. *Neurochem Res*. 2023;48(6):1663–1690. doi: 10.1007/s11064-023-03875-2
8. Lifshitz GV, Zhdanov DD, Lokhonina AV, et al. Ex vivo expanded regulatory T cells CD4+CD25+FoxP3+CD127^{Low} develop strong immunosuppressive activity in patients with remitting-relapsing multiple sclerosis. *Autoimmunity*. 2016;49(6):388–396. doi: 10.1080/08916934.2016.1199020
9. Brea D, Agulla J, Rodríguez-Yáñez M, et al. Regulatory T cells modulate inflammation and reduce infarct volume in experimental brain ischaemia. *J Cell Mol Med*. 2014;18(8): 1571–1579. doi: 10.1111/jcmm.12304
10. Olson KE, Mosley RL, Gendelman HE. The potential for Treg-enhancing therapies in nervous system pathologies. *Clin Exp Immunol*. 2023;211(2):108–121. doi: 10.1093/cei/uxac084
11. Kleinschnitz C, Kraft P, Dreykluft A, et al. Regulatory T cells are strong promoters of acute ischemic stroke in mice by inducing dysfunction of the cerebral microvasculature. *Blood*. 2013;121(4):679–691. doi: 10.1182/blood-2012-04-426734
12. Selvaraj UM, Stowe AM. Long-term T cell responses in the brain after an ischemic stroke. *Discovery Med*. 2017;24(134):323.
13. Wu Y, Li J, Shou J, et al. Diverse functions and mechanisms of regulatory T cell in ischemic stroke. *Exp Neurol*. 2021; (343):113782. doi: 10.1016/j.expneurol.2021.113782
14. Malviya V, Yshii L, Junius S, et al. Regulatory T-cell stability and functional plasticity in health and disease. *Immunol Cell Biol*. 2023;101(2):112–129. doi: 10.1111/imcb.12613
15. Mao L, Li P, Zhu W, et al. Regulatory T cells ameliorate tissue plasminogen activator-induced brain haemorrhage after stroke. *Brain*. 2017;140(7):1914–1931. doi: 10.1093/brain/awx111
16. Yuan C, Shi L, Sun Z, et al. Regulatory T cell expansion promotes white matter repair after stroke. *Neurobiol Dis*. 2023;(179):106063. doi: 10.1016/j.nbd.2023.106063
17. Sarvari S, Moakedi F, Hone E, et al. Mechanisms in blood-brain barrier opening and metabolism-challenged cerebrovascular ischemia with emphasis on ischemic stroke. *Metab Brain Dis*. 2020;35(6):851–868. doi: 10.1007/s11011-020-00573-8
18. Nikolic D, Jankovic M, Petrovic B, Novakovic I. Genetic aspects of inflammation and immune response in stroke. *Int J Mol Sci*. 2020;21(19):7409. doi: 10.3390/ijms21197409
19. Rayasam A, Hsu M, Kijak JA, et al. Immune responses in stroke: How the immune system contributes to damage and healing after stroke and how this knowledge could be translated to better cures? *Immunology*. 2018;154(3):363–376. doi: 10.1111/imm.12918
20. Tobin MK, Bonds JA, Minshall RD, et al. Neurogenesis and inflammation after ischemic stroke: What is known and where we go from here. *J Cereb Blood Flow Metab*. 2014;34(10): 1573–1584. doi: 10.1038/jcbfm.2014.130
21. Rustenhoven J, Drieu A, Mamuladze T, et al. Functional characterization of the dural sinuses as a neuroimmune interface. *Cell*. 2021;184(4):1000–1016.e27. doi: 10.1016/j.cell.2020.12.040
22. Fan X, Chen H, Jiang F, et al. Comprehensive analysis of cuproptosis-related genes in immune infiltration in ischemic stroke. *Front Neurol*. 2023;(13):1077178. doi: 10.3389/fneur.2022.1077178
23. Tuo QZ, Zhang ST, Lei P. Mechanisms of neuronal cell death in ischemic stroke and their therapeutic implications. *Med Res Rev*. 2022;(42):259–305. doi: 10.1002/med.21817
24. Yang K, Zhang Z, Liu X, et al. Identification of hypoxia-related genes and exploration of their relationship with immune cells in ischemic stroke. *Sci Rep*. 2023;13(1):10570. doi: 10.1038/s41598-023-37753-2
25. Mao R, Zong N, Hu Y, et al. Neuronal death mechanisms and therapeutic strategy in ischemic stroke. *Neurosci Bull*. 2022;38(10):1229–1247. doi: 10.1007/s12264-022-00859-0
26. Samoilova EM, Yusubalieva GM, Belopasov VV, et al. Infections and inflammation in the development of stroke. *J Neurology Psychiatry named after S.S. Korsakov*. 2021;121(8-2):11-21. (In Russ). doi: 10.17116/jnevro202112108211
27. Han L, Wang Z, Yuan J, et al. Circulating leukocyte as an inflammatory biomarker: Association with fibrinogen and neuronal damage in acute ischemic stroke. *J Inflamm Res*. 2023;(16):1213–1226. doi: 10.2147/JIR.S399021
28. Seifert HA, Vandenbark AA, Offner H. Regulatory B cells in experimental stroke. *Immunology*. 2018;154(2):169–177. doi: 10.1111/imm.12887
29. Ito M, Komai K, Mise-Omata S, et al. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. *Nature*. 2019;565(7738):246–250. doi: 10.1038/s41586-018-0824-5
30. Astarita JL, Dominguez CX, Tan C, et al. Treg specialization and functions beyond immune suppression. *Clin Exp Immunol*. 2023;211(2):176–183. doi: 10.1093/cei/uxac123
31. Wang Y, Sadike D, Huang B, et al. Regulatory T cells alleviate myelin loss and cognitive dysfunction by regulating neuroinflammation and microglial pyroptosis via TLR4/MyD88/NF-κB pathway in LPC-induced demyelination. *J Neuroinflammation*. 2023;20(1):41. doi: 10.1186/s12974-023-02721-0
32. Bluestone JA, McKenzie BS, Beilke J, Ramsdell F. Opportunities for Treg cell therapy for the treatment of human disease. *Front Immunol*. 2023;(14):1166135. doi: 10.3389/fimmu.2023.1166135
33. Li Y, McBride DW, Tang Y, et al. Immunotherapy as a treatment for stroke: Utilizing regulatory T cells. *Brain Hemorrhages*. 2023. doi: 10.1016/j.hest.2023.02.003
34. Zhang Y, Liesz A, Li P. Coming to the rescue: Regulatory T cells for promoting recovery after ischemic stroke. *Stroke*. 2021;52(12):e837–e841. doi: 10.1161/STROKEAHA.121.036072
35. Qiao C, Liu Z, Qie S. The Implications of microglial regulation in neuroplasticity-dependent stroke recovery. *Biomolecules*. 2023;13(3):571. doi: 10.3390/biom13030571
36. Ruhnau J, Schulze J, von Sarnowski B, et al. Reduced numbers and impaired function of regulatory T cells in peripheral blood of ischemic stroke patients. *Mediators Inflamm*. 2016;2016:2974605. doi: 10.1155/2016/2974605
37. Thornton AM, Lu J, Korty PE, et al. Helios+ and Helios- Treg subpopulations are phenotypically and functionally distinct and express dissimilar TCR repertoires. *Eur J Immunol*. 2019;49(3):398–412. doi: 10.1002/eji.201847935
38. Thornton AM, Korty PE, Tran DQ, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *J Immunol*. 2010;184(7):3433–3441. doi: 10.4049/jimmunol.0904028
39. Reinhardt J, Sharma V, Stavridou A, et al. Distinguishing activated T regulatory cell and T conventional cells by single-cell technologies. *Immunology*. 2022;166(1):121–137. doi: 10.1111/imm.13460
40. Mishra S, Srinivasan S, Ma C, Zhang N. CD8+ regulatory T cell: A mystery to be revealed. *Front Immunol*. 2021;(12):708874. doi: 10.3389/fimmu.2021.708874
41. Zhang B, Zhang X, Tang FL, et al. Clinical significance of increased CD4+ CD25- Foxp3+ T cells in patients with new-onset systemic lupus erythematosus. *Ann Rheum Dis*. 2008;67(7):1037–1040. doi: 10.1136/ard.2007.083543
42. Liston A, Dooley J, Yshii L. Brain-resident regulatory T cells and their role in health and disease. *Immunol Letters*. 2022;(248): 26–30. doi: 10.1016/j.imlet.2022.06.005
43. Khantakova JN, Bulygin AS, Sennikov SV. The regulatory T-cell memory phenotype: What we know. *Cells*. 2022;11(10):1687. doi: 10.3390/cells11101687
44. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+ CD25+ regulatory T cells. *Nature Immunol*. 2003;4(4):330–336. doi: 10.1038/ni904

45. Wan YY, Flavell RA. Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature*. 2007;445(7129):766–770. doi: 10.1038/nature05479
46. Husebye ES, Anderson MS, Kämpe O. Autoimmune polyendocrine syndromes. *N Engl J Med*. 2018;378(12):1132–1141. doi: 10.1056/NEJMra1713301
47. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science*. 2003;299(5609):1057–1061. doi: 10.1126/science.1079490
48. Hsieh CS, Lee HM, Lio CW. Selection of regulatory T cells in the thymus. *Nat Rev Immunol*. 2012;12(3):157–167. doi: 10.1038/nri3155
49. Allan SE, Passerini L, Bacchetta R., et al. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. *J Clin Invest*. 2005;115(11):3276–3284. doi: 10.1172/JCI24685
50. Mailer RK, Falk K, Rotzschke O. Absence of leucine zipper in the natural FOXP3D2D7 isoform does not affect dimerization but abrogates suppressive capacity. *PLoS One*. 2009;4(7):e6104. doi: 10.1371/journal.pone.0006104
51. Donetskova AD, Litvina MM, Smirnov DS, et al. Comparison of the expression of isoforms of the FOXP3 molecule by regulatory T cells of peripheral blood in allergic and lymphoproliferative diseases. *Russ Med J*. 2020;4(1):4–9. (In Russ). Донецкова А.Д., Литвина М.М., Смирнов Д.С., и др. Сравнение экспрессии изоформ молекулы FOXP3 регуляторными Т-клетками периферической крови при аллергических и лимфопролиферативных заболеваниях // *Русский медицинский журнал*. 2020. Т. 4, № 1. С. 4–9.
52. Bushnell CD, Chaturvedi S, Gage KR, et al. Sex differences in stroke: Challenges and opportunities. *J Cereb Blood Flow Metab*. 2018;38(12):2179–2191. doi: 10.1177/0271678X18793324
53. Arruvito L, Sanz M, Banham AH, Fainboim L. Expansion of CD4+CD25+ and FOXP3+ Regulatory T cells during the follicular phase of the menstrual cycle: Implications for human reproduction. *J Immunol*. 2014;178(4):2572–2578. doi: 10.4049/jimmunol.178.4.2572
54. Lee JH, Lydon JP, Kim CH. Progesterone suppresses the mTOR pathway and promotes generation of induced regulatory T cells with increased stability. *Eur J Immunol*. 2012;(42):2683–2696. doi: 10.1002/eji.201142317
55. Brown MA, Su MA. An inconvenient variable: Sex hormones and their impact on T cell responses. *J Immunol*. 2019;202(7):1927–1933. doi: 10.4049/jimmunol.1801403
56. McCullough LD, Mirza MA, Xu Y, et al. Stroke sensitivity in the aged: Sex chromosome complement vs. gonadal hormones. *Aging (Albany NY)*. 2016;8(7):1432. doi: 10.18632/aging.100997
57. Savage PA, Klawon DE, Miller CH. Regulatory T cell development. *Annu Rev Immunol*. 2020;(38):421–453. doi: 10.1146/annurev-immunol-100219-020937
58. De Lafaille MA, Lafaille JJ. Natural and adaptive FOXP3+ regulatory T cells: More of the same or a division of labor? *Immunity*. 2009;30(5):626–635. doi: 10.1016/j.immuni.2009.05.002
59. Lal G, Zhang N, van der Touw W, et al. Epigenetic regulation of Foxp3 expression in regulatory T cells by DNA methylation. *J Immunol*. 2009;182(1):259–273. doi: 10.4049/jimmunol.182.1.259
60. Magg T, Mannert J, Ellwart JW, et al. Subcellular localization of FOXP3 in human regulatory and nonregulatory T cells. *Eur J Immunol*. 2012;42(6):1627–1638. doi: 10.1002/eji.201141838
61. Yang W, Yu T, Cong Y. CD4+ T cell metabolism, gut microbiota, and autoimmune diseases: Implication in precision medicine of autoimmune diseases. *Precis Clin Med*. 2022;5(3):pbac018. doi: 10.1093/pcmedi/pbac018
62. Angelin A, Gil-de-Gómez L, Dahiya S, et al. Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metabol*. 201725(6):1282–1293.e7. doi: 10.1016/j.cmet.2016.12.018
63. André S, Tough DF, Lacroix-Desmazes S, et al. Surveillance of antigen-presenting cells by CD4+ CD25+ regulatory T cells in autoimmunity: Immunopathogenesis and therapeutic implications. *Am J Pathol*. 2009;174(5):1575–1587. doi: 10.2353/ajpath.2009.080987
64. Wing K, Onishi Y, Prieto-Martin P, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*. 2008;322(5899):271–275. doi: 10.1126/science.1160062
65. Qiu M, Zong JB, He QW, et al. Cell heterogeneity uncovered by single-cell RNA sequencing offers potential therapeutic targets for ischemic stroke. *Aging Dis*. 2022;13(5):1436–1454. doi: 10.14336/AD.2022.0212
66. Miragaia RJ, Gomes T, Chomka A, et al. Single-cell transcriptomics of regulatory T cells reveals trajectories of tissue adaptation. *Immunity*. 2019;50(2):493–504.e7. doi: 10.1016/j.immuni.2019.01.001
67. Szabo PA, Levitin HM, Miron M, et al. Single-cell transcriptomics of human T cells reveals tissue and activation signatures in health and disease. *Nat Commun*. 2019;10(1):4706. doi: 10.1038/s41467-019-12464-3
68. Dolati S, Ahmadi M, Khalili M, et al. Peripheral Th17/Treg imbalance in elderly patients with ischemic stroke. *Neurol Sci*. 2018;39(4):647–654. doi: 10.1007/s10072-018-3250-4
69. Santamaría-Cadavid M, Rodríguez-Castro E, Rodríguez-Yáñez M, et al. Regulatory T cells participate in the recovery of ischemic stroke patients. *BMC Neurol*. 2020;20(1):68. doi: 10.1186/s12883-020-01648-w
70. Chan A, Yan J, Csurhes P, et al. Circulating brain derived neurotrophic factor (BDNF) and frequency of BDNF positive T cells in peripheral blood in human ischemic stroke: Effect on outcome. *J Neuroimmunol*. 2015;(286):42–47. doi: 10.1016/j.jneuroim.2015.06.013
71. Pang X, Qian W. Changes in regulatory T-cell levels in acute cerebral ischemia. *J Neurol Surg A Cent Eur Neurosurg*. 2017;78(4):374–379. doi: 10.1055/s-0037-1599055
72. Booth NJ, McQuaid AJ, Sobande T, et al. Different proliferative potential and migratory characteristics of human CD4+ regulatory T cells that express either CD45RA or CD45RO. *J Immunol*. 2010;184(8):4317–4326. doi: 10.4049/jimmunol.0903781
73. Deng G, Tang Y, Xiao J, et al. Naïve-memory regulatory T cells ratio is a prognostic biomarker for patients with acute ischemic stroke. *Front Aging Neurosci*. 2023;(15):1072980. doi: 10.3389/fnagi.2023.1072980
74. Li S, Huang Y, Liu Y, et al. Change and predictive ability of circulating immunoregulatory lymphocytes in long-term outcomes of acute ischemic stroke. *J Cereb Blood Flow Metab*. 2021;41(9):2280–2294. doi: 10.1177/0271678X21995694
75. Yan J, Greer JM, Etherington K, et al. Immune activation in the peripheral blood of patients with acute ischemic stroke. *J Neuroimmunol*. 2009;206(1-2):112–117. doi: 10.1016/j.jneuroim.2008.11.001
76. Wang M, Thomson AW, Yu F, et al. Regulatory T lymphocytes as a therapy for ischemic stroke. *Semin Immunopathol*. 2023;45(3):329–346. doi: 10.1007/s00281-022-00975-z
77. Xiao S, Jin H, Korn T, et al. Retinoic acid increases Foxp3+ regulatory T cells and inhibits development of Th17 cells by enhancing TGF-beta-driven Smad3 signaling and inhibiting IL-6 and IL-23 receptor expression. *J Immunol*. 2008;181(4):2277–2284. doi: 10.4049/jimmunol.181.4.2277
78. Manicassamy S, Pulendran B. Retinoic acid-dependent regulation of immune responses by dendritic cells and macrophages. *Seminars Immunol*. 2009;21(1):22–27. doi: 10.1016/j.smim.2008.07.007
79. Patent RUS RU 2791738 C1. Shardina KYu, Zamorina SA, Bochkova MS, et al. A method for obtaining autologous regulatory T-lymphocytes by culturing ex vivo in the presence of chorionic gonadotropin. (In Russ). Патент РФ № RU 2791738 C1. Шардина К.Ю., Заморина С.А., Бочкова М.С., и др. Способ получения аутологичных регуляторных Т-лимфоцитов путем культивирования ex vivo в присутствии хорионического гонадотропина. Режим доступа: https://patents.google.com/patent/RU2791738C1/ru. Дата обращения: 15.08.2023.
80. Golovina TN, Mikheeva T, Brusko TM, et al. Retinoic acid and rapamycin differentially affect and synergistically promote the

https://doi.org/10.17816/clinpract568210

journal of clinical practice 2023 Vol 14 №3

ex vivo expansion of natural human T regulatory cells. *PloS One*. 2011;6(1):e15868. doi: 10.1371/journal.pone.0015868
81. Battaglia M, Stabilini A, Roncarolo MG. Rapamycin selectively expands CD4+CD25+FoxP3+regulatory T cells. *Blood*. 2005; 105(12):4743–4748. doi: 10.1182/blood-2004-10-3932
82. Chapman NM, Zeng H, Nguyen TL, et al. mTOR coordinates transcriptional programs and mitochondrial metabolism of activated Treg subsets to protect tissue homeostasis. *Nat Commun*. 2018;9(1):2095. doi: 10.1038/s41467-018-04392-5
83. Chan MW, Chang CB, Tung CH, et al. Low-dose 5-aza-2'-deoxycytidine pretreatment inhibits experimental autoimmune encephalomyelitis by induction of regulatory T cells. *Mol Med*. 2014;20(1):248–256. doi: 10.2119/molmed.2013.00159
84. Polansky JK, Kretschmer K, Freyer J, et al. DNA methylation controls Foxp3 gene expression. *Eur J Immunol*. 2008; 38(6):1654–1663. doi: 10.1002/eji.200838105
85. Zhang H, Xia Y, Ye Q, et al. In vivo expansion of regulatory T cells with IL-2/IL-2 antibody complex protects against transient ischemic stroke. *J Neurosci*. 2018;38(47):10168–10179. doi: 10.1523/JNEUROSCI.3411-17.2018
86. Greilach SA, McIntyre LL, Nguyen QH, et al. Presentation of human neural stem cell antigens drives regulatory T cell induction. *J Immunol*. 2023;210(11):1677–1686. doi: 10.4049/jimmunol.2200798
87. Xia Y, Hu G, Chen Y, et al. Embryonic stem cell derived small extracellular vesicles modulate regulatory T cells to protect against ischemic stroke. *ACS Nano*. 2021;15(4):7370–7385. doi: 10.1021/acsnano.1c00672
88. Guo S, Luo Y. Brain Foxp3+ regulatory T cells can be expanded by Interleukin-33 in mouse ischemic stroke. *Int Immunopharmacol*. 2020;(81):106027. doi: 10.1016/j.intimp.2019
89. Shu L, Xu C, Yan ZY, et al. Post-Stroke microglia induce Sirtuin2 expression to suppress the anti-inflammatory function of infiltrating regulatory T cells. *Inflammation*. 2019;42(6): 1968–1979. doi: 10.1007/s10753-019-01057-3
90. Pan Z, Ma G, Kong L, Du G. Hypoxia-inducible factor-1: Regulatory mechanisms and drug development in stroke. *Pharmacol Res*. 2021;(170):105742. doi: 10.1016/j.phrs.2021.105742
91. Yu HH, Ma XT, Ma X, et al. Remote limb ischemic postconditioning protects against ischemic stroke by promoting regulatory T cells thriving. *J Am Heart Assoc*. 2021;10(22):e023077. doi: 10.1161/JAHA.121.023077
92. Zhang Y, Liesz A, Li P. Coming to the rescue: Regulatory T cells for promoting recovery after ischemic stroke. *Stroke*. 2021;52(12):e837–e841. doi: 10.1161/STROKEAHA.121.036072
93. Liu C, Li N, Liu G. The role of MicroRNAs in regulatory T cells. *J Immunol Res*. 2020;2020:3232061. doi: 10.1155/2020/3232061
94. Kadir RR, Alwjwaj M, Bayraktutan U. MicroRNA: An emerging predictive, diagnostic, prognostic and therapeutic strategy in ischaemic stroke. *Cell Mol Neurobiol*. 2022;42(5):1301–1319. doi: 10.1007/s10571-020-01028-5
95. Xu W, Gao L, Zheng J, et al. The roles of MicroRNAs in stroke: Possible therapeutic targets. *Cell Transplant*. 2018;27(12): 1778–1788. doi: 10.1177/0963689718773361
96. Copsel SN, Malek TR, Levy RB. Medical treatment can unintentionally alter the regulatory T cell compartment in patients with widespread pathophysiologic conditions. *Am J Pathol*. 2020;190(10):2000–2012. doi: 10.1016/j.ajpath.2020.07.012
97. Poluektov MG, Narbut AM, Shuvakhina NA. The use of melatonin as a neuroprotector in ischemic stroke. *Medical Council*. 2019;(18):18–24. (In Russ). Полуэктов М.Г., Нарбут А.М., Шувахина Н.А. Применение мелатонина в качестве нейропротектора при ишемическом инсульте // *Медицинский совет*. 2019. № 18. С. 18–24.
98. Ren W, Liu G, Chen S, et al. Melatonin signaling in T cells: Functions and applications. *J Pineal Res*. 2017;62(3):e12394. doi: 10.1111/jpi.12394
99. Medrano-Campillo P, Sarmiento-Soto H, Álvarez-Sánchez N, et al. Evaluation of the immunomodulatory effect of melatonin on the T-cell response in peripheral blood from systemic lupus erythematosus patients. *J Pineal Res*. 2015;58(2):219–226. doi: 10.1111/jpi.12208
100. Sharma S, Nozohouri S, Vaidya B, Abbruscato T. Repurposing metformin to treat age-related neurodegenerative disorders and ischemic stroke. *Life Sci*. 2021;(274):119343. doi: 10.1016/j.lfs.2021.119343
101. Lee SK, Park MJ, Jhun JY, et al. Combination treatment with metformin and tacrolimus improves systemic immune cellular homeostasis by modulating Treg and Th17 imbalance. *Front Immunol*. 2021;(11):581728. doi: 10.3389/fimmu.2020.581728
102. Wang Z, Kawabori M, Houkin K. FTY720 (Fingolimod) ameliorates brain injury through multiple mechanisms and is a strong candidate for stroke treatment. *Curr Med Chem*. 2020; 27(18):2979–2993. doi: 10.2174/0929867326666190308133732
103. Malone K, Diaz Diaz AC, Shearer JA, et al. The effect of fingolimod on regulatory T cells in a mouse model of brain ischaemia. *J Neuroinflammation*. 2021;18(1):37. doi: 10.1186/s12974-021-02083-5
104. Noh MY, Lee WM, Lee SJ, et al. Regulatory T cells increase after treatment with poly (ADP-ribose) polymerase-1 inhibitor in ischemic stroke patients. *Int Immunopharmacol*. 2018;(60): 104–110. doi: 10.1016/j.intimp.2018.04.043
105. Luo X, Nie J, Wang S, et al. Poly(ADP-ribosyl)ation of FOXP3 protein mediated by PARP-1 protein regulates the function of regulatory T cells. *J Biol Chem*. 2015;290(48):28675–82. doi: 10.1074/jbc.M115.661611
106. Arpaia N, Campbell C, Fan X, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504(7480):451–455. doi: 10.1038/nature12726
107. Akimova T, Ge G, Golovina T, et al. Histone/protein deacetylase inhibitors increase suppressive functions of human FOXP3+ Tregs. *Clin Immunol*. 2010;136(3):348–363. doi: 10.1016/j.clim.2010.04.018
108. Ao LY, Yan YY, Zhou L, et al. Immune cells after ischemic stroke onset: Roles, migration, and target intervention. *J Mol Neurosci*. 2018;66(3):342–355. doi: 10.1007/s12031-018-1173-4
109. Jickling GC, Liu D, Stamova B, et al. Hemorrhagic transformation after ischemic stroke in animals and humans. *J Cereb Blood Flow Metab*. 2014;34(2):185–199. doi: 10.1038/jcbfm.2013.203
110. Pinheiro MA, Kooij G, Mizee MR, et al. Immune cell trafficking across the barriers of the central nervous system in multiple sclerosis and stroke. *Biochim Biophys Acta*. 2016;1862(3): 461–471. doi: 10.1016/j.bbadis.2015.10.018
111. Zhang D, Ren J, Luo Y, et al. T cell response in ischemic stroke: From mechanisms to translational insights. *Front Immunol*. 2021;(12):707972. doi: 10.3389/fimmu.2021.707972
112. Stubbe T, Ebner F, Richter D, et al. Regulatory T cells accumulate and proliferate in the ischemic hemisphere for up to 30 days after MCAO. *J Cereb Blood Flow Metab*. 2013;33(1):37–47. doi: 10.1038/jcbfm.2012.128
113. Yu H, Cai Y, Zhong A, et al. The «dialogue» between central and peripheral immunity after ischemic stroke: Focus on spleen. *Front Immunol*. 2021;(12):5194. doi: 10.3389/fimmu.2021.792522
114. Belopasov VV, Zhuravleva EN, Nugmanova NP, Abdrashitova AT. Postcovid neurological syndromes. *Clin Pract*. 2021;12(2):69–82. (In Russ). Белопасов В.В., Журавлева Е.Н., Нугманова Н.П., Абдрашитова А.Т. Постковидные неврологические синдромы // *Клиническая практика*. 2021. Т. 12, № 2. С. 69–82. doi: 10.17816/clinpract71137
115. Ahmad SJ, Feigen CM, Vazquez JP, et al. Neurological Sequelae of COVID-19. *J Integr Neurosci*. 2022;21(3):77. doi: 10.31083/j.jin2103077
116. Belopasov VV, Yachou Y, Samoilova EM, Baklaushev VP. The nervous system damage in COVID-19. *J Clin Pract*. 2020. Vol. 11, N 2. P. 60–80. doi: 10.17816/clinpract34851
117. Katsanos AH, Palaiodimou L, Zand R, et al. The impact of SARS-CoV-2 on stroke epidemiology and care: A meta-analysis. *Ann Neurol*. 2021;89:380–388. doi: 10.1002/ana.25967
118. Sadeghi A, Tahmasebi S, Mahmood A, et al. Th17 and Treg cells function in SARS-CoV-2 patients compared with

healthy controls. *J Cell Physiol*. 2021;236(4):2829–2839. doi: 10.1002/jcp.30047
119. Wang H, Wang Z, Cao W, et al. Regulatory T cells in COVID-19. *Aging Dis*. 2021;12(7):1545–1553. doi: 10.14336/AD.2021.0709
120. Wang HY, Ye JR, Cui LY, et al. Regulatory T cells in ischemic stroke. *Acta Pharmacol Sin*. 2022;43(1):1–9. doi: 10.1038/s41401-021-00641-4
121. Dhawan M, Rabaan AA, Alwarthan S, et al. Regulatory T Cells (Tregs) and COVID-19: Unveiling the mechanisms, and therapeutic potentialities with a special focus on long COVID. *Vaccines (Basel)*. 2023;11(3):699. doi: 10.3390/vaccines11030699
122. Haunhorst S, Bloch W, Javelle F, et al. A scoping review of regulatory T cell dynamics in convalescent COVID-19 patients: Indications for their potential involvement in the development of Long COVID? *Front Immunol*. 2022;(13):1070994. doi: 10.3389/fimmu.2022
123. Meckiff BJ, Ramírez-Suástegui C, Fajardo V, et al. Imbalance of regulatory and cytotoxic SARS-CoV-2-Reactive CD4+ T Cells in COVID-19. *Cell*. 2020;183(5):1340–1353.e16. doi: 10.1016/j.cell.2020.10.001
124. Hoffmann AD, Weinberg SE, Swaminathan S, et al. Unique molecular signatures sustained in circulating monocytes and regulatory T cells in convalescent COVID-19 patients. *Clin Immunol*. 2023;(252):109634. doi: 10.1016/j.clim.2023.109634
125. Rizzo PA, Bellavia S, Scala I, et al. COVID-19 vaccination is associated with a better outcome in acute ischemic stroke patients: A retrospective observational study. *J Clin Med*. 2022;11(23):6878. doi: 10.3390/jcm11236878

AUTHORS' INFO

The author responsible for the correspondence:
Oksana A. Zhukova, Research Associate;
address: 28 Orekhovy boulevard, 115682 Moscow, Russia;
ORCID: 0000-0002-0907-0078;
e-mail: Oksana.saprikina82@mail.ru

Co-authors:
Daria A. Chudakova, PhD, Senior Researcher;
ORCID: 0000-0002-9354-6824;
eLibrary SPIN: 1410-9581;
e-mail: daria.chudakova.bio@yandex.ru

Vladimir V. Belopasov, MD, PhD, Dr. Sci. (Med.), Professor;
ORCID: 0000-0003-0458-0703;
eLibrary SPIN: 6098-1321; e-mail: belopasov@yandex.ru

Elena V. Shirshova, MD, PhD, Dr. Sci. (Med.), Professor;
ORCID: 0000-0002-3557-5424;
eLibrary SPIN: 7491-0434; e-mail: shirshova.ev@fnkc-fmba.ru

Vladimir P. Baklaushev, MD, PhD, Dr. Sci. (Med.), Assistant Professor;
ORCID: 0000-0003-1039-4245;
eLibrary SPIN: 3968-2971;
e-mail: baklaushev.vp@fnkc-fmba.ru

Gaukhar M. Yusubalieva, MD, PhD;
ORCID: 0000-0003-3056-4889;
eLibrary SPIN: 1559-5866; e-mail: gaukhar@gaukhar.org

ОБ АВТОРАХ

Автор, ответственный за переписку:
Жукова Оксана Анатольевна, н.с.;
адрес: Россия, 115682, Москва, Ореховый б-р, д. 28;
ORCID: 0000-0002-0907-0078;
e-mail: Oksana.saprikina82@mail.ru

Соавторы:
Чудакова Дарья Александровна, к.б.н., с.н.с.;
ORCID: 0000-0002-9354-6824;
eLibrary SPIN: 1410-9581;
e-mail: daria.chudakova.bio@yandex.ru

Белопасов Владимир Викторович, д.м.н., профессор;
ORCID: 0000-0003-0458-0703;
eLibrary SPIN: 6098-1321; e-mail: belopasov@yandex.ru

Ширшова Елена Вениаминовна, д.м.н., профессор;
ORCID: 0000-0002-3557-5424;
eLibrary SPIN: 7491-0434; e-mail: shirshova.ev@fnkc-fmba.ru

Баклаушев Владимир Павлович, д.м.н., доцент;
ORCID: 0000-0003-1039-4245;
eLibrary SPIN: 3968-2971;
e-mail: baklaushev.vp@fnkc-fmba.ru

Юсубалиева Гаухар Маратовна, к.м.н.;
ORCID: 0000-0003-3056-4889;
eLibrary SPIN: 1559-5866; e-mail: gaukhar@gaukhar.org

https://doi.org/10.17816/clinpract568210