Chapter 6

Regulatory T Lymphocytes (Treg): Modulation and Clinical Application

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Abstract

Treg cells CD4+CD25+FOXP3+ have a specific function in the tolerance of autoantigens and regulation of the immune response. Modulation of differentiation pathways and the use of Treg cells in cell therapy have been reported in autoimmune diseases, systemic lupus erythromatosis, autoimmune hepatitis, type 1 diabetes mellitus, multiple sclerosis, rheumatoid arthritis, graft-versus-host disease, bone marrow transplantation and solid organs. The expansion of Treg cells *in vivo* occurs through low-dose IL-2 treatment. However, because of the heterogeneity and variability of Treg cells, the isolation of peripheral blood cells, through the technique of leucopheresis by GMP (good manuring practice), for *in vitro* expansion is difficult, necessitating a large combination of specific and reliable cellular markers. Currently, two specific markers, Helios and neuropilin-1, are being studied to facilitate the differentiation of thymus Treg cells and peripheral Treg cells. However, Treg cells induced in vitro are unstable. Modulation of the FOXP3 gene in the CNS1 and CNS2 region is an alternative to maintaining the stability of expanded Treg cells *in vitro*.

Keywords: autoimmune tolerance, cell therapy, heterogeneity treg, FOXP3+, regulatory T

1. Introduction

Regular T lymphocytes (Treg) were first described in the year 1970 in murine models [1]. They are subpopulations of T lymphocytes defined by the expression of CD4+ and CD25+ molecules,

as well as by the transcription factor FOXP3 (*forkhead box P3*). Treg cells maintain self-tolerance and immune homeostasis through immune responses against self and non-self antigens and in fetal-maternal self-tolerance. Regulation of the immune response occurs through suppression of effector T cells, minimizing the production of cells of adaptive immunity and innate immunity [2, 3]. The suppressor function of Treg cells is directed by the transcription factor FOXP3, occurring in a non-random manner. However, a deregulation in the Treg cells can make them autoreactive with the recognition of autoantigens, developing autoimmune diseases [4]. The organism acts naturally against self-reactive T cells through the process of negative selection by inactivation or clonal deletion in the thymic tissue, and genetic mutations contribute to self-tolerance in the thymus [5].

FOXP3 expression is initiated through a combination of antigen recognition, microenvironmental influences, and epigenetic factors. FOXP3 is present in about 10–20% of the T cells. Peripheral induction of FOXP3 expression may occur in the colon and placenta [6, 7]. Treg cells exhibit high expression of the IL-2α receptor (CD25) and low expression of CD127 [8]. Changes or loss of FOXP3 is associated with the development of collagenases and vasculitis, rheumatoid arthritis, mixed connective tissue disease, Kawasaki disease, Wegener's granulomatosis, systemic lupus erythematosus and Sjögren's syndrome, enteropathies, type 1 diabetes, thyroiditis and eczema [9]. The various clinical alterations of which Treg cells are present, the use of Treg as cell therapy with *in vitro* and *ex vivo* expansion has been a research alternative for certain treatments with the development of tolerance and autoimmunity [10].

2. Treg cell heterogeneity

Treg cells account for 5–10% of peripheral CD4+ T cells in humans and rats. Treg cells that grow in the thymus are called natural (nTreg) or thymic (tTreg) Tregs, and Treg cells that develop at the periphery by specific stimuli of conventional CD4+ T cells are termed pTreg cells. When Treg cells are induced *in vitro* are called iTreg [11]. Treg cell generation in the thymus and peripheral tissues occurs in response to T cell receptor (TCR) and cytokine receptor signaling. Natural Treg cells are generated during the period of positive selection of CD4+ T cells by expression of the transcription factor of the FOXP3 gene in the thymus. FOXP3 expression is controlled by conserved noncoding sequences (CNS) in the promoter region of the gene and by intronic regulatory sequences [12]. TGF-β, IL-2, and TCR are required for FOXP3 gene expression during cell differentiation [13]. The promoter region of the FOXP3 gene is activated by the NF-kB pathway, NFAT (nuclear factor of activated T), the transcription factor SMAD-3, the retinoic acid produced by dendritic cells and epithelial cells, rapamycin and NR4As proteins [14–16].

However, there is a small portion of Treg that does not express FOXP3 is known as regulatory T type 1 (Tr1) with phenotype CD41+CD49b1+LAG-31+CD2261+FOXP3−. These cells are induced by the chronic activation of CD4+ by antigens in the presence of IL-10 and are responsible for peripheral immunotolerance. It is possible to distinguish the Tr1 from other CD4+ populations from the expression of the cytokines: IL-10++TGF-b+IFN-γ+IL-5+IL-4

IL-2$^{-low/neg}$ [10]. T cells expressing FOXP3 circulate through the secondary lymphoid tissues as "central" Tregs. Activation signals involving T cell receptors (TCR), co-stimulation of CD28 and/or interleukin-2 (IL-2) induce positive regulation of interferon regulating factor 4 (IRF4) expression, promoting the differentiation of Treg cells "central" in "effector" Treg cells. Unknown stimuli induce the polarization of Treg cells by upregulation of transcription factors that may act in conjunction with FOXP3 to induce the expression of chemokine receptors that mediate recruitment to tissues or sites of inflammation [17, 18]. During activation of TCR in a cytokine medium, T CD4+ cells can differentiate into various T cell lines, T helper (Th), including Th1, Th2, Th17, Th17, and iTreg, as defined by their production standard and function of cytokines [19].

Thymic Treg cells are generated in the medulla and/or the medullo-cortex junctions in the thymus and arise from a thimocyte CD4+CD8−. TCR plays an essential role in the differentiation of Treg cells, but it does not act alone. TCR signaling along with co-stimulation of nuclear factor activated T-cell (NF-AT), an activator of protein-1 (AP-1) and CARMA1/Bcl10/Malt1 NF-kB, acts on the FOXP3 gene to induce its expression. In addition, signaling via tumor growth factor β (TGF-β), interleukin-2 (IL-2) and transcription activator-5 (STAT5) induce signs of stimuli for expression of the Foxp3 gene, so that differentiation of naive T occurs Treg. Through stimuli by TGF-β tTreg can differentiate into pTreg [20–22]. The deficiency of TCR signaling mediators such as TAK1, Bcl10, CARMA1, PKCh, and IKKb reduces the number of Treg cells generated in the thymus without affecting the generation of conventional T cells. This decrease in Treg compromises immunotolerance and equilibration of the immune system [23].

2.1. Treg cell subtypes

Treg naive cells are recognized by the phenotype FOXP3loCD45RA+CD45RO−, and Treg cells are FOXP3hiCD45RA−CD45RO+ and express both the Fas receptor (CD95) and cytotoxic T-lymphocyte antigen (CTLA-4). A small part of Treg has a phenotype ICOS+IL-10+. Treg cells expressing FOXP3 exhibit their immunoregulatory activity by a variety of effector mechanisms such as CTLA-4 uptake, IL-2 uptake, IL-10, TGF-β, IL-35 and galactin-1 production. The environment in which Treg cells are found alters the mechanisms by which they exert suppressive activity. Identification of CD45RA or CD45RO molecules, when combined with CD25 and/or FOXP3, is useful for identifying naive reg T cells. Human CD45RO+CD25hiCD4+Treg cells are similar to rat Treg cells in CD25 expression. The Treg CD45RA+FOXP3+ and CD45RO+FOXP3+ cells are functionally different but are related to the development of immunosuppression. Expression of the transcription factor of the B lymphocyte-induced maturation protein (Blimp-1) is common to all Treg cells [15, 24–26].

Differentiation of naive Treg gives rise to subtypes of Treg CD4+CD25FOXP3+: tTreg, pTreg, and iTreg. With the use of Treg cells in the therapy of human diseases, it is important to distinguish between cell subtypes. Expression in the Helios molecule can be effective in differentiating between the subtypes of tTreg and iTreg/pTreg [21]. Helios is expressed in the thymus, so it may be a marker to identify tTreg from the other populations of Treg, and it acts as upregulation in FOXP3 protein [27–29]. However, the Helios molecule can be found

in iTregs and pTregs depending on the type of antigen presenting cells and signals found. Cells that super-expression Helios have a superior effect of peripheral immunosuppression. This feature can be used in treatments for autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) [30]. Through *in vitro* induction of Helios in Treg cells, it was found that CD103 and GITR are expressed at high levels in a subset of cells Treg Helios+. These markers together can be used to differentiate Treg subtypes from each other [21, 31]. Another alternative, the Nrp1 protein (neuropilin-1) is expressed in tTreg cells as opposed to iTreg and pTreg cells [31].

The pTreg cells are present in the intestine and mucosa. Dendritic cells present in the mucosa, especially CD103+ induce FOXP3+ Tregs through the production of TGF-β and retinoic acid [32]. Retinoic acid binds to its receptor, RAR, generating induction signals in the CNS1 region of the FOXP3 gene. This signal induction leads to increased histone acetylation in the region of the CNS1, the SMAD3 binding sites, and increased phosphorylated SMAD3 binding, inducing expression of FOXP3, and originating pTreg cells in the gut. The microbiota also promotes cell differentiation of TCD4+ in Treg CD4+FOXP3+. Most of the Tregs in the intestine coexpress FOXP3 and RORgt, a Th17 regulator, as well as T-bet, GATA3 or IRF4, proteins that present suppression role in the Th1/Th2 response, maintaining the immunological tolerance and acting in the resistance to pathological infections originated microorganisms present in the mucosae [33].

2.2. FOXP3 and autoimmunity

FOXP3 is the main labeled Treg cell. The key role of FOXP3 in the immune response of Treg has been described in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) and in rats scurfy [34, 35]. Patients and scurfy mice present monogenic mutations in the FOXP3 gene, which confers a phenotype with dysregulation of the immune system with the development of autoimmunity due to lack of Treg cells [36, 37]. Patients with IPEX present mutations in a Phe367 residue of the F367V protein, F367L, and F367C, which make up the FOXP3 protein [38]. Polymorphic changes in the promoter region of the FOXP3 gene contribute to loss of function and dysregulation of FOXP3, as well as the development of polygenic autoimmune diseases [39]. However, there is no dominant mechanism for the development of autoimmunity. Factors affecting FOXP3 function, effects of genetic alterations, altered signaling, and expression of FOXP3 messenger RNA (mRNA) or mature protein are related to the development of autoimmunity with an imbalance between conventional Treg cells and pathogenic Tregs [40].

IPEX is a rare genetic disease resulting from the lack of functional Treg cells due to the loss of functional mutations in FOXP3. It affects men exclusively because of its recessive pattern of heredity linked to the X chromosome and is often fatal in the first year of life unless it is rescued with bone marrow transplantation. Clinically, IPEX presents a triad of autoimmune enteropathy, autoimmune endocrinopathy, and eczematous dermatitis. The most common manifestation is enteropathy followed by endocrinopathy, especially type 1 insulin-dependent diabetes mellitus. Additional manifestations described include immune-mediated cytopenia, which may present as neutropenia, anemia and/or thrombocytopenia, and autoimmune

nephropathy, hepatitis, and pulmonary disease. Food allergy with high serum IgE and eosinophilia. Patients with IPEX generally have a wide range of autoantibodies due to adaptive immune dysregulation. The only curative treatment available for this disease is the allogeneic hematopoietic stem cell transplantation with chemotherapy of reduced intensity. Prior to transplantation, patients require nutritional support and immunosuppressive therapy, which may include glucocorticoids and/or steroid-sparing agents, such as calcineurin inhibitors, rapamycin inhibitor (mTOR) [11].

Single nucleotide polymorphism (SNP) 7340C>T is related to the development of autoimmune diseases and allergies in children. This polymorphism alters the stability of FOXP3 mRNA by preventing translation. In addition to SNPs in FOXP3 itself, SNPs in three other loci indirectly affect FOXP3 expression and are associated with autoimmunity: CD25, PTPN2, and PTPN22. All three genes are involved in the response to IL-2. Tregs do not produce IL-2, but this interleukin is essential for its survival and function. The low expression of CD25 is associated as STAT5 after exposure to IL-2, with decreased Tregs throughout life in patients with type 1 diabetes and multiple sclerosis. The polymorphism rs3761549 G/A is related to the development of Graves' disease in children. These changes related to FoxP3 protein are commonly known as IPEX-type changes [40–43].

3. Treg-cell therapy

The use of Treg cells in clinical practice was made possible after isolation and enhancement of GMP (good manuring practice) CD4+CD25+ Treg cells with a yield of approximately 90% Treg, allowing the cryopreservation and expansion of these cells. Cell therapy with Treg has a purpose in the treatment of diseases, which result in a decompensated or undesired suppressed Treg activity in cancer, immunoglobulin deficiency, autoimmune or inflammatory diseases, and deleterious consequences of immunosuppression after organ transplantation. Therefore, the manipulation of Treg cells can control the progression of cancer through cell configurations, solid organ transplantation and hematopoietic cells, transplant rejection, and autoimmune diseases. The concept of cellular immunotherapy with Treg is to give the patient Treg cells to decrease the exaggerated immune response to autoimmune diseases, organ transplants and bone marrow [44–47]. The nTreg cells, *in vitro*, can be expanded by antigenic stimulation in the presence of a high concentration of IL-2. *In vivo*, low-dose IL-2 treatment increases Treg expansion and is used in the treatment of graft versus host disease (GVHD) and hepatitis C virus-induced cryoglobulinemic vasculitis and, together with rapamycin. Low dose IL-2 was chosen to preferentially expand Treg cells without also expanding activated effector T cells. Modulation of IL-2 homeostasis is an important mechanism by which Treg modulates effector differentiation of CD8+ under strongly immunogenic conditions [48, 49]. Some authors suggest the use of rapamycin for Treg expansion *in vivo* with an approximately 75–80% yield of pure cells and total depletion of CD8 and CD19 [50–52].

Three categories of GMP-grade clinical Treg can define: first generation (CD4+CD25+); second generation, bone fide Treg (CD4+CD25+CD127$^{low/-}$) and third generation naive Treg (CD4+CD25+CD127$^{low/-}$CD45Ra+). These three types of Treg can be isolated and expanded

by Il-2 [46]. The main obstacle to Tregs expansion in the laboratory is iTregs instability. The use of the Nrp1 protein reduces the phosphorylation of the Akt protein, promoting cell stability. Demethylation of the CpG islands in the CNS2 region at the FOXP3 locus recruits transcription factors, including STAT5, NFAT, Runx1/Cbfb, CREB and FOXP3 itself, making the tTregs stable. However, demethylation in the CNS2 region of FOXP3 is known to render iTregs unstable. The control of the methylation/demethylation processes of the CNS1 and CNS2 regions of the FOXP3 gene of the iTregs is still not possible with 100% efficacy in the laboratory. In this way, it compromises clonal expansion *in vitro*, and several transcription factors are involved [47, 53].

3.1. Graft-versus-host disease (GVHD)

Allogeneic stem cell transplantation presents a series of problems, among which we can highlight GVHD, with high mortality rates of 15–30% in transplanted patients and 50% of morbidity. Treg cells are a novel approach based on cellular immunotherapy to reduce the risk of severe acute lesions of graft versus host disease (aGVHD). These lesions may occur within 100 days after transplantation. Chronic GVHD takes about 2–5 years for the signs and symptoms of the diseases to appear in the transplanted patient due to the presence of effector T cells in the marrow receptor tissue. To combat this reaction, aggressive immunosuppressive therapies are started, often unsuccessful. Despite the advances in GVHD treatment, the high rates of death are still high [54–56]. The development of acute and chronic forms of GVHD is different with signs and symptoms because it involves cells cytotoxic TCD8+ and helper TCD4+, and these cells activate different pathways in the autoimmune response. The pathway involving donor TCD8+ cells is activated when binding of TCR to major class I histocompatibility complex (MHC-I) peptides occurs, and interaction the patient antigen-presenting cells (APCs), with the release of granzins, perforins, and production of inflammatory cytokines. Although activation of donor TCD4+ cells results in the activation of a Th1 inflammatory response with high production of INF-Y, IL-12, and IL-2, or a Th2-mediated inflammatory response with extensive production of IL-4, IL-5, IL-6 and IL-10 [54, 57].

After the haematopoietic stem cell transplantation (HSCT), reconstituted Treg cells express markers of recent thymic emigrants. These markers increase the number of native Treg cells in the population of graft-derived Treg cells after HSCT. Second, Treg Helios+ cells from patients receiving HSCT express higher levels of naive markers (such as CD45RA and CD31) than those from patients with active systemic lupus erythromatosus. This increase in Treg controls the immune responses of Th1 and Th2. Infusion of Treg cells into HSCT has been explored in murine and human models. Infusion of Treg cells during allogenic HSCT reduces acute and chronic graft-versus-host disease [58]. Patients with HSCT demonstrated that the use of IL-2 at the dose of 1×10^6 IU per square meter decreases the chances of developing GVHD, in which the number of Treg increased due to activation of the FOXP3+ gene by IL-2, and the amount of Tcon decreased [51]. The immunological reaction of cGVHD exhibits phosphorylation of the transcription factor STAT5, increase of IL-17, IL-15 and deficiency or decrease of IL-2, The IL-2 therapy increased cell proliferation in the thymic and decrease apoptosis by activates phosphorylation of STAT5 [59]. Another alternative for Treg expansion is CD28 stimulation

of pTregs, which results in a polyclonal expansion and preservation of a Treg phenotype and function as indicated by the high level FOXP3/Helios expression, reduced prokaryotic cytokine expression, inflammatory and powerful suppressive function [60]. Tr1 cells express CD49b and Lag3, producing IL-10. Tr1 cells play a role in tolerance but are distinct from FOXP3+ Tregs. Studies to examine these cells in organ transplantation are being initiated. The association between immunosuppressive drugs that increase the serum concentration of IL-2 to induce Treg has also been gaining strength in the treatment of GVHD [61–65].

3.2. Type 1 diabetes

Type 1 diabetes mellitus (T1DM) is a chronic disease that results from the autoimmune destruction of insulin-producing pancreatic beta cells. May be associated with the development of IPEX [66]. The death of β cells occurs due to exposure of their antigens to MHC class II complex APC cells and presentation to TCD4+ lymphocytes in the lymphatic modules of the pancreas. After presentation, TCD4+ cells differentiate into self-reactive effector TCD4+ (Teffs). The fractions of the complement system C3a and C5a facilitate the expansion and the function of Teff. In pancreatic islets, activated Teffs release cytokines including IFN-γ and IL-2, resulting in the recruitment of cytotoxic T lymphocytes and TCD8+ lymphocytes. Cytotoxic inflammatory cells eventually infiltrate and destroy the islet cells in a process called "insulite", with the release of perforins and granzins AND release of IFN-γ, TNF α, IL-1β by macrophages. Chemokines released by the injured β-cells promote recruitment of additional mononuclear cells and the release of additional autoantigens allows the expansion and propagation of the self-reactive Teff response [67–71]. In the pathogenesis of T1DM, the immune response is exaggerated against its own antigens. There is an imbalance between Tregs and effector T cells. Isolation and expansion ex vivo of Tregs CD25+CD127$^{low/-}$CD25+ showed improved function and retained their diversity of T cell receptors, then these cells were used in T1DM patients. The infusions were well tolerated and with good safety. The use of pharmacotherapy including anti-CD3 therapy, glutamic acid decarboxylase (GAD) injection, hematopoietic stem cell transplantation (HSCT), autologous umbilical cord blood transfusion and stem cell educator therapy has demonstrated efficacy with increased levels of C-peptides and decrease in the daily dose of insulin [72, 73].

The subpopulations of Tregs in T1DM are different when compared to healthy individuals. The proportion of cells CD25$^{low/-}$ between cells Treg CD4+FOXP3+In T1DM patients was higher than in healthy patients. Low or no CD25 expression implies a decrease in Treg cell differentiation with decreased peripheral suppressor activity and increased Teff cell growth [74, 75]. The difficulty in using expanded Tregs ex vivo in patients with T1DM is found in the cells themselves since these cells express CD45RO+ memory phenotype. Another issue is the expression of Helios by lymphocytes in peripheral blood. Expanded lymphocytes in vitro have a lower expression of the molecule on their surface when compared to their own lymphocytes. However, the activity of suppressing autologous or allogeneic TCD8+ effector cells is maintained. Thus, it becomes a good alternative in the treatment of T1DM in the long term. Some studies have conflicting results on the use of Treg cells in the treatment of T1DM since several mechanisms are involved in the T1DM pathology; however, they are in line with

lack, dysregulation or deficiency of local and peripheral Treg [76]. Studies with the use of IL-2 in the treatment of T1DM have demonstrated effective results in the expansion of Treg [77].

3.3. Autoimmune hepatitis and systemic lupus erythematosus

The inflammatory response of autoimmune hepatitis (AIH) involves the B-lymphocytes, T cells, Th1, Th17 and cytotoxic T cells. Studies have shown that the function of the mature FOXP3 protein shows inactivity in T CD4+ cells. Cell therapy, such as the infusion of autologous, antigen-specific and hepatic regulatory T cells to restore hepatic immune tolerance, may soon be a potential future treatment for patients with AIH [78, 79]. Hepatic tolerance is involved in the pathogenesis of autoimmune hepatitis, with an imbalance of immune responses. There are controversies in scientific research regarding the number of Treg present in AHI. Though the modulating function of the cell appears to be compromised [80, 81]. However, in treated patients with IL-2, the Treg number is higher than untreated patients. This fact supports the hypothesis of treating patients with autologous Tregs expanded *ex vivo* and may lead to tolerance to hepatic antigens during the development of chronic disease, with remission of the clinical signs and symptoms of AIH. The use of IL-2 for Treg expansion *in vivo* is an option for treatment in patients with reduced Treg numbers [82–84].

SLE is a chronic autoimmune disease characterized by the production of antinuclear autoantibodies of the IgG type. Symptoms of the disease include light hypersensitivity, impaired joints, thyroid dysfunction, changes in the central nervous system and renal filtration [85]. There is growing evidence that Th1, Th2, Th9, and Th17 cells are associated with the pathogenesis of SLE. Disorders related to the amount, function of Treg show a worse evolution in the disease and decrease the production of IL-2. But studies that demonstrate this commitment are not homogeneous [86]. The use of IL-2 in the treatment of SLE demonstrated decreased exaggerated inflammatory response and increased proliferation of Tregs *in vivo* [87–90]. Treg cells expressing Helios are used with functional suppressive capacity and migratory potential in inflamed tissues is expanded in active SLE, presumably by γ-chain signaling cytokines and TCR stimulation, to compensate for autoreactive effector responses [91]. Treatment with melatonin increases the frequency of CD3+CD4+FOXP3+ cells and the mean fluorescence intensity of FOXP3 in patients with SLE [92].

3.4. Rheumatoid arthritis and multiple sclerosis

Rheumatoid arthritis is characterized by chronic inflammation of the joints, with severe pain and in the long term, loss of movement of the affected joint. In the development of rheumatoid arthritis, Treg cells are unable to suppress inflammatory responses, with an imbalance between Treg effector cells and TCD4+ cells. Low doses of IL-2 increase Treg stimulation in rheumatoid arthritis [93, 94]. In rats, depletion of Treg cells results in the onset of a variety of autoimmune diseases, including arthritis. Treg's cellular replacement relieves the symptoms of the disease. The importance of Treg cells in rheumatoid arthritis is supported by the efficacy of CTLA4-Ig therapy, an increased ratio of Treg cells/effector

T cells after treatment with anti-IL-6R or anti-TNF-α and the identification of CTLA-4 associated with rheumatoid arthritis. FOXP3+ T cells are able to convert into pathogenic Th17 cells. Th17 cells are increased in rheumatoid arthritis, being responsive for the production of inflammatory cytokines and the activation of inflammation in severe cases. The modulation between Treg/Th17 is an alternative for the immunocellular treatment of rheumatoid arthritis [95–97]. Tregs play a key role in protecting individuals against autoimmunity. Many studies suggest that the amount of Treg may be a protective factor against the development of multiple sclerosis. The use of TGF-β may be an alternative aid in the treatment of multiple sclerosis, since Treg and effector T cells are defective. The causes of multiple sclerosis are still unknown, but the immune system plays a central role in the development of the disease [98, 99].

4. Conclusion

The success of Treg cell therapy depends initially on the isolation and characterization of cells. New studies are emerging, with the discovery of new cell markers for the identification of Treg. However, current research does not use a universally applicable standard for Treg identification. This gap in identification leads to conflicting and doubtful research results. The cellular variability of Treg is wide. It is important to characterize the phenotype and suppressor function of each subtype of Treg present in the periphery or in the thymus. The deregulation of these cells leads to the development of autoimmune diseases or the worsening of the clinical picture of these diseases. The FOXP3 protein is responsive to Treg cell suppressor activity, together with other molecules. Understanding the modulation pathway to activate the FOXP3 gene is important for the expansion of stable Tregs in vitro and that in the future we will have effective cellular therapy without damage to the organism.

Conflict of interests

The authors declare that there is no conflict of interest

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References

[1] Gershon RK, Kondo K. Cell interactions in the induction of tolerance: The role of thymic lymphocytes. Immunology. 1970;**18**(5):723-737

[2] Sakaguchi S, Yamaguchi T, Nomura T. and Ono M. Regulatory T cells and imune tolerance. Cell. 2008;**133**:775-787

[3] Ghaebi M, Nouri M, Ghasemzadeh A, Farzadi L, Jadidi-Niaragh F, Ahmadi M, Yousefi M. Immune regulatory network in successful pregnancy and reproductive failures. Biomedicine & Pharmacotherapy. 2017;**88**:61-73. DOI: 10.1016/j.biopha.2017.01.016

[4] Rosenblum MD, Way SS, Abbas AK. Regulatory T cell memory. Nature Reviews Immunology. 2016;**16**(2):90-101. DOI: 10.1038/nri.2015.1

[5] Paiva RS, Lino AC, Bergman M-L, et al. Recent thymic emigrants are the preferential precursors of regulatory T cells differentiated in the periphery. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(16):6494-6499. DOI: 10.1073/pnas.1221955110

[6] Schumacher A, Zenclussen AC. Regulatory T cells: regulators of life. American Journal of Reproductive Immunology. 2014;**72**(2):158-170. DOI: 10.1111/aji.12238

[7] Churchman SM, El-Jawhari JJ, Burska AN, et al. Modulation of peripheral T-cell function by interleukin-7 in rheumatoid arthritis. Arthritis Research & Therapy. 2014;**16**(6):511. DOI: 10.1186/s13075-014-0511-3

[8] Li X, Zheng Y. Regulatory T cell identity: Formation and maintenance. Trends in immunology. 2015;**36**(6):344-353. DOI: 10.1016/j.it.2015.04.006

[9] Gomez-Tourino I, Arif S, Eichmann M, Peakman M. T cells in type 1 diabetes: Instructors, regulators and effectors: A comprehensive review. Journal of Autoimmunity. 2016;**66**:7-16. DOI: 10.1016/j.jaut.2015.08.012

[10] Zeng H, Zhang R, Jin B, Chen L. Type 1 regulatory T cells: A new mechanism of peripheral immune tolerance. Cellular & Molecular Immunology. 2015;**12**(5):566-571. DOI: 10.1038/cmi.2015.44

[11] Alroqi FJ, Chatila TA. T Regulatory cell biology in health and disease. Current Allergy and Asthma Reports. 2016;**16**(4):27. DOI: 10.1007/s11882-016-0606-9

[12] Wing JB, Sakaguchi S. Foxp3^{+} T(reg) cells in humoral immunity. International Immunology. 2014;**26**(2):61-69. DOI: 10.1093/intimm/dxt060

[13] Kim CH. FOXP3 and its role in the immune system. Advances in Experimental Medicine and Biology. 2009;**665**:17-29

[14] Won HY, Hwang ES. Transcriptional modulation of regulatory T cell development by novel regulators NR4As. Archives of Pharmacal Research. 2016;**39**(11):1530-1536

[15] Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. Nature Reviews Immunology. 2010;**10**(7):490-500. DOI: 10.1038/nri2785

[16] Sekiya T, Kashiwagi I, Inoue N, Morita R, Hori S, Waldmann H, et al. The nuclear orphan receptor Nr4a2 induces Foxp3 and regulates differentiation of CD4+ T cells. Nature Communications. 2011;**2**:269. DOI: 10.1038/ncomms1272

[17] Lu L, Zhou X, Wang J, Zheng SG, Horwitz DA. Characterization of protective human CD4CD25 FOXP3 regulatory T cells generated with IL-2, TGF-β and retinoicacid. PLoS One. 2010;**5**(12):e15150. DOI: 10.1371/journal.pone.0015150

[18] Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4 + T cells expressing the FoxP3 transcription factor. Immunity. 2009;**30**(6):899-911. DOI: 10.1016/j.immuni.2009.03.019

[19] Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations (*). Annual Review of Immunology. 2010;**28**:445-489. DOI**:** 10.1146/annurev-immunol-030409-101212

[20] Chen W, Konkel JE. The development of thymic Foxp3+ regulatory T cells: TGF-β matters. European Journal of Immunology. 2015;**45**(4):958-965. DOI: 10.1002/eji.201444999

[21] Yuan X, Cheng G, Malek TR. The importance of regulatory T-cell heterogeneity in maintaining self-tolerance. Immunological Reviews. 2014;**259**(1):103-114. DOI: 10.1111/imr.12163

[22] Hall BM, Tran GT, Robinson CM, Hodgkinson SJ. Induction of antigen specific CD4(+) CD25(+)Foxp3(+)T regulatory cells from naïve natural thymic derived T regulatory cells. International Immunopharmacology. 2015;**28**(2):875-886. DOI: 10.1016/j.intimp.2015.03.049

[23] Oh H, Ghosh S. NF-κB: Roles and regulation in different CD4(+) T-cell subsets. Immunological Reviews. 2013;**252**(1):41-51. DOI: 10.1111/imr.12033

[24] Cretney E, Kallies A, Nutt SL. Differentiation and function of Foxp3(+) effector regulatory T cells. Trends in Immunology. 2013;**34**(2):74-80. DOI: 10.1016/j.it.2012.11.002

[25] Ohkura N, Sakaguchi S. Regulatory T cells: Roles of T cell receptor for their development and function. Seminars in Immunopathology. 2010;**32**(2):95-106. DOI: 10.1007/s00281-010-0200-5

[26] Beyer M, Schultze JL. Plasticity of T(reg) cells: Is reprogramming of T(reg) cells possible in the presence of FOXP3? International Immunopharmacology. 2011;**11**(5):555-560. DOI: 10.1016/j.intimp.2010.11.024

[27] John LB, Ward AC. The Ikaros gene family: Transcriptional regulators of hematopoiesis and immunity. Molecular Immunology. 2011;**48**(9-10):1272-1278. DOI: 10.1016/j.molimm.2011.03.006

[28] Shevach EM, Thornton AM. tTregs, pTregs, and iTregs: Similarities and differences. Immunological reviews. 2014;**259**(1):88-102. DOI: 10.1111/imr.12160

[29] Grzanka J, Leveson-Gower D, Golab K, Wang XJ, Marek-Trzonkowska N, Krzystyniak A, et al. FoxP3, Helios, and SATB1: Roles and relationships in regulatory T cells. International Immunopharmacology. 2013;**16**(3):343-347. DOI: 10.1016/j.intimp.2013.02.004

[30] Müller M, Herrath J, Malmström V. IL-1R1 is expressed on both Helios(+) and Helios(−) FoxP3(+) CD4(+) T cells in the rheumatic joint. Clinical & Experimental Immunology. 2015;**182**(1):90-100. DOI: 10.1111/cei.12668

[31] Zabransky DJ, Nirschl CJ, Durham NM, Park BV, Ceccato CM, Bruno TC, et al. Phenotypic and functional properties of Helios+ regulatory T cells. PLoS One. 2012;**7**(3):e34547. DOI: 10.1371/journal.pone.0034547

[32] Hadaschik EN, Enk AH. TGF-β1-induced regulatory T cells. Human Immunology. 2015;**76**(8):561-564. DOI: 10.1016/j.humimm.2015.06.015

[33] Kanamori M, Nakatsukasa H, Okada M, Lu Q, Yoshimura A. Induced regulatory T cells: Their development, stability, and applications. Trends in Immunology. Nov 2016;**37**(11):803-811. DOI: 10.1016/j.it.2016.08.012

[34] Bacchetta R, Barzaghi F, Roncarolo MG. From IPEX syndrome to FOXP3 mutation: A lesson on immune dysregulation. Annals of the New York Academy of Sciences. 2016. 1-18. DOI: 10.1111/nyas.13011

[35] Gambineri E, Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. Current Opinion in Rheumatology. 2003;**15**(4):430-435

[36] Zhan H, Sinclair J, Adams S, Cale CM, Murch S, Perroni L, et al. Immune reconstitution and recovery of FOXP3 (forkhead box P3)-expressing T cells after transplantation for IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome. Pediatrics. 2008;**121**(4):e998-e1002. DOI: 10.1542/peds.2007-1863

[37] Blanco Quirós A, Arranz Sanz E, Bernardo Ordiz D, Garrote Adrados JA. Fromautoimmune enteropathy to the IPEX (immune dysfunction, polyendocrinopathy, enteropathy, X-linked) syndrome. Allergologia et Immunopathologia. 2009;**37**(4):208-215. DOI: 10.1016/j.aller.2009.04.002

[38] Colobran R, Álvarez de la Campa E, Soler-Palacín P, Martín-Nalda A, Pujol-Borrell R, de la Cruz X, et al. Clinical and structural impact of mutations affecting the residue Phe367 of FOXP3 in patients with IPEX syndrome. Clinical Immunology. 2016;**163**:60-65. DOI: 10.1016/j.clim.2015.12.014

[39] Jmaa MB, Abida O, Bahloul E, Toumi A, Khlif S, Fakhfakh R, Elloumi N, Sellami K, Masmoudi A, Turki H, Masmoudi H. Role of FOXP3 gene polymorphism in the susceptibility to Tunisian endemic Pemphigus Foliaceus. Immunology Letters. 2017;**184**:105-111. DOI:10.1016/j.imlet.2017.02.005

[40] Pesenacker AM, Cook L, Levings MK. The role of FOXP3 in autoimmunity. Current Opinion in Immunology. 2016;**43**:16-23. DOI: 10.1016/j.coi.2016.07.004

[41] Parackova Z, Kayserova J, Danova K, Sismova K, Dudkova E, Sumnik Z, et al. T regulatory lymphocytes in type 1 diabetes: Impaired CD25 expression and IL-2 induced STAT5 phosphorylation in pediatric patients. Autoimmunity. 2016;**49**(8):523-531

[42] Cerosaletti K, Schneider A, Schwedhelm K, Frank I, Tatum M, Wei S, et al. Multiple autoimmune-associated variants confer decreased IL-2R signaling in CD4+ CD25(hi) T cells of type 1 diabetic and multiple sclerosis patients. PLoS One. 2013;**8**(12):e83811. DOI: 10.1371/journal.pone.0083811

[43] Bossowski A, Borysewicz-Sańczyk H, Wawrusiewicz-Kurylonek N, Zasim A, Szalecki M, Wikiera B, et al. Analysis of chosen polymorphisms in FoxP3 gene in children and adolescents with autoimmune thyroid diseases. Autoimmunity. 2014;**47**(6):395-400. DOI: 10.3109/08916934.2014.910767

[44] Peters JH, Preijers FW, Woestenenk R, Hilbrands LB, Koenen HJ, Joosten I. Clinical grade Treg: GMP isolation, improvement of purity by CD127 Depletion, Treg expansion, and Treg cryopreservation. PLoS One. 2008;**3**(9):e3161. DOI: 10.1371/journal.pone.0003161

[45] Hoffmann P, Boeld TJ, Eder R, Albrecht J, Doser K, Piseshka B, Dada A, Niemand C, Assenmacher M, Orsó E, Andreesen R, Holler E, Edinger M. Isolation of CD4+CD25+ regulatory T cells for clinical trials. Biology of Blood and Marrow Transplantation. 2006;**12**(3):267-274

[46] Jeffery HC, Braitch MK, Brown S, Oo YH. Clinical potential of regulatory T cell therapy in liver diseases: An overview and current perspectives. Frontiers in Immunology. 2016;**7:**334. DOI: 10.3389/fimmu.2016.00334

[47] Li Z, Li D, Tsun A, Li B. FOXP3+ regulatory T cells and their functional regulation. Cellular and Molecular Immunology. 2015;**12**(5):558-565. DOI: 10.1038/cmi.2015.10

[48] Horch M, Nguyen VH. Regulatory T-cell immunotherapy for allogeneic hematopoietic stem-cell transplantation. Therapeutic Advances in Hematology. 2012;**3**(1):29-44. DOI: 10.1177/2040620711422266

[49] Miyara M, Ito Y, Sakaguchi S. TREG-cell therapies for autoimmune rheumatic diseases. Nature Reviews Rheumatology. 2014;**10**(9):543-551. DOI: 10.1038/nrrheum.2014.105

[50] Velaga S, Alter C, Dringenberg U, Thiesler CT, Kuhs S, Olek S, et al. Clinical-grade regulatory T cells: Comparative analysis of large-scale expansion conditions. Experimental Hematology. 2017;**45**:27-35.e1. DOI: 10.1016/j.exphem.2016.09.008

[51] Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP 3rd, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. The New England Journal of Medicine. 2011;**365**(22):2055-2066. DOI: 10.1056/NEJMoa1108188

[52] Brunstein CG, Miller JS, Cao Q, McKenna DH, Hippen KL, Curtsinger J, et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: Safety profile and detection kinetics. Blood. 2011;**117**(3):1061-1070. DOI: 10.1182/blood-2010-07-293795

[53] Mackay LK, Kallies A. Transcriptional regulation of Tissue-Resident lymphocytes. Trends in Immunology. 2017;**38**(2):94-103. DOI: 10.1016/j.it.2016.11.004

[54] Schroeder MA, DiPersio JF. Mouse models of graft-versus-host disease: advances and limitations. Disease Models & Mechanisms. 2011;**4**(3):318-333. DOI: 10.1242/dmm.006668

[55] Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, et al. Long-term survival and late deaths after allogeneic hematopoietic cell transplantation. Journal of Clinical Oncology. 2011;**29**(16):2230-2239. DOI: 10.1200/JCO.2010.33.7212

[56] Battiwalla M, Hashmi S, Majhail N, Pavletic S, Savani BN, Shelburne N. National institutes of health hematopoietic cell transplantation late effects initiative: Developing recommendations to improve survivorship and long-term outcomes. Biology of Blood and Marrow Transplantation. 2017;**23**(1):6-9. DOI: 10.1016/j.bbmt.2016.10.020

[57] Hülsdünker J, Zeiser R. Insights into the pathogenesis of GvHD: What mice can teach us about man. Tissue Antigens. 2015;**85**(1):2-9. DOI: 10.1111/tan.12497

[58] Swart JF, Delemarre EM, van Wijk F, Boelens JJ, Kuball J, van Laar JM, Wulffraat NM. Haematopoietic stem cell transplantation for autoimmune diseases. Nature Reviews Rheumatology. 2017. DOI: 10.1038/nrrheum.2017.7

[59] Matsuoka K, Koreth J, Kim HT, Bascug G, McDonough S, Kawano Y, et al. Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. Science Translational Medicine. 2013;**5**(179):179ra43. DOI: 10.1126/scitranslmed.3005265

[60] He X, Smeets RL, van Rijssen E, Boots AM, Joosten I, Koenen HJ. Single CD28 stimulation induces stable and polyclonal expansion of human regulatory T cells. Scientific Reports. 2017;**7**:1-11. DOI: 10.1038/srep43003

[61] Bacchetta R, Lucarelli B, Sartirana C, Gregori S, Lupo Stanghellini MT, Miqueu P, et al. Immunological outcome in Haploidentical-HSC transplanted patients treated with IL-10-Anergized donor T cells. Frontiers in Immunology. 2014;**5**:16. DOI: 10.3389/fimmu.2014.00016

[62] Andolfi G, Fousteri G, Rossetti M, Magnani CF, Jofra T, Locafaro G, et al. Enforced IL-10 expression confers type 1 regulatory T cell (Tr1) phenotype and function to human CD4(+) T cells. Molecular Therapy. 2012;**20**(9):1778-1790. DOI: 10.1038/mt.2012.71

[63] Hippen KL, Riley JL, June CH, Blazar BR. Clinical perspectives for regulatory T cells in transplantation tolerance. Seminars in Immunology. 2011;**23**(6):462-468. DOI: 10.1016/j.smim.2011.07.008

[64] McDonald-Hyman C, Turka LA, Blazar BR. Advances and challenges in immunotherapy for solid organ and bone marrow transplantation. Science Translational Medicine. 2015;**7**(280):280rv2. DOI: 10.1126/scitranslmed.aaa6853

[65] Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. Nature Reviews Immunology. 2012;**12**(6):443-58. DOI: 10.1038/nri3212

[66] Charbonnier LM, Janssen E, Chou J, Ohsumi TK, Keles S, Hsu JT, et al. Regulatory T-cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. Journal of Allergy and Clinical Immunology. 2015;**135**(1):217-227. DOI: 10.1016/j.jaci.2014.10.019

[67] Cabrera SM, Rigby MR, Mirmira RG. Targeting regulatory T cells in the treatment of type 1 diabetes mellitus. Current Molecular Medicine. 2012;**12**(10):1261-1272

[68] Barcala Tabarrozzi AE, Castro CN, Dewey RA, Sogayar MC, Labriola L, Perone MJ. Cell-based interventions to halt autoimmunity in type 1 diabetes mellitus. Clinical & Experimental Immunology. 2013;**171**(2):135-146. DOI: 10.1111/cei.12019

[69] Bordignon C, Canu A, Dyczko A, Leone S, Monti P. T-cell Metabolism as a Target to control autoreactive T cells in β-cell autoimmunity. Current Diabetes Reports. 2017;**17**(5):24. DOI: 10.1007/s11892-017-0848-5

[70] Frumento D, Nasr MB, Essawy BE, D'Addio F, Zuccotti GV, Fiorina P. Immunotherapy for type 1 diabetes. Journal of Endocrinological Investigation. 2017. DOI: 10.1007/s40618-017-0641-y

[71] Papadopoulou-Marketou N, Chrousos GP, Kanaka-Gantenbein C. Diabetic nephropathy in type 1 diabetes: A review of early natural history, pathogenesis, and diagnosis. Diabetes/Metabolism Research and Reviews. 2017;**33**(2). DOI: 10.1002/dmrr.2841

[72] Gitelman SE, Bluestone JA. Regulatory T cell therapy for type 1 diabetes: May the force be with you. Journal of Autoimmunity. 2016;**71**:78-87. DOI: 10.1016/j.jaut.2016.03.011

[73] Tan T, Xiang Y, Chang C, Zhou Z. Alteration of regulatory T cells in type 1 diabetes mellitus: a comprehensive review. Clinical Reviews in Allergy & Immunology. 2014;**47**(2):234-243. DOI: 10.1007/s12016-014-8440-0

[74] Zóka A, Barna G, Somogyi A, Műzes G, Oláh Á, Al-Aissa Z, et al. Extension of the $CD4^{+}Foxp3^{+}CD25(-/low)$ regulatory T-cell subpopulation in type 1 diabetes mellitus. Autoimmunity. 2015;**48**(5):289-297. DOI: 10.3109/08916934.2014.992518

[75] Putnam AL, Brusko TM, Lee MR, Liu W, Szot GL, Ghosh T, et al. Expansion of human regulatory T-cells from patients with type 1 diabetes. Diabetes. 2009;**58**(3):652-662. DOI: 10.2337/db08-1168

[76] Du W, Shen YW, Lee WH, Wang D, Paz S, Kandeel F, et al. Foxp3+ Treg expanded from patients with established diabetes reduce Helios expression while retaining normal function compared to healthy individuals. PLoS One. 2013;**8**(2):e56209. DOI: 10.1371/journal.pone.0056209

[77] Todd JA, Evangelou M, Cutler AJ, Pekalski ML, Walker NM, Stevens, et al. Regulatory T cell responses in participants with type 1 diabetes after a single dose of Interleukin-2: A non-randomised, open label, adaptive dose-finding trial. PLoS Medicine. 2016;**13**(10):e1002139. DOI: 10.1371/journal.pmed.1002139

[78] Than NN, Jeffery HC, Oo YH. Autoimmune hepatitis: Progress from global immunosuppression to personalised regulatory T cell therapy. Canadian Journal of Gastroenterology and Hepatology. 2016;2016:7181685. DOI: 10.1155/2016/7181685

[79] Manns MP, Lohse AW, Vergani D. Autoimmune hepatitis—Update 2015. Journal of Hepatology. 2015;**62**:S100-S111. DOI: 10.1016/j.jhep.2015.03.005

[80] Sebode M, Lohse AW. Future perspective: Immunomodulatory therapy for autoimmune hepatitis. Digestive Diseases and Sciences. 2014;**32**(5):502-506. DOI: 10.1159/000360493

[81] Peiseler M, Sebode M, Franke B, Wortmann F, Schwinge D, Quaas A, et al. FOXP3+ regulatory T cells in autoimmune hepatitis are fully functional and not reduced in frequency. Journal of Hepatology. 2012;**57**(1):125-132. DOI: 10.1016/j.jhep.2012.02.029

[82] Taubert R, Hardtke-Wolenski M, Noyan F, Wilms A, Baumann AK, Schlue J, et al. Intrahepatic regulatory T cells in autoimune hepatitis are associated with treatment response and depleted with current therapies. Journal of Hepatology. 2014;**61**(5):1106-1114. DOI: 10.1016/j.jhep.2014.05.034

[83] Christen U, Hintermann E. Immunopathogenic mechanisms of autoimmune hepatitis: How much do we know from animal models? Haybaeck J, editor. International Journal of Molecular Sciences. 2016;**17**(12):2007. DOI: 10.3390/ijms17122007

[84] Cassim S, Bilodeau M, Vincent C, Lapierre P. Novel immunotherapies for autoimmune hepatitis. Frontiers in Pediatrics. 2017;**5**:8. DOI: 10.3389/fped.2017.00008

[85] Edens C, Robinson AB. Systemic lupus erythematosus, bone health, and osteoporosis. Current Opinion in Endocrinology, Diabetes and Obesity. 2015;**22**(6):422-431. DOI: 10.1097/MED.0000000000000197

[86] Shu Y, Hu Q, Long H, Chang C, Lu Q, Xiao R. Epigenetic variability of CD4+CD25+ tregs contributes to the pathogenesis of autoimmune diseases. Clinical Reviews in Allergy & Immunology. Apr 2017;**52**(2):260-272. DOI: 10.1007/s12016-016-8590-3

[87] Mizui M, Tsokos GC. Low-Dose IL-2 in the treatment of lupus. Current Rheumatology Reports. 2016;**18**(11):68

[88] Von Spee-Mayer C, Siegert E, Abdirama D, Rose A, Klaus A, Alexander T, et al. Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. Annals of the Rheumatic Diseases. 2016;**75**(7):1407-1415. DOI: 10.1136/annrheumdis-2015-207776

[89] Jiang SH, Shen N, Vinuesa CG. Posttranscriptional T cell gene regulation to limit Tfh cells and autoimmunity. Current Opinion in Immunology. 2015;**37**:21-27. DOI: 10.1016/j.coi.2015.09.003

[90] Ohl K, Tenbrock K. Regulatory T cells in systemic lupus erythematosus. European Journal of Immunology. 2015;**45**(2):344-355. DOI: 10.1002/eji.201344280

[91] Alexander T, Sattler A, Templin L, Kohler S, Groß C, Meisel A, et al. Foxp3+ Helios+ regulatory T cells are expanded in active systemic lupus erythematosus. Annals of the Rheumatic Diseases. 2013;**72**(9):1549-1558. DOI: 10.1136/annrheumdis-2012-202216

[92] Ren W, Liu G, Chen S, Yin J, Wang J, et al. Melatonin signaling in T cells: Functions and applications. Journal of Pineal Research. 2017;**62**(3):1-15. DOI: 10.1111/jpi.12394

[93] Byng-Maddick R, Ehrenstein MR. The impact of biological therapy on regulatory T cells in rheumatoid arthritis. Rheumatology (Oxford). 2015;**54**(5):768-775. DOI: 10.1093/rheumatology/keu487

[94] Kosmaczewska A, Ciszak L, Swierkot J, Szteblich A, Kosciow K, Frydecka I. Exogenous IL-2 controls the balance in Th1, Th17, and Treg cell distribution in patients with progressive rheumatoid arthritis treated with TNF-alpha inhibitors. Inflammation. 2015;**38**(2):765-774. DOI: 10.1007/s10753-014-9987-x

[95] Komatsu N, Takayanagi H. Regulatory T cells in Arthritis. Progress in Molecular Biology and Translational Science. 2015;**136**:207-215. DOI: 10.1016/bs.pmbts.2015.07.021

[96] Wang T, Sun X, Zhao J, Zhang J, Zhu H, Li C, et al. Regulatory T cells in rheumatoid arthritis showed increased plasticity toward Th17 but retained suppressive function in peripheral blood. Annals of the Rheumatic Diseases. 2015;**74**(6):1293-1301. DOI: 10.1136/annrheumdis-2013-204228

[97] Asnagli H, Martire D, Belmonte N, et al. Type 1 regulatory T cells specific for collagen type II as an efficient cell-based therapy in arthritis. Arthritis Research & Therapy. 2014;**16**(3):R115. DOI: 10.1186/ar4567

[98] Noori-Zadeh A, Mesbah-Namin SA, Bistoon-Beigloo S, Bakhtiyari S, Abbaszadeh HA, Darabi S, et al. Regulatory T cell number in multiple sclerosis patients: A meta-analysis. Multiple Sclerosis and Related Disorders. 2016;**5**:73-76. DOI: 10.1016/j.msard.2015.11.004

[99] Lee PW, Severin ME, Lovett-Racke AE. TGF-β regulation of encephalitogenic and regulatory T cells in multiple sclerosis. European Journal of Immunology. 2017;**47**(3):446-453. DOI: 10.1002/eji.20164

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