Review Article

The Generation and Regulation of Tissue-Resident Tregs and Their Role in Autoimmune Diseases

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Regulatory T cells (Tregs), as an important subset of T cells, play an important role in maintaining body homeostasis by regulating immune responses and preventing autoimmune diseases. In-depth research finds that Tregs have strong instability and plasticity, and according to their developmental origin, Tregs can be classified into thymic-derived Tregs (tTregs), endogenous-induced Tregs (pTregs), which are produced by antigen-stimulated T cells in the periphery in vivo, and induced Tregs (iTregs), which differentiate from naïve T cells in vitro. In recent years, studies have found that Tregs are divided into lymphatic and tissue-resident Tregs according to their location. Research on the generation and function of lymphoid Tregs has been more comprehensive and thorough, but the role of tissue Tregs is still in the exploratory stage, and it has become a research hot spot. In this review, we discuss the instability and plasticity of Tregs and the latest developments of tissue-resident Tregs in the field of biology, including adipose tissue, colon, skeletal muscle, and other Tregs that have been recently discovered as well as their production, regulation, and function in specific tissues and their role in the pathogenesis of autoimmune diseases.

1. Introduction

In the early 1970s, Gershon and Kondo found a group of inhibitory cells in the spleen of mice [1], but the specific classification and marking of these cells were not clear until 1995, when research by Sakaguchi et al. confirmed that a type of suppressive T cell subgroup highly expresses the cytokine interleukin- (IL-) 2 receptor alpha chain (CD25) and the transcription factor Foxp3, which is a member of the fork-shaped transcription factor family [2], and named them regulatory T cells (Tregs). However, controversy exists on the use of CD25 as a characteristic marker of Tregs because conventional T cells can also express CD25 after activation [3]. Therefore, Foxp3 is commonly used as a characteristic molecule of Tregs.

Tregs are essential for maintaining autoimmune tolerance, and their dysfunction can lead to severe autoimmune diseases [2]. For example, Foxp3 mutations can cause the Scurfy phenotype in mice [4]. Scurfy mice have X-linked recessive mutations, and the symptoms are squamous skin,

lymphoid tissue hyperplasia, hyperglobulinemia, lymphadenopathy, anaemia, atrophy, and premature death, among others. [5]. Similarly, symptoms have been observed in patients with IPEX syndrome, which is caused by human Foxp3 mutations. Powell et al. first described this disease in 1982 [6, 7]. These findings suggest that Foxp3 might be a key characteristic molecule that determines the functions of Tregs. Alexander et al. confirmed that the three conserved noncoding DNA sequences (CNS1, CNS2, and CNS3) of the Foxp3 gene control the development and differentiation of mouse Tregs. CNS2 is involved in the genetic maintenance of Foxp3 expression, and CNS3 has the effect of promoting the production of thymus and peripheral Tregs. CNS1, which contains a TGF- β -NFAT response element, has no obvious effect on the differentiation and development of tTregs but has an important role in the development of pTregs [5, 8].

In addition, studies indicate that Tregs are unstable and plastic, and their phenotype and function change with the specific environment. In addition to Foxp3, a second condition is also required to establish Treg function, i.e., specific

epigenetic characteristics acquired during development [9, 10]. Under inflammatory conditions, a certain proportion of Tregs might lose Foxp3 expression and become unstable, or the expression of Foxp3 can be maintained, but its overall epigenetic characteristics change, such as the methylation of Foxp3, which leads to reduced Foxp3 function and secretion of proinflammatory cytokines. This is also the reason for the abnormal plasticity observed in several autoimmune environments [3]. In the T-helper Type 1 (Th1) inflammatory state, Tregs express the transcription factor T-bet and the chemokine CXCR3. At this time, Tregs have Th1-like cell characteristics. Under different conditions, Tregs can also acquire Th2-like or Th17-like properties. During the period of inflammatory regression, these plastic Tregs can also become “normal” Tregs [3, 6]. As an important subgroup of Tregs with immunomodulatory function, previous studies have primarily focused on the Tregs distributed in central and peripheral lymphoid tissues. However, recent studies have indicated that Treg subgroups are also found in non-lymphoid tissues such as adipose tissue, colonic tissue, and skeletal muscle tissue, and thus, they are referred to as tissue-resident Tregs. Therefore, this article reviews the research progress in the production and regulation of tissue Tregs and its role in autoimmune diseases.

2. Instability and Plasticity of Tregs

The phenotype and function of Tregs are unstable. It is known that as the most important regulator of Treg phenotype and function [3], Foxp3 plays a vital role in the development of Tregs. The conserved noncoding sequence CNS2 described above contains a conserved CPG island (TSDR) structure, which is specifically hypomethylated in Tregs, and CNS2 demethylation in Tregs is mediated by Tet-dependent oxidation. This process is helpful in recruiting multiple transcription factors core-binding factor, beta-subunit (Cbf β), runt-related transcription factor 1 (Runx1), signal transducers, and activators of transcription 5 (STAT5) and Foxp3 to CNS2, which further ensures the stable expression of Foxp3 [11]. Studies have shown that conditional deletion of Foxp3 makes the Treg lineage unstable, and CNS2-deficient mice can spontaneously develop autoimmune diseases and chronic inflammation [12, 13]. Previous fate-mapping studies have also shown that under autoimmune inflammation, a small portion of Tregs becomes unstable. These previously Foxp3-expressing (ex-Foxp3) T cells have a memory T cell phenotype and produce inflammatory cytokines. Different models have been proposed to explain the generation of ex-Foxp3 cells, and among these models, the reprogramming model suggests that remethylation of the CNS2 region can make Tregs become unstable under inflammatory conditions. Therefore, Tregs lose their Foxp3 expression and are reprogrammed into Th-cells [11].

Tregs have various plasticity and can lose Foxp3 expression under certain conditions or pathological conditions, transforming from immunosuppressive Treg cells into cells with other functions. This process enables them to make adaptive adjustments in the local microenvironment, which is constantly changing. The most typical plasticity of Tregs

is the acquisition of Th1-like Treg characteristics. In patients with autoimmune diseases such as type 1 diabetes, multiple sclerosis, and autoimmune hepatitis, the proportion of Interferon- γ (IFN- γ)⁺ Tregs in peripheral blood increases and shows lower inhibitory ability than Tregs in healthy individuals in the same batch [14]. Th1-like Tregs promote IFN- γ secretion by upregulating the expression of T-bet, and Th1-like Tregs also upregulate the expression of other Th1 cell markers such as CXCR3 [3]. In patients with multiple sclerosis, Tregs can be plasticized into Th2-like Tregs, and the frequency of Th2-like Tregs in the skin increases. At the same time, Th2-like Tregs are also observed in the peripheral blood of allergy-susceptible mutant mice (Il4raF709) and food allergy patients [3]. Th2-like Tregs are characterized by upregulation of interferon regulatory factor 4 (IRF4) and Gata-3 and increased secretion of IL-4 and IL-13 [3, 15]. Tregs can also be plasticized into Th17 cells. In homeostasis, Tregs in the peripheral blood of a small group of people can be transformed into Th17 cells [16, 17]. In addition, Th17-like Tregs were also observed in the synovium of patients with active rheumatoid arthritis [18] and in the skin tissue of patients with psoriasis [19]. Th17-like Tregs secrete IL-17 and upregulate the gene that encodes the transcription factor Retinoic Acid Receptor-Related Orphan Receptor γ t (ROR γ t), and their inhibitory ability is reduced [16]. Studies have shown that IL-1 β and IL-6 can promote Tregs to secrete IL-17 [3], other environmental factors, such as the immunomodulatory enzymes indoleamine 2,3-dioxygenase (IDO) and Toll-like receptor (TLR)2. IDO mainly inhibits the production of IL-6 in dendritic cells (DCs) in a general control nonderepressible 2 (GCN2) kinase-dependent manner, thereby inhibiting the transformation of Tregs into Th17-like Tregs [20]. Nyirenda et al. used flow cytometry to sort Tregs in the conditions of the presence or absence of TLR2 ligand Pam3Cys incubated on a culture plate that combines anti-CD3 and CD28 for 72 hours. It was found that the expression of IL-17 in Tregs increased significantly in the presence of Pam3Cys [21].

In contrast, under certain conditions, Th-cells can also be transformed into Tregs. You et al. [22] used two models, a CD28-deficient NOD mouse model and a T cell remodelled NOD-SCID mouse model. After anti-CD3 treatment, a transforming growth factor β - (TGF- β -) dependent CD4⁺CD25⁺Treg subgroup was found in the spleen of the model mice but not in normal mice. When the ratio of CD4⁺CD25⁺ Tregs was tested, no significant change was observed, indicating that these cells are not derived from the expansion of thymus Tregs but from the transformation of other cells. Later, Laurence et al. [23] used a graft-versus-host response (GVHR) mouse model in research in which the fates of Foxp3⁺ Th-cells isolated from the control group and the STAT3-deficient group were tracked, showing that the proportion of Foxp3⁺ Tregs in the STAT3-deficient group was much higher than that in the control group. This result indicates that the initial Th-cells that are deficient in STAT3 might tend to transform into Tregs. In addition, it has also been observed that Th-like Tregs have a demethylated TSDR structure in the Foxp3 locus, which indicates that their phenotype might be reversible [3, 24].

3. Various Tissue-Resident Tregs

Because Tregs are highly plastic, they might display different phenotypes and functions in different environments. In recent years, studies have shown that in addition to Tregs derived from lymphoid tissues, tissue-resident Tregs also appear in a variety of specific tissues/organs, such as the adipose tissue, colon, skeletal muscle, and skin.

Tregs of different organizations have different functions. Adipose tissue Tregs mainly regulate the balance between immunity and metabolism. Colon tissue Tregs maintain intestinal homeostasis and protect the body from pathogenic microorganisms. Skeletal muscle Tregs primarily maintain skeletal muscle homeostasis and promote skeletal muscle regeneration under inflammatory conditions. Skin Tregs plays a role in promoting hair regeneration. Although these tissue Tregs have different phenotypes and functions, studies have found that they are all closely related to autoimmune diseases (Table 1). The Tregs of different tissues are introduced in detail below.

3.1. Adipose Tissue Tregs: A Bridge between Immunity and Metabolism. Adipose tissue is distributed throughout the body, including visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT). Adipose tissue acts as a “warehouse” for storing calories and is a loose connective tissue that is mainly divided into white adipose tissue (WAT) and brown adipose tissue (BAT). WAT stores energy as fat, and the metabolic function of BAT is to oxidize lipids to generate heat [25]. Although adipose tissue is characterized by adipose cells, these are not the only cells it contains. Adipose tissue also includes vascular endothelial cells, stem cells, neutrophils, macrophages, and lymphocytes [25]. The balance between these cells is closely related to tissue homeostasis in the body.

Studies have confirmed that a certain proportion of Tregs exist in VAT and SAT. VAT Foxp3⁺ CD4⁺ T cells are considered to be a typical tissue Treg population [5]. Feuerer et al. identified adipose-resident Tregs in mouse adipose tissue. When mice are born, the proportion of Tregs in the two types of adipose tissue is relatively low and similar until 20 weeks after birth. The proportion of SAT Tregs is almost unchanged while the proportion of Tregs in VAT is increasing. The proportion of Foxp3⁺ Tregs in VAT can reach more than half that of CD4⁺ T cells, which is much higher than the proportion of Tregs in lymphoid tissue and SAT [26]. Visceral WAT Tregs are derived from the thymus [5]. These cells appear in the thymus during the first week of life [27]. The origin of subcutaneous WAT Tregs and BAT Tregs is not yet clear.

VAT is the site of cellular and molecular interaction between the metabolic system and the immune system [28]. Studies have shown that adipose-derived proinflammatory cytokines, such as tumor necrosis factor- α (TNF α), IL-6, and type 1 IFN, are considered to be the cause of insulin resistance and metabolic syndrome [5, 29]. It is speculated that adipose tissue Tregs play an important role in controlling adipose inflammation and affect the overall metabolic homeostasis in the body [5]. Therefore, the following ques-

tion arises: what is the molecular mechanism that regulates the production and function of Tregs in adipose tissue?

3.1.1. Regulatory Molecules of Adipose Tissue Treg Production and Function. Gene expression analysis showed that peroxisome proliferator-activated receptor γ (PPAR- γ) is the main regulator of adipocyte differentiation and the main molecular coordinator of VAT Treg phenotype and function. Coimmunoprecipitation experiments confirmed that Foxp3 and PPAR- γ interact directly or as a component of the complex [5, 30, 31]. To determine the importance of PPAR- γ for the VAT Treg phenotype in vivo, Cipolletta and others crossed mice expressing Cre recombinase under the control of Foxp3 promoter/enhancer elements with mice carrying “floxed” PPAR- γ , thereby knocking out the expression of PPAR- γ in Tregs. It was found that the number of Tregs in the adipose tissue of the mutant was significantly less than that in the wild-type (WT) control group at the 25th week. The results also showed that the expression of Foxp3 was reduced while the expression of Foxp3 in the total Tregs in the mutant lymphoid organs was normal [30]. In a mouse model of obesity induced by a high-fat diet, the number of VAT Tregs was significantly reduced. The PPAR- γ agonist pioglitazone was injected into mice. Pioglitazone improves insulin sensitivity by interacting with PPAR- γ and affecting lipid metabolism. This method of administration changed the metabolic parameters of WT mice, increased the number of VAT Tregs, and improved the local inflammatory response, indicating that PPAR- γ plays an important role in the production and function of VAT Tregs [30–32].

In addition, research shows that IL-33 binds to the suppression of tumorigenicity 2 receptor (ST2) to activate myeloid differentiation factor 88 (MyD88). This pathway is essential for the development and maintenance of adipose tissue Tregs, and it is also significant for transcriptional feature maintenance of adipose tissue Tregs. Molofsky et al. found that the number of VAT Tregs in an obese mouse model that received IL-33 increased dramatically. IL-33 can directly promote the proliferation, activation, and function of VAT Treg cells, and IL-33 can also activate innate lymphoid cell 2 (ILC2) by upregulating ICOSL on ILC2 and subsequently combining with ICOS on Tregs to induce the proliferation of Tregs in VAT [33]. Vasanthakumar et al. found that IL-33 can also drive the proliferation of VAT Tregs in WT mice and can increase the number of VAT Tregs in genetically obese mice and obese mice induced by a high-fat diet. IL-33 induces higher expression of Foxp3 and GATA-3 in T cells, which further proves that IL-33 can promote Treg proliferation and maintain its transcriptional characteristics [34]. IL-33 affects VAT Tregs through the adaptor protein MyD88. In experiments, the number of Tregs in adipose tissue with the MyD88-specific defect was observed to decrease, and therefore, it can be said that differentiation of VAT Tregs requires signal transduction mediated by downstream MyD88 of IL-33 [34]. Flow cytometry showed that compared with nonexpression of ST2 in the spleen, lymph nodes, small intestine, and lungs, VAT Tregs showed high expression of ST2. The expression of ST2 in VAT Tregs

TABLE 1: Types, regulatory molecules, functions, and related autoimmune diseases of tissue Tregs.

Tissue Treg name	Major regulators	Functions	Related diseases	References
Adipose tissue Tregs	PPAR- γ IL-33 BATF IRF4 Id2 MHCII IL-10	Maintain adipose tissue homeostasis, regulate the balance between the immune system and metabolic system	Hashimoto's thyroiditis, atherosclerosis, diabetes	[4] [26–39]
Colonic tissue Tregs	TGF- β ST2/IL-33 KLRG1	Inhibit inflammation and maintain intestinal homeostasis	Multiple sclerosis	[4] [40–47]
Skeletal muscle Tregs	IL-33 Areg	Promote repair of skeletal muscle damage and inhibit inflammation	Idiopathic inflammatory myopathy	[48–55]
Skin tissue Tregs	CCR6 EGFR	Promote hair growth and repair skin tissue damage	Alopecia areata, psoriasis	[4] [56–60]
Lung Tregs	IL-18	Tissue repair	—	[61]
Liver Treg	—	—	Primary biliary cholangitis, primary sclerosing cholangitis, autoimmune hepatitis	[62]
Placenta Treg	—	Promote maternal-foetal tolerance	—	[4, 31]
Oral mucosa Tregs	CD69 CD103 CTLA4, CD44 Nrp-1	Inhibit local inflammation	—	[63]

—: Undefined.

in 20–25-week-old lean mice exceeds 80%, and its expression is closely related to VAT Treg markers such as Killer Cell lectin-like Receptor Subfamily G Member 1 (KLRG), C-C chemokine receptor type 2 (CCR2), Ly6C, CD69, and programmed cell death protein 1 (PD-1) [31, 34, 35].

The transcription regulators B-cell-activating transcription factor (BATF) and IRF4 can regulate the differentiation of VAT Tregs by regulating the expression of PPAR- γ . To determine the roles that BATF and IRF4 play in the development of VAT Tregs, Vasanthakumar et al. [34] found that VAT Tregs almost completely disappeared in the BATF-deficient mice. Similarly, Tregs are nearly nonexistent in VAT of IRF4-deficient mice. In addition, the spleen Tregs of WT mice and IRF4-deficient mice were directly separated or cultured with CD3 and CD28, and it was found that the expression of PPAR- γ in WT mice Tregs was significantly higher than that in directly isolated cells after culture. However, for IRF4-deficient mice, no obvious difference was noted before and after culturing [34]. The results show that IRF4 is a key molecule that induces PPAR- γ transcription in Tregs. These studies prove that both BATF and IRF4 are key components of the transcriptional programme of induced Tregs, which facilitates the regulation of the development of VAT Tregs. IRF4 (as a target of the NF- κ B pathway) and BATF are both downstream molecules of IL-33 signal transduction, which also shows that IL-33 can promote the expression of its own receptors by maintaining high expression of IRF4 and BATF [34].

Transcription factor inhibitor of DNA binding 2 (Id2) is also necessary for the survival of adipose tissue Tregs, and the

deletion of Id2 results in a significant reduction of the adipose tissue Treg population. Studies have found that the expression level of Id2 mRNA in adipose tissue Tregs is approximately four times that in the spleen, and Id2 expression is necessary for adipose tissue Tregs to produce cytokines. The absence of Id2 in Tregs can lead to systemic inflammation, infiltration of inflammatory macrophages and CD8⁺ effector T cells, and increased glucose intolerance. Id2-deficient adipose tissue Tregs reduce the production of IL-10 and IL-13, and the production of IL-13 can promote resolution of inflammation by secretion of IL-10 and endocytosis of macrophages. All of these observations indicate that Id2 is essential for maintaining the survival and function of adipose tissue by regulating adipose tissue Tregs [36].

The T cell receptor (TCR) signal is essential for maintaining VAT Tregs. Vasanthakumar et al. found that VAT Tregs express higher levels of nerve growth factor-induced gene B (NGFI-B) and lower levels of transcription factor 7 (TCF7) than splenic Tregs. NGFI-B is an orphan nuclear receptor, also known as Nur77. The transcription factor Nur77 is upregulated in response to TCR signals, and TCF7 is downregulated in response to TCR signals. Further studies have also shown that the TCR signal induces the transcription regulators BATF and IRF4 and subsequently initiates the expression of the two key regulators PPAR- γ and ST2 of VAT Tregs [34]. Therefore, these results indicate that the TCR signal is crucial for the maintenance and development of VAT Tregs. In addition, MHC class II- (MHCII-) mediated antigen presentation

is necessary for the development and maintenance of VAT Tregs [34, 35]. In mice lacking MHCII, the number of VAT Tregs decreases dramatically [35].

IL-10, also known as cytokine synthesis inhibitory factor (CSIF), is a pleiotropic cytokine that can exert immunosuppressive or immunostimulatory effects in many types of cells. Feuerer et al. [26] showed that the IL-10 transcript level in abdominal fat cells is extremely high. Compared with the lung and spleen, a 136-fold augmentation of IL-10 transcripts in fat was estimated from RT-PCR quantitation. In addition, pathway analysis shows that VAT Tregs not only produce large amounts of IL-10 but also seem to respond to it, because compared with LN Tregs, many genes downstream of IL-10R are upregulated in adipose Tregs. This suggests that IL-10 may have an important regulatory role for VAT Tregs (Figure 1).

3.1.2. Adipose Tissue Tregs in Autoimmune Diseases. The abnormality of VAT Tregs is related to the occurrence of Hashimoto's thyroiditis (HT). HT is a common organ-specific autoimmune disease, and insulin sensitivity decreases in patients with HT [37]. Min et al. established HT model mice and found that in addition to decreased insulin sensitivity, the number of VAT Tregs also decreased. In that study, the Tregs in the peripheral blood of mice were separated and made to express GFP. Known as GFP-CD25⁺Foxp3⁺ Tregs, the adoptive transfer of these constructs to HT model mice can effectively reverse impaired insulin sensitivity. However, after the anti-CD25 antibody was administered three days later, GFP-CD25⁺Foxp3⁺ Tregs were almost undetectable in the peripheral blood, but they were detected in VAT. These adoptively transferred Tregs migrate to adipose tissue and play a role in local tissues. Therefore, this discovery proves the specific role of VAT Tregs in HT insulin resistance [37].

Adipose tissue Tregs not only are used to maintain the homeostasis of adipose tissue in the body but also have a beneficial effect on systemic metabolic abnormalities related to obesity [38]. Obesity is related to a series of pathological conditions (cardiovascular disease, atherosclerosis, diabetes, fatty liver, and increased risk of cancer). A new view posits that obesity is an autoimmune disease, and obesity is associated not only with T2D but also with a higher incidence of psoriasis, and the risk of autoimmune thyroiditis and multiple sclerosis is also higher. Reports show that insulin resistance, which is closely related to obesity, is also related to the development of autoimmune T1D. One of the common features of these diseases is the low level of Tregs [39], illustrating the interrelationship between obesity and autoimmune diseases. The study of adipose tissue Tregs might offer new ideas for the treatment of these diseases.

3.2. Colonic Tissue Tregs. The colon is the location with the largest number of gut microbiota. The main function of the colon is to extract water and salt from solid waste and to carry out microbial fermentation for unabsorbed substances. In histology, the intestine contains four layers: mucosa, submucosa, muscularis propria, and adventitia or serosa. The mucosa is where most immune processes occur and consists

of the epithelial layer, the lamina propria, and the muscularis mucosa. The lamina propria is composed of connective tissues such as collagen and elastin, blood and lymphatic vessels, myofibroblasts, and nerve endings, and it contains many immune cells such as monocytes, plasma cells, B lymphocytes, and T lymphocytes. Among them, T lymphocytes include Tregs, eosinophils, macrophages, and mast cells [5]. These cells play an important role in resisting intestinal microbial infections and maintaining immune homeostasis, and colonic Tregs are considered to be highly important cell types and are the key performers of colon immune balance.

The number of Tregs in the colon accounts for approximately 25-35% of the number of CD4⁺ T cells [40, 41]. Based on the expression of ROR γ t and endothelial transcription factor 3 (GATA3), colonic Foxp3⁺ Tregs can be divided into three different subtypes of Foxp3⁺ ROR γ t⁻ Tregs, Foxp3⁺ ROR γ t⁺ Tregs, and Foxp3⁺ GATA3⁺ Tregs (Figure 2). Among these, Foxp3⁺ ROR γ t⁻ accounted for approximately 50% of colonic Tregs, and their functions in the colon are not described in detail, but these cells might mediate tolerance of food antigens. The second subtype is Foxp3⁺ ROR γ t⁺ Tregs, which account for approximately 15% of colonic Tregs. These cells are primarily involved in microbial tolerance. The third subtype is Foxp3⁺ GATA3⁺ Tregs, which account for approximately one-third of colonic Tregs. These cells can express ST2 receptors, help to inhibit inflammation, and can secrete amphiregulin to promote tissue repair during inflammation [5].

3.2.1. Regulatory Molecules of Colonic Treg Production and Function. Foxp3⁺ ROR γ t⁺ Tregs are primarily involved in microbial tolerance, and studies have shown that the existence of microorganisms is closely related to their production. A possible mechanism includes short-chain fatty acids produced by microbial fermentation that can induce naïve T cells to differentiate into Foxp3⁺ ROR γ t⁺ Tregs in the colonic lamina propria. Butyrate, especially through its histone deacetylase inhibitory function, can promote the expression of Foxp3. All-trans retinoic acid is a vitamin A metabolite produced by TGF- β and DC that can also induce naïve T cells to differentiate into Tregs in the presence of microbial antigens [5]. Clostridium is the main category of symbiotic microorganisms and can induce naïve T cells in the colon to differentiate into Tregs. Clostridium also plays an important role in inhibiting inflammation and allergic reactions [42]. The data show that in the presence of complex microbiota in the intestinal organs, especially in the lamina propria of the colon, Tregs express ROR γ t in a high proportion, and most ROR γ t⁺ Tregs do not express Helios or Recombinant Neuropilin 1 (Nrp1). Function-related genes in Foxp3⁺ ROR γ t⁺ T cells, such as Foxp3, cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor (GITR), Eos, and Helios, show an epigenetic feature of significant demethylation, indicating that these cells have stable regulatory functions. During intestinal-specific immune response, ROR γ t promotes the inhibitory function of Tregs. Foxp3⁺ ROR γ t⁺ T cells are also highly stable in in vitro conditions, and only a small portion of them decrease the

FIGURE 1: The surface markers, secreted cytokines, and basic functions of VAT Treg cell. The VAT Tregs express phenotypic conventional Treg markers (FoxP3, CD25, and CTLA-4). They produce functional cytokines, IL-10 and IL-35. PPAR- γ and Id2 are the key regulators of these cells. Besides, a large set of chemokines and chemokine receptors is highly expressed by VAT Tregs, including CCR1, CCR2, and CCR9. The VAT Tregs are a bridge between immunity and metabolism, which play an important role in multiple autoimmune diseases.

FIGURE 2: Classification and basic functions of colonic Tregs. Based on the expression of ROR γ t and GATA3, colonic Foxp3⁺ Tregs can be divided into three different subtypes of Foxp3⁺ ROR γ t⁻ Tregs, Foxp3⁺ ROR γ t⁺ Tregs, and Foxp3⁺ GATA3⁺ Tregs.

expression of Foxp3 or ROR γ t. ROR γ t⁺ T cells failed to induce Foxp3 expression in vitro, indicating that Foxp3⁺ ROR γ t⁺ T cells are not developed from ROR γ t⁺ T cells [43].

The third type of cells is described above. Most of Foxp3⁺ GATA3⁺ Tregs express Helios, and they are of thymus origin. In the colon, ST2/IL-33 signalling can promote the

expression of Foxp3 and GATA3 in Tregs [44, 45]. Colonic GATA3⁺ Tregs express widely ST2, and thus, the production of this group of Tregs is closely related to the ST2/IL-33 signalling pathway. Chiering et al. [45] found that IL-33 signalling can lead to phosphorylation of GATA3, and phosphorylation of GATA3 recruits additional GATA3 and RNA polymerase II to the Foxp3 promoter, thereby enhancing Foxp3 expression. At the same time, GATA3 also recruits ST2 enhancers, indicating that in addition to inducing Foxp3, IL-33 also promotes the expression of ST2 in Tregs by regulating the direct transcription of the ST2 gene, thereby further enhancing Treg differentiation. At the same time, research by Chiering et al. [45] also confirmed that IL-33 can enhance TGF β 1-mediated Treg differentiation in vitro. Unlike the first type of Treg cells that depend on microorganisms, the ST2/IL-33 signal transduction effects are observed in a nondiseased environment and are not associated with external inflammation [44]. Currently, it is not yet known how the first types of cells, namely, Foxp3⁺ ROR γ ^t Tregs, are produced.

Intestinal Tregs express high levels of CD103 and recombinant human KLRG1, and these are two known heterotypic receptors of E-cadherin, both of which are related to Treg activation. KLRG1 is closely related to the effector-like Treg population that expresses GATA3 in the intestine. KLRG1 is an inhibitory receptor expressed by Tregs, but in contrast to the inhibitory receptor CTLA-4, not every Treg has expression, and its expression is only found in a subset of effector-like Tregs. Studies have shown that Tregs lacking in KLRG1 have the advantage of accumulating in the colon but not in the lymphoid organs, indicating that KLRG1 restricts the accumulation of intestinal Tregs, and KLRG1 plays an inherent role in colonic Treg homeostasis, but it does not change the total number of Tregs [46].

3.2.2. Colonic Tregs in Autoimmune Diseases. Multiple sclerosis (MS) is an autoimmune disease that affects the brain and spinal cord. Research shows that the intestinal microbiota of MS patients is moderately imbalanced. The use of the autoimmune encephalomyelitis (EAE) model has successfully proven that changes in the gut microbiota are potential risk factors for autoimmune diseases. Studies have shown that *Clostridium XIVa*, *Clostridium IV*, and bacteria-like organism from human faeces have the ability to induce colonic Tregs and can inhibit inflammation, such as in colitis and EAE. Another study showed that colonic Tregs express high levels of DKK-1 mRNA, and the specific decrease of DKK-1 in Tregs leads to the loss of control for CD4⁺ T cell proliferation, leading to autoreactive T cell-mediated colitis. These results suggest that we can correct microbial dysbiosis and change the microbiota to induce the production of colonic Tregs, which might be a method for preventing or treating MS [47].

3.3. Skeletal Muscle Tregs. Skeletal muscle is composed of muscle cells arranged in bundles, each of which has different lengths, and as arranged closely, the physiological function of skeletal muscle is to make the body move. Skeletal muscle has the characteristic that it shrinks under the condition of stim-

ulation, and the stimulation of skeletal muscles comes from nerves in the human body. When the skeletal muscle receives stimulation from the nerve that tells it to contract, it moves the bones around the joints, and thus, the body moves. The study found a unique group of Tregs. These cells accumulate in the skeletal muscle shortly after acute skeletal muscle injury, and skeletal muscle regeneration is caused by the activation and differentiation of myogenic stem cells (known as satellite cells) under the basal layer of myofibrils. Inflammation and immune cells play a vital role in the regeneration process. Tregs accumulate in damaged muscles in response to specific cytokines, such as IL-33, and Tregs promote muscle growth by releasing growth factors such as amphiregulin. Muscle repair during ageing is impaired due to the decrease of the number of Tregs, which can be restored by supplementing IL-33 [48].

3.3.1. Regulatory Molecules for Production and Function of Skeletal Muscle Tregs. IL-33 might be one of the molecules that induce Treg accumulation in damaged muscles because as an endogenous signal of tissue damage, IL-33 participates in the regulation of Tregs in different tissues. IL-33 is increased shortly after skeletal muscle damage and acts on Tregs containing the ST2 receptor, which is encoded by the *Il1rl1* gene. *Il1rl1* is one of the strongest genes in Tregs isolated from damaged muscles. Kuswanto et al. [49] crossed mice lacking ST2 in muscle Tregs (Treg-*Il1rl1*mut) with wild mice (*Il1rl1* WT). Using cardiotoxin (CTX) to induce muscle damage, the cellular immunofluorescence method showed that the two-month-old mutant mouse and WT mouse had similar numbers of Tregs in the muscle after CTX induction for one day, but over time, the Treg-*Il1rl1*mut mice showed muscle damage and delayed muscle regeneration. In elderly skeletal muscles with weak regenerative capacity and ST2-deficient Tregs, intramuscular injection of IL-33 can induce an increase in the number of skeletal muscle Tregs and affect muscle regeneration in elderly mice [49, 50]. However, evidence to the contrary exists with respect to the effect of IL-33 on skeletal muscle Tregs. Jin et al. [50] used toxoplasma to induce a mouse skeletal muscle infection model. In that study, at 30 days after infection, IL-33 was injected intramuscularly, followed by injection every other day for a total of three treatments. On the 7th day after the last treatment, it was found that the administration of IL-33 during chronic infection did not increase the number or frequency of skeletal muscle Tregs and did not promote Treg proliferation. This observation might be due to the continuous production of many other antagonistic (inflammatory) cytokines caused by chronic infection, which inhibits the effect of IL-33. Alternatively, excessive effector cells inhibited the effect of IL-33 directly.

Skeletal muscle Tregs exert an immune regulation function by producing amphiregulin (Areg) (Figure 3). Areg belongs to the family of epithelial growth factors, a growth factor overexpressed by muscle Tregs that can enhance muscle regeneration [51]. Areg transmits signals by binding to epidermal growth factor (EGF) receptors, and flow cytometry results showed that high levels of Areg protein were expressed in muscle Tregs while Areg

FIGURE 3: Skeletal muscle Treg phenotype and function. Skeletal muscle Tregs also express the conventional marker of Foxp3, CTLA-4, and IL-2R. This Treg responds to IL-33 produced after muscle injury and produces a lot of amphiregulin for subsequent repair.

expression in spleen Tregs was limited [50, 51]. Studies have shown that chronic *Toxoplasma gondii* infection in skeletal muscle disrupts the normal immune regulatory network, leading to pathological changes in Treg function. During this period, Tregs increase the bias towards inflammatory macrophages. Jin et al. [50] used Areg therapy in which Areg was injected intramuscularly into mice infected with *Toxoplasma gondii* for a long-term and Areg was injected intraperitoneally every other day for a total of three times. It was found that this treatment can restore the body's immune balance and promote muscle tissue repair, indicating that Areg plays an important role in mediating Tregs to regulate muscle tissue repair and immune balance. Skeletal muscle Tregs can also affect tissue regeneration by regulating local inflammation after injury. Skeletal Tregs can secrete several immunosuppressive cytokines, such as TGF β , IL-10, and IL-35, but important molecules expressed by Tregs can interact with other types of cells. For example, Tregs express CD95, an extracellular nucleotidase that can promote crosstalk between Tregs and CD73-expressing cells to hydrolyse adenosine-triphosphate (ATP). CD73 is released to the tissue damage area after apoptosis, and Tregs act on ATP through extracellular nucleotidases CD39 and CD73 to cause adenosine formation and activate adenosine receptors (mainly adenosine receptor A2A) and intracellular protein kinase A (PKA) through the second messenger cAMP to play the role of inhibiting inflammatory T cells and macrophages. In humans and mice, granzyme, another important molecule expressed by skeletal muscle Tregs, can also promote cell death in a perforin-dependent manner. Tregs express a large number of inhibitory molecules such as CTLA4, which competes with the costimulatory molecule CD28 to bind to CD80/CD86, thereby reducing the role of reactive T cells and antigen-presenting cells. In addition, Tregs can also increase the expression of indoleamine 2 and 3-dioxygenase (IDO) in DC through CTLA-4-induced signal transduction, leading to starvation of reactive T cells and cell cycle arrest [52].

3.3.2. Skeletal Muscle Tregs in Autoimmune Diseases. Idiopathic inflammatory myopathy (IIM) is a group of systemic autoimmune connective tissue diseases, and based on clinical and immunopathological characteristics, it can be divided into three subtypes: polymyositis (PM), dermatomyositis (DM), and inclusion body myositis (IBM). PM and DM are characterized by chronic muscle weakness and inflammation of muscle tissue, leading to disability, reduced quality of life, and shortened life expectancy. In histopathology, myopathy is characterized by immune cell infiltration, mainly T cells and macrophages, in skeletal muscle tissue [53]. Conceptually, Tregs in skeletal muscle can fight against destructive inflammatory components, thereby supplying anti-inflammatory or muscle-protecting properties [54]. Ali and Rosenblum [54] selected dermatomyositis patients as the research object, and the enrichment of Foxp3⁺ T cells in inflammatory muscle tissue was detected. It is speculated that this might be due to a local inflammatory response that induced the production or aggregation of Tregs in muscle tissue, which subsequently played the role of anti-inflammatory and tissue repair. This result offers the first basis for studying the role of skeletal muscle Tregs in inflammatory myopathy and supplies new ideas for the treatment of idiopathic myopathy.

3.4. Skin Tregs. The skin is the largest organ of the human body. Skin tissue includes three main layers: epidermis, dermis, and endothelium. The epidermis acts as a physical barrier, and the epidermis-dermis junction contains various sweat glands and sebaceous glands that act as a biochemical barrier. The cellular components of the epidermis include Langerhans cells and T-lymphocytes. The dermis includes NK-cells, B-cells, macrophages, and mast cells, as well as skin DC cells [5]. In the skin tissue of adult mice, Tregs account for approximately 20-60% of CD4⁺ T cells and approximately 20% in adult humans. The source of mouse skin Tregs might be the migration of thymus Tregs, which settle in the skin tissue in early life. However, similar to the intestine, symbiotic microorganisms also play selected roles in this process [54].

3.4.1. Regulatory Molecules of Skin Treg Production and Function. Tregs accumulate in the skin of newborns from day 6 to day 13 [55], and CCR6 plays an important role in the formation of skin Tregs. Scharschmidt et al. [56] isolated Tregs from the skin of mouse pups on the 13th day after birth and performed complete transcriptome RNA sequencing. The results showed that skin Tregs highly expressed CCR6. The skin of newborn SPF mice produces a large amount of CCR6 ligand-chemokine CCL20, suggesting that it might recruit Tregs to the skin tissue through CCR6. Therefore, Scharschmidt et al. [56] chose to use the adoptive transfer model to test the effect of CCR6 for skin Tregs to transfer CD4⁺ single-positive thymocytes from wild-type (WT) mice and CCR6-deficient (CCR6^{-/-}) mice to nuclear gene recombination activation gene- (RAG2-) deficient receptors. Two weeks after the transfer, the spleen, skin-draining lymph nodes, and skin were collected, and flow cytometry was used to evaluate the relative ability of WT and CCR6^{-/-} Tregs to

FIGURE 4: Skin Treg phenotype and function. Skin tissue Tregs are characterized with expression of phenotypic markers, CTLA-4, IL-2R, Foxp3, and CD103, and chemokine receptors, CCR4 and CCR6. These Tregs will be recruited to the damaged tissue with expression of homing molecules such as CD103 and CCR4 after UVB exposure. CCL22 derived from the damaged tissue, a chemokine binding to CCR4, will recruit these Tregs, which finally promote the tissue repair. In addition, EGFR expression on these cells can promote skin Treg activation and enhance skin Treg function.

migrate to these tissues. It was found that the proportion of Tregs in the skin of CCR6^{-/-} mice was significantly reduced, and CCR6^{-/-} and WT Tregs in the skin showed the same Ki-67 expression after the transfer. This result indicates that the lack of CCR6 on Tregs leads to a decrease in the ability to migrate to the skin rather than a decrease in local proliferation in the tissue. These results suggest that in newborns, CCR6 plays an important role in promoting Tregs to migrate to the skin. In addition, short-wave ultraviolet (UVB) irradiation can enrich and accumulate Tregs in the skin of mice. To study the possible effect of UVB on Treg homeostasis, Yamazaki et al. [57] attempted to determine the frequency of Foxp3⁺ Tregs in the skin, skin-draining lymph nodes, and spleen after UVB exposure. The study found that on the 4th day after UVB exposure, Tregs in the skin began to expand, and until day 7, the frequency of CD4⁺ Treg cells increased significantly and reached a peak that lasted until day 14. After 3 weeks of UVB exposure, the expanded Tregs in the skin migrated to the skin draining lymph nodes and spleen, and flow cytometry analysis showed that accumulated Tregs during this process express homing molecules such as CD103 and CCR4 so that they can migrate to the non-UVB irradiation component (Figure 4).

Skin Tregs can actively repair skin damage. In mice, certain highly activated Tregs accumulate in the skin in the early stage of the wound, and in the process of wound healing, the specific loss of skin Tregs weakens wound closure and re-epithelialization [58]. Considering that the epidermal growth factor receptor (EGFR) pathway plays an important role in wound healing and T cell function, to determine whether this pathway is involved in the process of Tregs to reduce wound-related inflammation and promote skin repair, Nosbaum et al. [58] used RT-qPCR to detect the expression of EGFR in Tregs isolated from skin-draining lymph nodes and skin before and after injury. No EGFR expression was detected in Tregs isolated from the skin or skin-draining lymph node (SDLN) before injury, but high expression of EGFR was detected in skin Tregs 3 days after injury. However, no EGFR

expression was observed in the Tregs of the skin-draining lymph nodes at any time after injury. This observation indicates that in the model, the induction of EGFR preferentially occurs on Tregs in an inflamed skin. Subsequently, compared with WT mice, EGFR-deficient mice in Tregs were found to have weakened early wound closure after trauma. It is possible that the lack of EGFR in Tregs leads to a decrease in the percentage and absolute number of Tregs in the skin at the early stage of the wound. In addition, the activation of Tregs is lower in the damaged skin of EGFR-deficient mice, and the accumulation of proinflammatory macrophages at the injury site increases. All of the above evidence proves the important role of EGFR in skin tissue repair, which can promote skin Treg activation and enhance skin Treg function [58].

3.4.2. Skin Tregs in Autoimmune Disease. Skin tissue-resident T cells are mainly located around hair follicles (HF). The epithelial cells of the hair follicle tissue can divide and multiply such that the hair is constantly replaced and grown, but if the function of the hair follicle gradually weakens, the appearance of hair loss occurs. Early studies found that Foxp3⁺ Treg-deficient mice, namely, Scurfy mice, died of fulminant systemic inflammation at a young age, and these mice had obvious symptoms of skin inflammation and hair loss. This observation suggests that Tregs might play a necessary and important role in inhibiting skin inflammation in the early stages of life. However, this model is a systemic Treg defect model, and it is not clear whether the lack of skin Tregs is involved in this process, but it suggests that we might use skin Tregs as a research idea. Alopecia areata is an autoimmune disease that has a feature of abnormal HF circulation. According to research, it has been observed in 80% of patients who have received treatment that successful hair regeneration is related to the increase of Tregs in the damaged head skin. Tregs play a role in the HF cycle and hair regeneration, and the proliferation and activation of skin Tregs are related to the stages of the HF cycle, thus illustrating the important role of skin Tregs in HF [54], which is of

great significance for the prevention of autoimmune alopecia. Psoriasis is a chronic, recurrent, and autoimmune skin disease that accounts for approximately 2-3% of the global population. Psoriasis mainly affects the skin barrier, and the skin of diseased mice shows the most serious erythema of scale [59]. To study the potential therapeutic effect of kaempferol on skin damage and inflammation in psoriasis, Liu et al. established a mouse model of psoriasis induced by imiquimod (IMQ). It was found that after eight days of treatment with kaempferol, the symptoms of skin lesions were reduced. The expression of Foxp3 in skin lesions was measured by immunohistochemical staining, and it was observed that Foxp3 expression was mainly located in the dermis and was significantly higher in the kaempferol treatment group than in the control group (the same dose of 10% PEG400 solution but without kaempferol). The result shows that skin Tregs play an important role in the treatment of psoriasis and also offers new ideas for the treatment of psoriasis.

3.5. Other Tissue Tregs. In addition to the above-mentioned tissue Tregs, certain studies have also found that selected other tissue Tregs appear in a variety of tissues, such as the lung, liver, and placental tissues. In a mouse model of intranasal influenza virus infection, Arpaia et al. found a population of Treg cells expressing high levels of proinflammatory cytokines IL-18 receptor (IL-18R) and ST2 in the lung. IL-18R⁺ Treg cells can produce a large amount of tissue repair protein amphiregulin to enhance tissue repair [60]. In the liver of patients with autoimmune liver diseases (AILD) such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and autoimmune hepatitis (AIH), dysfunction or lack of quantification of Tregs was found, suggesting that liver tissue Tregs play a unique role in AIH [61]. A Treg population also exists in the placenta, and its main function is to promote maternal-foetal tolerance. For female mice lacking the CNS1 segment of Foxp3, if they carry allogeneic but different gene offspring, foetal reabsorption and placental immune cell infiltration increase [5, 31]. In short, the main function of placental Tregs is to suppress effector immunity and support maternal vascular adaptation, thereby promoting trophoblast invasion and placental entry into the maternal blood supply.

Oral mucosa is an important barrier tissue to protect the oral from invading pathogens, and foreign antigen inflammation in the oral cavity is rarely observed, indicating that overt immune activation in this site is actively suppressed. Park et al. [63] found that the oral mucosa was highly enriched in Foxp3⁺ Tregs, which further showed a different phenotype from Tregs in other peripheral tissues. CD69 and CD103 are surface markers expressed by Foxp3⁺ Treg cells in the oral mucosa; most of the Foxp3⁺ Tregs in the oral mucosa are CD103⁺ Tregs. CD103 is expressed predominantly by conventional T cells in the lung. Integrin CD103 is a surface molecule that can bind to E-cadherin on epithelial cells, thereby retaining T cells in the barrier tissues. This is in sharp contrast with Foxp3⁺ Treg cells from other tissues. Treg cells that can be found across barrier tissues express CD69 that distinguishes them from their peripheral counterparts. CD69 can act as both an activation marker and a tissue

retention molecule of mucosal Treg. In the context of T cell migration, CD69 interferes with S1P1 signalling, thereby impairing T cell recycling. A large population of CD69⁺ cells coexpress CD103; so such CD69⁺CD103⁺ cells will be effectively encapsulated in the tissue, CD103 will cause tissue retention, and CD69 will inhibit tissue migration. In order to check whether the oral mucosal Tregs have other distinguishing characteristics, the expressions of CTLA4, CD44, and Nrp-1 were evaluated, which are commonly used markers for the activation and differentiation of Treg cells. Oral mucosal Tregs express a large amount of CTLA4 and CD44, and their expression level is significantly higher than that of Tregs in other tissues. However, the expression of Nrp-1 was greatly reduced. This is also a major feature that distinguishes it from other tissues [63]. In short, tissue Tregs can not only control local inflammation by inhibiting T cell activation but also control local inflammation by affecting the activity of neutrophils, macrophages, and other myeloid subpopulations. The oral mucosal tissue Tregs are also essential to control the local tissue immunity of the oral cavity.

4. Conclusions and Outlook

Tregs have a strong immunosuppressive ability that restricts the activation and proliferation of effector T cells and maintains immune tolerance. Treg immunotherapy is the main direction of autoimmune disease research [47]. Increasingly, studies have revealed that Tregs with different phenotypes exist in different tissues and are closely related to many diseases, including autoimmune diseases. However, many questions with respect to this group of cells remain to be clarified, such as whether Tregs in different tissues have their own specific phenotypic characteristics, the source of tissue Tregs, whether it settles in the tissue after the development of the thymus in the neonatal period, whether it is recruited from lymphatic tissue under pathological conditions such as inflammation, whether Tregs can be induced by naïve T cells or other cells in local tissues, whether tissue Tregs have the same immune regulation function as lymphoid Tregs, whether their mechanisms of action are consistent, the plasticity and instability of the tissue Treg, and whether the epigenetic regulation of tissue Tregs is the same as that of lymphoid Tregs. However, all of these problems assume that we must clear up the characteristic phenotype of tissue Tregs in the first place to effectively isolate this type of cell subgroup for an in-depth study.

In short, the characteristics and functional diversity of tissue Tregs have been gradually revealed. Therefore, it appears feasible to use this feature and diversity to selectively target and treat autoimmune diseases and other diseases. In this process, many difficulties must be overcome to promote the application of Treg therapy in the field of immunotherapy.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- [1] R. K. Gershon and K. Kondo, "Infectious immunological tolerance," *Immunology*, vol. 21, no. 6, pp. 903–914, 1971.
- [2] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, "Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases," *Journal of Immunology*, vol. 155, no. 3, p. 1151, 1995.
- [3] D. V. Margarita and D. A. Hafler, "Regulatory T cells in autoimmune disease," *Nature Immunology*, vol. 19, no. 7, pp. 665–673, 2018.
- [4] M. E. Brunkow, E. W. Jeffery, K. A. Hjerrild et al., "Disruption of a new forkhead/winged-helix protein, scurfy, results in the fatal lymphoproliferative disorder of the scurfy mouse," *Nature Genetics*, vol. 27, no. 1, pp. 68–73, 2001.
- [5] A. Sharma and D. Rudra, "Emerging functions of regulatory T cells in tissue homeostasis," *Frontiers in Immunology*, vol. 9, p. 883, 2018.
- [6] C. L. Bennett, J. Christie, F. Ramsdell et al., "The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3," *Nature Genetics*, vol. 27, no. 1, pp. 20–21, 2001.
- [7] B. R. Powell, N. R. Buist, and P. Stenzel, "An X-linked syndrome of diarrhea, polyendocrinopathy, and fatal infection in infancy," *The Journal of Pediatrics*, vol. 100, no. 5, pp. 731–737, 1982.
- [8] Y. Zheng, S. Josefowicz, A. Chaudhry, X. P. Peng, K. Forbush, and A. Y. Rudensky, "Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate," *Nature*, vol. 463, no. 7282, pp. 808–812, 2010.
- [9] H. Morikawa and S. Sakaguchi, "Genetic and epigenetic basis of Treg cell development and function: from a FoxP3-centered view to an epigenome-defined view of natural Treg cells," *Immunological Reviews*, vol. 259, no. 1, pp. 192–205, 2014.
- [10] N. Ohkura, M. Hamaguchi, H. Morikawa et al., "T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development," *Immunity*, vol. 37, no. 5, pp. 785–799, 2012.
- [11] Z. Zhang, W. Zhang, J. Guo, Q. Gu, X. Zhu, and X. Zhou, "Activation and functional specialization of regulatory T cells lead to the generation of Foxp3 instability," *The Journal of Immunology*, vol. 198, no. 7, pp. 2612–2625, 2017.
- [12] M. Kanamori, H. Nakatsukasa, M. Okada, Q. Lu, and A. Yoshimura, "Induced regulatory T cells: their development, stability, and applications," *Trends in Immunology*, vol. 37, no. 11, pp. 803–811, 2016.
- [13] S. L. Bailey-Bucktrout, M. Martinez-Llordella, X. Zhou et al., "Self-antigen-driven activation induces instability of regulatory T cells during an inflammatory autoimmune response," *Immunity*, vol. 39, no. 5, pp. 949–962, 2013.
- [14] S. A. McClymont, A. L. Putnam, M. R. Lee et al., "Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes," *Journal of Immunology*, vol. 186, no. 7, pp. 3918–3926, 2011.
- [15] M. N. Rivas, O. T. Burton, P. Wise et al., "Regulatory T cell reprogramming toward a Th2-cell-like lineage impairs oral tolerance and promotes food allergy," *Immunity*, vol. 42, no. 3, pp. 512–523, 2015.
- [16] M. Ayyoub, F. Deknuydt, I. Raimbaud et al., "Human memory FOXP3⁺ Tregs secrete IL-17 ex vivo and constitutively express the TH17 lineage-specific transcription factor ROR γ t," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 21, pp. 8635–8640, 2009.
- [17] G. Beriou, C. M. Costantino, C. W. Ashley et al., "IL-17-producing human peripheral regulatory T cells retain suppressive function," *Blood*, vol. 113, no. 18, pp. 4240–4249, 2009.
- [18] N. Komatsu, K. Okamoto, S. Sawa et al., "Pathogenic conversion of Foxp3⁺ T cells into TH17 cells in autoimmune arthritis," *Nature Medicine*, vol. 20, no. 1, pp. 62–68, 2014.
- [19] H. J. Bovenschen, P. C. van de Kerkhof, P. E. van Erp, R. Woestenrenk, I. Joosten, and H. J. P. M. Koenen, "Foxp3⁺ regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin," *Journal of Investigative Dermatology*, vol. 131, no. 9, pp. 1853–1860, 2011.
- [20] M. D. Sharma, D. Y. Hou, Y. Liu et al., "Indoleamine 2,3-dioxygenase controls conversion of Foxp3⁺ Tregs to TH17-like cells in tumor-draining lymph nodes," *Blood*, vol. 113, no. 24, pp. 6102–6111, 2009.
- [21] M. H. Nyirenda, L. Sanvito, P. J. Darlington et al., "TLR2 stimulation drives human naive and effector regulatory T cells into a Th17-like phenotype with reduced suppressive function," *The Journal of Immunology*, vol. 187, no. 5, pp. 2278–2290, 2011.
- [22] S. You, B. Leforban, C. Garcia, J. F. Bach, J. A. Bluestone, and L. Chatenoud, "Adaptive TGF- β -dependent regulatory T cells control autoimmune diabetes and are a privileged target of anti-CD3 antibody treatment," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 15, pp. 6335–6340, 2007.
- [23] A. Laurence, S. Amarnath, J. Mariotti et al., "STAT3 transcription factor promotes instability of nTreg cells and limits generation of iTreg cells during acute murine graft-versus-host disease," *Immunity*, vol. 37, no. 4, pp. 209–222, 2013.
- [24] U. Baron, S. Floess, G. Wiczorek et al., "DNA demethylation in the human FOXP3 locus discriminates regulatory T cells from activated FOXP3⁺ conventional T cells," *European Journal of Immunology*, vol. 37, no. 9, pp. 2378–2389, 2007.
- [25] M. Esteve Ràfols, "Adipose tissue: cell heterogeneity and functional diversity," *Endocrinología y Nutrición (English Edition)*, vol. 61, no. 2, pp. 100–112, 2014.
- [26] M. Feuerer, L. Herrero, D. Cipolletta et al., "Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters," *Nature Medicine*, vol. 15, no. 8, pp. 930–939, 2009.
- [27] J. R. DiSpirito, D. Zemmour, D. Ramanan et al., "Molecular diversification of regulatory T cells in nonlymphoid tissues," *Science Immunology*, vol. 3, no. 27, p. eaat5861, 2018.
- [28] O. Osborn and J. M. Olefsky, "The cellular and signaling networks linking the immune system and metabolism in disease," *Nature Medicine*, vol. 18, no. 3, pp. 363–374, 2012.

- [29] D. Ganguly, "Do type I interferons link systemic autoimmunities and metabolic syndrome in a pathogenetic continuum?," *Trends in Immunology*, vol. 39, no. 1, pp. 28–43, 2018.
- [30] D. Cipolletta, M. Feuerer, A. Li et al., "PPAR- γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells," *Nature*, vol. 486, no. 7404, pp. 549–553, 2012.
- [31] M. Panduro, C. Benoist, and D. Mathis, "Tissue Tregs," *Annual Review of Immunology*, vol. 34, no. 1, p. 609, 2015.
- [32] U. Smith, "Pioglitazone: mechanism of action," *International Journal of Clinical Practice. Supplement*, vol. 55, no. 121, pp. 13–18, 2001.
- [33] A. B. Molofsky, F. Van Gool, H.-E. Liang et al., "Interleukin-33 and interferon- γ counter-regulate group 2 innate lymphoid cell activation during immune perturbation," *Immunity*, vol. 43, no. 1, pp. 161–174, 2015.
- [34] A. Vasanthakumar, K. Moro, A. Xin et al., "Erratum: the transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells," *Nature Immunology*, vol. 16, no. 5, p. 544, 2015.
- [35] D. Kolodin, N. van Panhuys, C. Li et al., "Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice," *Cell Metabolism*, vol. 21, no. 4, pp. 543–557, 2015.
- [36] A. B. Frias Jr., E. J. Hyzny, H. M. Buechel et al., "The transcriptional regulator Id2 is critical for adipose-resident regulatory T cell differentiation, survival, and function," *The Journal of Immunology*, vol. 203, no. 3, pp. 658–664, 2019.
- [37] M. Yang, L. Su, Q. Tao, C. Zhang, Y. Wu, and J. Liu, "Depletion of regulatory T cells in visceral adipose tissues contributes to insulin resistance in Hashimoto's thyroiditis," *Frontiers in Physiology*, vol. 9, p. 136, 2018.
- [38] H. S. Schipper, B. Prakken, E. Kalkhoven, and M. Boes, "Adipose tissue-resident immune cells: key players in immunometabolism," *Trends in Endocrinology and Metabolism*, vol. 23, no. 8, pp. 407–415, 2012.
- [39] C. Procaccini, F. Carbone, M. Galgani et al., "Obesity and susceptibility to autoimmune diseases," *Expert Review of Clinical Immunology*, vol. 7, no. 3, pp. 287–294, 2014.
- [40] K. S. Kim, S. W. Hong, D. Han et al., "Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine," *Science*, vol. 351, no. 6275, pp. 858–863, 2016.
- [41] E. Sefik, N. Geva-Zatorsky, S. Oh et al., "Individual intestinal symbionts induce a distinct population of $\text{ROR}^{\gamma+}$ regulatory T cells," *Science*, vol. 349, no. 6251, pp. 993–997, 2015.
- [42] K. Atarashi, T. Tanoue, K. Oshima et al., "Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota," *Nature*, vol. 500, no. 7461, pp. 232–236, 2013.
- [43] B.-H. Yang, S. Hagemann, P. Mamareli et al., "Foxp3 $^{+}$ T cells expressing ROR γ t represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation," *Mucosal Immunology*, vol. 9, no. 2, pp. 444–457, 2016.
- [44] G. Brad and P. Sophie, "The ST2/IL-33 axis in immune cells during inflammatory diseases," *Frontiers in Immunology*, vol. 8, p. 475, 2017.
- [45] C. Schiering, T. Krausgruber, A. Chomka et al., "The alarmin IL-33 promotes regulatory T-cell function in the intestine," *Nature*, vol. 513, no. 7519, pp. 564–568, 2014.
- [46] H. Meinicke, A. Bremser, M. Brack, K. Schrenk, H. Pircher, and A. Izcue, "KLRG1 impairs regulatory T-cell competitive fitness in the gut," *Immunology*, vol. 152, no. 1, pp. 65–73, 2017.
- [47] A. M. Schubert, H. Sinani, and P. D. Schloss, "Antibiotic-induced alterations of the murine gut microbiota and subsequent effects on colonization resistance against Clostridium difficile," *Mbio*, vol. 6, no. 4, 2015.
- [48] S. Schiaffino, M. G. Pereira, S. Ciciliot, and P. Rovere-Querini, "Regulatory T cells and skeletal muscle regeneration," *FEBS Journal*, vol. 284, no. 4, p. 517, 2016.
- [49] W. Kuswanto, D. Burzyn, M. Panduro et al., "Poor repair of skeletal muscle in aging mice reflects a defect in local, interleukin-33-dependent accumulation of regulatory T cells," *Immunity*, vol. 44, no. 2, pp. 355–367, 2016.
- [50] R. M. Jin, J. Warunek, and E. A. Wohlfert, "Therapeutic administration of IL-10 and amphiregulin alleviates chronic skeletal muscle inflammation and damage induced by infection," *Immunohorizons*, vol. 2, no. 5, pp. 142–154, 2018.
- [51] D. Burzyn, W. Kuswanto, D. Kolodin et al., "A special population of regulatory T cells potentiates muscle repair," *Cell*, vol. 155, no. 6, pp. 1282–1295, 2013.
- [52] H. Lei, K. Schmidt-Bleek, A. Dienelt, P. Reinke, and H.-D. Volk, "Regulatory T cell-mediated anti-inflammatory effects promote successful tissue repair in both indirect and direct manners," *Frontiers in Pharmacology*, vol. 6, p. 184, 2015.
- [53] A. Waschbisch, N. Schwab, T. Ruck, M. P. Stenner, and H. Wiendl, "FOXP3 $^{+}$ T regulatory cells in idiopathic inflammatory myopathies," *Journal of Neuroimmunology*, vol. 225, no. 1–2, pp. 137–142, 2010.
- [54] N. Ali and M. D. Rosenblum, "Regulatory T cells in skin," *Immunology*, vol. 152, no. 3, pp. 372–381, 2017.
- [55] T. C. Scharschmidt, K. S. Vasquez, H.-A. Truong et al., "A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes," *Immunity*, vol. 43, no. 5, pp. 1011–1021, 2015.
- [56] T. C. Scharschmidt, K. S. Vasquez, M. L. Pauli et al., "Commensal microbes and hair follicle morphogenesis coordinately drive Treg migration into neonatal skin," *Cell Host & Microbe*, vol. 21, no. 4, pp. 467–477.e5, 2017.
- [57] S. Yamazaki, A. Nishioka, S. Kasuya et al., "Homeostasis of thymus-derived Foxp3-regulatory T cells is controlled by ultraviolet B exposure in the skin," *The Journal of Immunology*, vol. 193, no. 11, pp. 5488–5497, 2014.
- [58] A. Nosbaum, N. Prevel, H. A. Truong et al., "Cutting edge: regulatory T cells facilitate cutaneous wound healing," *Journal of Immunology*, vol. 196, no. 5, pp. 2010–2014, 2016.
- [59] C. Liu, H. Liu, C. Lu et al., "Kaempferol attenuates imiquimod-induced psoriatic skin inflammation in a mouse model," *Clinical & Experimental Immunology*, vol. 198, no. 3, pp. 403–415, 2019.
- [60] N. Arpaia, J. A. Green, B. Molledo et al., "A distinct function of regulatory T cells in tissue protection," *Cell*, vol. 162, no. 5, pp. 1078–1089, 2015.
- [61] H. C. Jeffery, L. E. Jeffery, P. Lutz et al., "Low-dose interleukin-2 promotes STAT-5 phosphorylation, Treg survival and CTLA-4-dependent function in autoimmune liver diseases," *Clinical & Experimental Immunology*, vol. 188, no. 3, pp. 394–411, 2017.

- [62] J.-Y. Park, H. Chung, D. T. DiPalma, X. Tai, and J.-H. Park, "Immune quiescence in the oral mucosa is maintained by a uniquely large population of highly activated Foxp3 + regulatory T cells," *Mucosal Immunology*, vol. 11, no. 4, pp. 1092–1102, 2018.
- [63] J. M. Pandya, I. Loell, M. S. Hossain et al., "Effects of conventional immunosuppressive treatment on CD244⁺ (CD28null) and FOXP3⁺ T cells in the inflamed muscle of patients with polymyositis and dermatomyositis," *Arthritis Research & Therapy*, vol. 18, no. 1, p. 80, 2016.