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REVIEW

Tissue adaptation of regulatory T cells in adipose tissue

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$Foxp3^+$ regulatory T (Treg) cells critically suppress over-activated immune responses and therefore maintain immune homeostasis. Adipose tissue-resident Treg (AT Treg) cells are known for modulating immunity and metabolism in adipose tissue microenvironment through various physiological signals, as well as their heterogeneous subsets, which potentially play disparate roles in aging and obesity. Recent single-cell studies of Treg cells have revealed specialized trajectories of their tissue adaptation and development in lymphoid tissues and at barrier sites. Here, we reviewed a T Cell Receptor (TCR)-primed environmental cue-boosted model of adipose Treg cells' tissue adaptation, especially in response to IL-33, IFN-α, insulin, and androgen signals, which trigger sophisticated transcriptional cascades and ultimately establish unique transcriptional modules in adipose Treg cell subsets. In addition, we further discuss potential therapeutic strategies against aging and obesity by blocking detrimental environmental cues, strengthening the functions of specific AT Treg subsets and modifying the communications between AT Treg subsets and adipocytes.

Keywords: adipose tissue · Foxp3 · metabolic disease · obesity · regulatory T cell

Introduction

Regulatory T (Treg) cells, mainly characterized by the expression of CD25 and Foxp3 [1], have been proven to function in a series of physiological and pathologic processes and have been considered as a potential therapeutic target since they have been first identified by the expression of these markers 20 years ago [2, 3]. For the majority of primary studies, researchers are inclined to analyze the circulating Treg cells and those located in peripheral lymphoid organs in mice and humans due to their massive numbers and accessibility, while research on tissue-resident Treg cells is a recently emerging field [4, 5]. The naive Treg cells ($CD62L^{hi}CCR7^+$ or $CD45RA^{hi}CD25^{low}$ Treg cells), reside in secondary lymphoid organs, and form the majority of Treg cells, which function as a baseline suppressor of the immune system; and activated Treg cells ($CD45RA^{low}CD25^{hi}$ or $CD62L^{low}CCR7^{low}CD44^{hi}KLRG1^+CD103^+$ Treg cells) mainly circulate through the blood, play critical roles in enhanced immunosuppression and are able to encounter newly emerging antigens [4, 5]. However, tissue-resident Treg cells show various phenotypes and functions according to specific immune processes and local environments, which not only differ them from the previous two Treg cell categories but also contribute to the heterogeneity of themselves [6]. Thus, except locations, different subsets of tissue-resident Treg cells can also be identified by specific cell markers and transcription factors [7, 8].

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Adipose tissues (ATs) mainly consist of three categories: classical brown, beige, and white adipose tissue (WAT) [9]. Among these, Treg cells residing in visceral adipose tissue (VAT) merit special attention because they are the first identified tissue-resident Treg cell population and are the one most researchers are focused on [10]. In both in vivo and in vitro studies, AT Treg cells show distinctive characters, such as specific chemokine receptors, transcription factors, adhesion molecules, distinct T cell antigen receptor repertoires, mechanisms of action, targets, and migration patterns. For instance, AT Treg cells express a unique TCR repertoire, complementarity-determining region 3α (CDR3α), which is responsible for specific antigen binding. AT Treg cells also express higher amounts of transcription factor PPARγ and IL-33 receptor ST2 [11–13], which are involved in the regulation of insulin sensitivity [6, 14]. This review focuses on the specific features of AT Treg cells by describing the developmental trajectories and their regulators, as also summarizes their functions in various physiological processes.

Developmental trajectory of AT Treg cells

It is widely acknowledged that classical Treg cells are derived either from the thymus or from conventional $CD4^+$ T cells (Tconvs) in the periphery [15]. Several studies have suggested that VAT Treg cells might be derived from the thymus [11, 16]. Using TCR-seq in Limited (LTD) mouse line, Feuerer et al. have shown that the TCRs of VAT Treg cells are different from Tconvs residing in the lymph nodes (LN) and fat [10]. Further transcriptomic analysis, DNA methylation patterns, and transfer experiments have also revealed the differences between VAT Treg cells and Tconvs from fat and lymph nodes (LN) [11, 12, 16]. In addition, most VAT Treg cells have a $Helios^{hi}$ $Nrp\text{-}1^{hi}$ phenotype with $Helios^+$ appearing in nearly 100% VAT Treg cells, while peripheral Treg cells (pTreg cells) own a $Helios^{lo}$ $Nrp\text{-}1^{lo}$ phenotype [11, 17]. These findings indicate that VAT Treg cells may not be derived from Tconvs in the periphery.

Several lines of evidence suggest that the differentiation of AT Treg cells, from birthplace thymus to adipose tissue, is a multi-step and environmental cue-boosted process. Using vTreg53 (VAT Treg) TCR-tg and Pparg-Tdtomato reporter mouse, Li and colleagues have reported that the acquisition of VAT Treg phenotype is a two-step, two-site process with splenic $PPAR\gamma^{lo}$ Treg cells acting as an intermediate state between $PPAR\gamma^-$ Treg cells in thymus and $PPAR\gamma^+$ counterparts in VAT [12]. Physiological signals such as TCR interaction, Foxp3, and IL-33 play active roles in promoting the accumulation of splenic $PPAR\gamma^{lo}$ Treg cells [12, 18]. Furthermore, the splenic tissue-Treg precursor population consists of two transcriptionally divergent subpopulations, called X and Y cells. X cells express more genes encoding adhesion and migration-related proteins, while Y cells express higher level of protein-coding genes for cell activation and differentiation, indicating that X and Y cells are derived separately and might preferentially turn into Treg cells in different non-lymphoid tissues (NLTs) [19].

Similarly, the multi-step process has also been verified by different expression levels of markers such as Id3 [20], Klrg1, and Nfil3 [21]. Treg cells in secondary lymphoid organs of mice could be divided into three subsets, which are $Id3^+CD44^{lo}CD62L^{hi}$ central memory Treg (cTreg) cells, $Id3^+CD44^{hi}CD62L^{lo}$ effector Treg (eTreg) cells, and $Id3^-CD44^{hi}CD62L^{lo}$ eTreg cells in a developmental order [20]. $Id3^-$ eTreg cell is likely to be a terminally differentiated Treg cell population in spleen and has an apparent tendency towards NLTs including fat (the adipose tissue in this article is described as “fat”, while there is no clue as to what type of adipose tissue it is) [20]. Additionally, the same conclusion has been drawn by the upregulation of Klrg1 and downregulation of Nfil3 in a stepwise manner (in terms of VAT Treg cells), which are regulated by transcription factor BATF [21] and BLIMP1 [22], respectively. In adipose tissue, Treg meta-cells (in both SAT and VAT) are classified into two clusters: $CD73^{hi}$ $ST2^{lo}$ and $CD73^{lo}$ $ST2^{hi}$ Treg cells. The former expresses more lymphoid tissue-related markers and are also enriched in secondary lymphoid tissue, while the latter expresses more non-lymphoid tissue genes and are only observed in adipose tissue [23]. It is identified that Treg cells can be converted from the $CD73^{hi}$ $ST2^{lo}$ subset to $CD73^{lo}$ $ST2^{hi}$ subset in adipose tissue upon insulin stimulation, which might indicate the activation of adipose Treg cells [23]. In other words, Treg cells derived from secondary lymphoid tissue are transformed into an adipose-resident Treg cell phenotype under the influence of adipose tissue microenvironment (Figure 1) [23]. From the perspective of epigenomics, the interpretating of ATAC-seq and scRNA-seq data show the priming and establishing of pan-Treg Open Chromatin Regions (OCRs) in the spleen, which enables the rapid transcriptional turn-on of tissue-specific genes as soon as Treg cells arrive at a particular NLT such as VAT, injured skeletal muscle, and colonic lamina propria [24].

Physiological signals and AT Treg cells

In this section, we review some of the key regulators that modulate the accumulation, function, and further differentiation of AT Treg cells (Figure 2).

PPARγ

Peroxisome proliferator-activated receptor (PPAR)-γ is a member of the PPAR nuclear hormone receptor subfamily, functioning as a ligand-gated transcription factor [25]. It is identified as the “master regulator” of adipocyte formation and differentiation [13].

Moreover, PPARγ is also a key molecular regulator for VAT Treg cells’ accumulation, phenotyping, and function [13]. By analyzing the gene expression profiles of Treg cells from mouse visceral fat and lymphoid organ, it has been found that the level of transcripts encoding PPARγ is higher in the former [13]. Treg cells from mouse visceral fat also have elevated co-clustered transcripts functioning in leukocyte migration and extravasation (Ccr1, Ccr3, Cxcr6, Cxcl2, and Cxcl3), lipid metabolism (Pcyt1a and Dgat1),

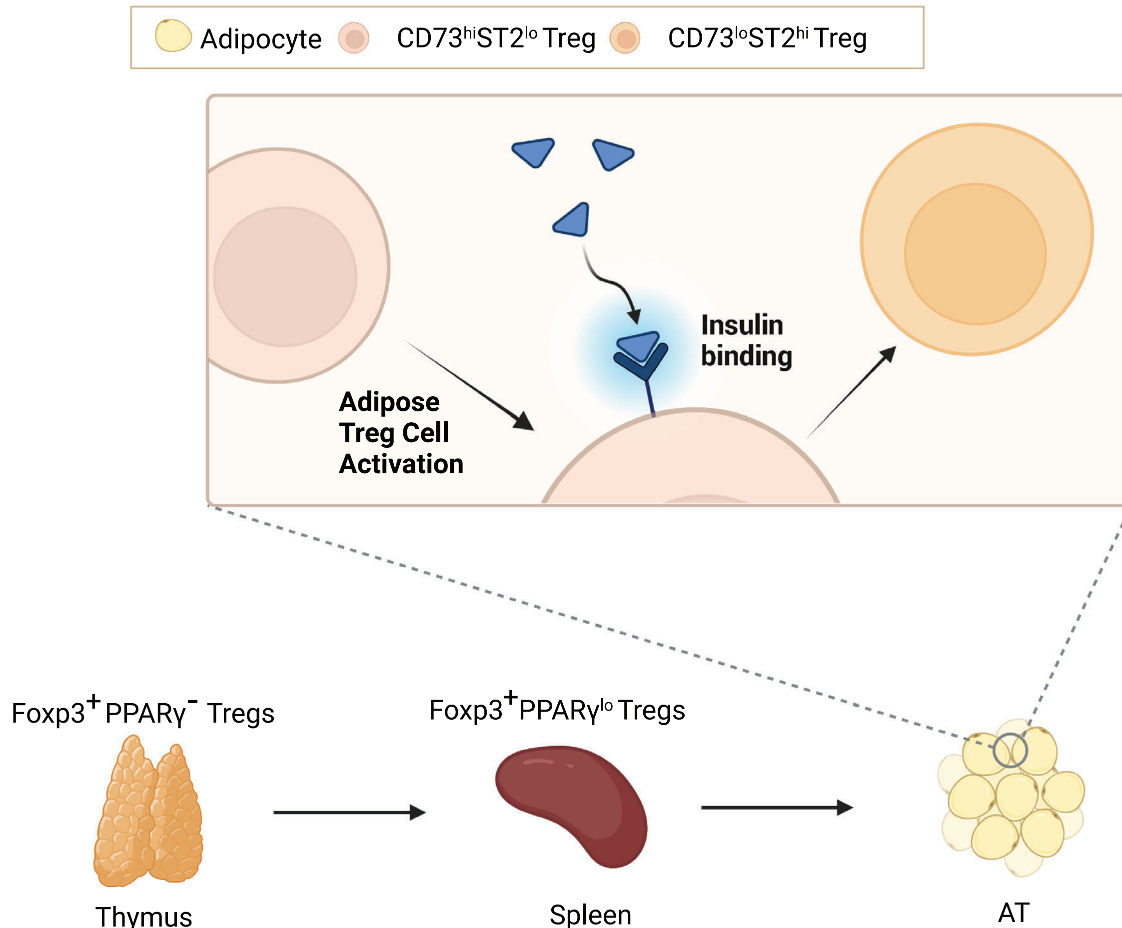

Figure 1. Environmental cue-boosted tissue adaptation of AT Treg cells. After leaving the thymus, Treg cells first differentiate in the spleen, expressing low levels of PPARγ, which is driven by a couple of priming events. After the stimulation with cytokines and tissue-specific antigens as well as epigenetic reprogramming, they further migrate to adipose tissue. In adipose tissue, the $CD73^{hi}$ $ST2^{lo}$ Treg cells are converted to $CD73^{lo}$ $ST2^{hi}$ counterparts through insulin signals, whose receptor is primed possibly as a result of adipose Treg cell activation.

and anti-inflammation (IL-10) [13]. There are two isoforms of PPARγ, PPARγ1 and PPARγ2, with PPARγ2 having additional 30 amino acids at the N-terminal [26, 27]. Both isoforms are expressed in VAT Treg cells [13]. According to cell gene signature, they can induce the upregulation of most genes in VAT Treg cells [28]. However, only PPARγ1 induces the down-regulation of gene expression in VAT Treg cells [13, 28]. For example, both PPARγ isoforms can promote VAT Treg cells up-signature in conjunction with Foxp3, but only PPARγ1 drives the down-signature [13].

Researchers have found a strong correlation between obesity and transcriptional changes of VAT Treg cells, caused by the absence of PPARγ. This suggests that obesity can be regulated by PPARγ in some way [28, 29]. High-fat diet activates CDK5 in obese mice, which induces phosphorylation of serine at 273 loci in PPARγ, resulting in reduced PPARγ vitality [30]. Therefore, the blockade of CDK5 might serve as a promising therapy [30, 31]. Recently, PPARγ is shown to repress the expression of CD73 (encoded by *Nt5e*) in adipose Treg cells, whereas ablation of *Nt5e* in Treg cells not only suppresses the induction of thermogenic and lipolytic genes but also inhibits insulin sensitivity, indicating the overexpression of PPARγ may exert several undesirable side effects [23, 32].

TCR

Adipose-resident Treg cells display a tissue-antigen specific TCR repertoire that recognizes local cognate antigens [12]. The comparison of complementarity-determining region 3α (CDR3α) has revealed that AT Treg cells have a highly restricted distribution of TCR repertoire, which is distinct from Treg cells residing in other organs like spleen and lymph nodes [10]. It is reported that the TCR:MHCII interaction markedly contributes to the accumulation and maintenance of AT Treg cells, which provokes circulating Treg cells to exit the lymph organs and invade the fat, as well as restimulates Treg cell filtration in adipose tissue [10]. In MHCII-deficient mice, the number of AT Treg cells decreases dramatically and few remaining AT Treg cells express Gata3 (a typical AT Treg cell marker) [11]. In addition, CD1d, a non-classical MHC-like

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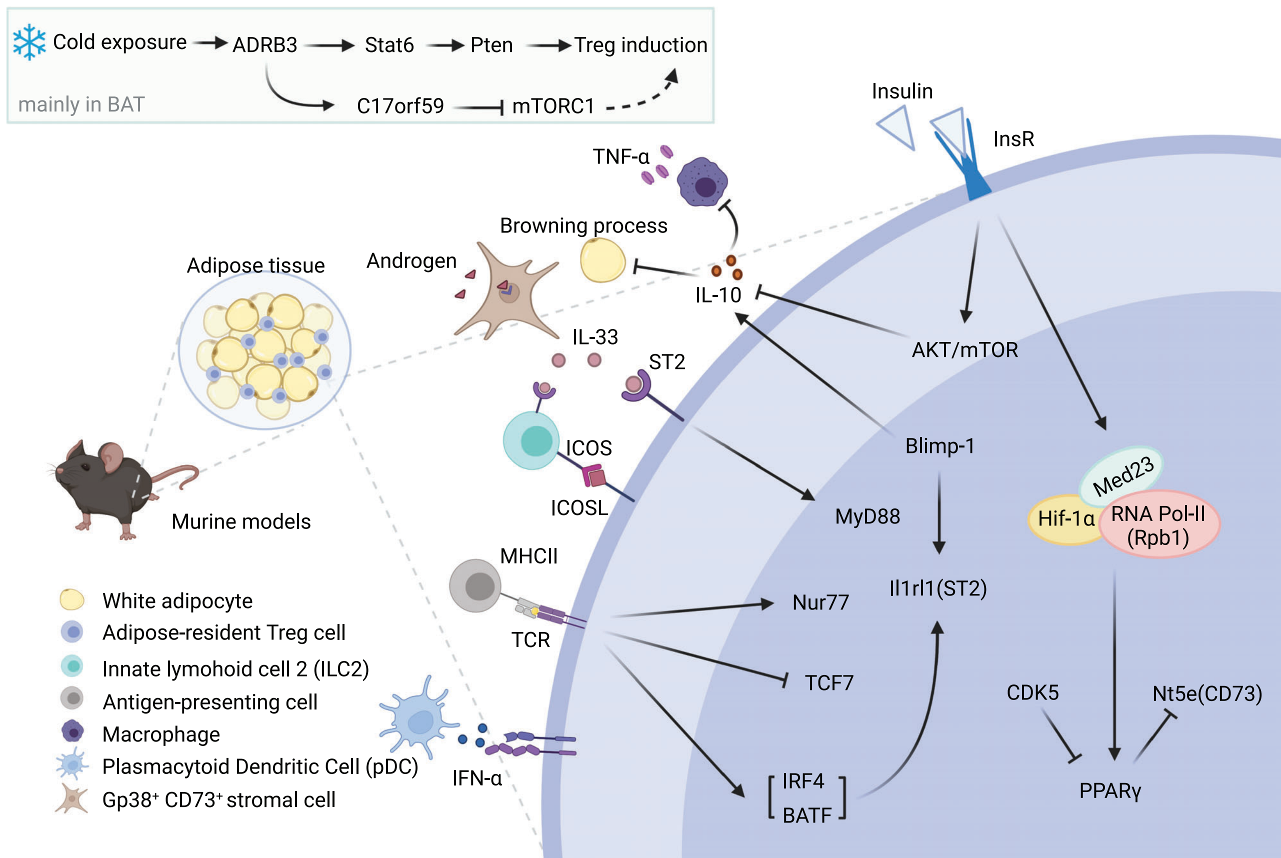

Figure 2. Physiological signaling pathways that regulate tissue adaptation of AT Treg cells. In murine WAT Treg cells, insulin can activate AKT-mTORC1 pathway, resulting in the reduction of IL-10 secretion. Besides, insulin induces PPARγ expression through HIF-1α-Med23-RNA-Pol-II complex, which down-regulates the expression of Nt5e. IL-10, driven by transcription factor Blimp-1, inhibits the secretion of TNF-α in macrophages and the browning of adipocytes. IL-33 is produced by $Gp38^{+}CD73^{+}$ stromal cells and is positively associated with androgen. IL-33-ST2 pathway not only promotes Treg cell proliferation via adaptor protein MyD88, but also induces Treg cell through the ICOS-ICOSL interaction between ILC2 and VAT Treg cells. TCR: MHC interaction induces transcriptional regulators BATF and IRF4, which promote the expression of ST2. ST2, can also be induced by Blimp-1, is a key regulator for VAT Treg cell phenotyping. The TCR:MHC interaction also upregulates Nur77 and downregulates TCF7 expression. In obese mice, CDK5 is activated and induces the phosphorylation of PPARγ at serine 273, resulting in reduced activity of PPARγ. In long-term HFD (High Fat Diet) model, IFN-α, secreted by pDCs, is directly toxic to $PPAR\gamma^{+}$ VAT Treg cells, while the detailed mechanism is still unclear. Cold exposure activates ADRB3, and induces Pten by overexpressing Stat6, resulting in Treg cell induction mainly in BAT (Brown Adipose Tissue). The activation of ADRB3 also upregulates C17orf59 and suppresses mTOR activity, thus leading to the induction of Treg cells.

molecule presenting lipid antigen to $CD4^{+}$ T cells, may also play a role in presenting antigens to stimulate VAT Treg cells [11].

A study has reported that VAT Treg cells express a higher level of Nur77 and lower level of TCF7 than splenic Treg cells in response to TCR signaling [33]. Besides, TCR signals activate transcriptional regulators BATF and IRF4, which promote the expression of ST2, a key regulator for VAT Treg cell phenotype [33]. Furthermore, the most classical T cell co-stimulators (e.g., B7 molecules, CD80 and CD86) can also keep Treg cell number steady in adipose, liver and lymphoid tissues and thus reduce HFD-induced inflammation [34, 35]. These results suggest that TCR signals are critical in maintaining AT Treg cells. According to several studies, some antigen-presenting cell (APC) populations, like macrophages and dendritic cells, are essential to sustain Treg cells in VAT, which reflects the significance of TCR:MHCII interaction in regulating AT Treg cells [11, 36, 37].

IL-33/ST2

IL-33 is a member of the IL-1 family, which is abundantly expressed in endothelial cells, epithelial cells, and fibroblast-like cells [38, 39]. It is often regarded as a potential alarmin of excess inflammation for it is released upon cell injury or tissue damage [40, 41]. It targets resident immune cells in tissue expressing its receptor: the suppression of tumorigenicity 2 (ST2; the *Il1rl1* gene product) [42].

ST2 is supposed to be the only functional receptor for IL-33 [41, 43] and is expressed in various kinds of cells in AT, including adipocytes, mast cells, ILC2s, Th2 cells, and Treg cells [44]. It has two main splice variants and both variants bind to IL-33. The membrane-bound full-length form (ST2L) binds to IL-33 through the ST2L and IL-1RAcP receptor complex, which promotes NF-κB signaling. While the soluble form (sST2) binds directly to IL-33 and acts as a decoy receptor competing with membrane-bound

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ST2 [45, 46]. ST2 is a crucial factor for the maintenance and function of AT Treg cells. In VAT, the number of ST2$^+$ Treg cells increases with age and achieves nearly 90% in mice at the age of 30 weeks, while only a small number of them is detected in spleen [11]. The maintenance of ST2 expression in AT Treg cells depends on IL-33 [33], which stimulates DC to produce IL-2 (selective regulator for ST2$^+$ Treg cell expansion) [47]. Besides, the IL-33-ST2 axis participates in the transformation of CD4$^+$Foxp3$^-$ cells to CD4$^+$Foxp3$^+$ Treg cells [48], through which the immunomodulatory function of AT Treg cells is activated.

The IL-33-ST2 axis is reported to be a positive regulator of VAT Treg cells during obesity-associated insulin resistance and inflammation. In more detail, ST2 or IL-33-deficient mice fed with a normal diet have reduced number of VAT Treg cells [11, 33, 49] and the injection of IL-33 into either lean or obese mice leads to a prominent expansion of VAT Treg cells [11, 33, 50]. It is shown in obese mice that such expansion of VAT Treg cells, caused by IL-33 treatment, can alleviate inflammation and improve metabolic health by reducing fasting blood glucose levels and improving insulin tolerance [50, 51]. However, the expression of IL-33 is slightly elevated in obese mice, suggesting the existence of a distinct mechanism [52]. Several pieces of evidence support the perspective that the reduction of Treg cells and the aberrant decrease of IL-33 are associated with increased sST2, which is released by adipocytes and regarded as a pathogenic factor in obesity [52]. As mentioned above, sST2 acts as a decoy receptor that inhibits IL-33 function. The expression and secretion of sST2, by AT cells, are stimulated by TNF-α expression and antagonized by Zbtb7b expression. The ablation of Zbtb7b aggravates insulin resistance during obesity [52].

Both direct and indirect mechanisms have been proposed for IL-33 in regulating the proliferation and function of Treg cells. IL-33 promotes the expansion and activation of ILC2 cells, which is essential for the accumulation of Treg cells through the inducible T-cell co-stimulator (ICOS) and ICOS ligand (ICOSL) interactions [49]. Besides, in Treg-intrinsic way, also regarded as the direct mechanism, adaptor protein MyD88 (deficiency causes lack of most Treg cells in the adipose tissue) together with transcription regulators BATF and IRF4 (influence the expression of both *Il1rl1* and *Pparg*) regulate Treg proliferation and development through IL-33-ST2 pathway [33].

Insulin

Since their discovery, AT Treg cells have been proven to play important roles in influencing the inflammatory state of adipose tissue and insulin resistance by affecting adipose tissue's synthesis of inflammatory mediators and glucose uptake [10, 53]. In obese mice, AT Treg cells function as immunosuppressive factors and a preserver of insulin sensitivity [53]. However, in aged mice the depletion of PPARγ$^+$ or ST2^{hi} AT Treg cells improves insulin sensitivity [54].

Insulin signaling promotes inflammation in vivo by supporting the optimal activation and function of conventional CD4$^+$ T cells and other immune cell types. It is shown that diet-induced hyperinsulinemia causes AT Treg cell dysfunction, by reducing the expression of IL-10 and restraining the expansion of ST2$^+$ Treg cells [55], which is potentially mediated by the Akt/mTOR pathway as insulin activates AKT signaling in Treg cells [56]. Thus, excessive insulin signals for Treg cells may lead to undesirable metabolic outcomes and the interception of insulin signals might be an approach to relieve the detrimental effects of hyperinsulinemia. Besides, the role of IL-6 and the balance of Th17/Treg have been proven to take part in insulin sensitivity in rat model [57].

As mentioned above, AT Treg meta-cells are classified into the CD73^{hi} ST2^{lo} and CD73^{lo} ST2^{hi} clusters [23]. The transformation of the two clusters is suggested to be a successive, time and environment-dependent program [23]. It is shown that insulin signals, mediated by HIF-1α-Med23-RNA-Pol-II complex, are critical in the induction of PPARγ expression and the generation of CD73^{lo}ST2^{hi} Treg cells. The fact that HIF-1α-Med23-RNA-Pol-II complex influences AT Treg cell homeostasis, makes it a potential therapeutic target for age or diet-induced insulin resistance [23].

Other Regulators

In addition to regulators mentioned above, AT Treg cells can also be regulated by cytokines such as IL-21, IL-2; transcription factors including IRF4, STAT3, Foxp3, STAT6, and ID2; as well as other stimuli like cold exposure, ADRB3 stimulation, diet, and sex hormones [2, 14, 58, 59].

IL-21 plays important roles in inducing and maintaining chronic inflammatory processes as well as negatively regulating Treg cell differentiation and activity [58]. Signal transducer and activator of transcription 3 (STAT3) also plays crucial roles in skewing adaptive immunity in VAT and contributing to diet-induced obesity and insulin resistance [60]. Compared with wild-type mice fed with HFD, *Il21* or *Stat3* knockout mice fed with HFD have increased accumulation of VAT Treg cells and improved insulin sensitivity but decreased adipose inflammation [58, 60]. Further studies have suggested that in obesity state, the level of interferon regulatory factor 4 (IRF4, a transcriptional regulator of fasting lipolysis in adipose tissue [61, 62]) is negatively regulated by IL-21 and it might correlate with the decrease of VAT Treg cells [58, 61].

IL-2 is mainly produced by activated CD4$^+$ T cells, activated CD8$^+$ T cells, NK cells, dendritic cells, and macrophages [63]. Treg cells express all three chains (CD25, CD122, CD132) of IL-2 receptors and depend on IL-2 for survival and function [63]. Stimulation of IL-2 diminishes the activity of AKT pathway, which is of great importance to the development and function of Treg cells [58, 59]. As for its role in VAT Treg cells, some animal experiments use IL-2 to increase the fraction of Treg cells in VAT and spleen [10, 28]. Another study has shown that regulatory iNKT cells could regulate VAT Treg cells' enrichment by producing IL-2 [64]. Foxp3 is identified as the major transcription factor of Treg cells [1, 2, 65]. Recent studies have found that besides working

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as a marker to distinguish Treg cells from Tconv cells, Foxp3 also promotes Treg cells to accumulate in VAT [12].

In addition, IFN-α-expressing plasmacytoid dendritic cells (pDCs) are reported to be directly toxic to PPARγ^+ VAT Treg cells [66]. The long-term HFD induced regression of VAT Treg cells is associated with pDCs' production of IFN-α, which usually inhibits their accumulation [66]. Therefore, pDCs and type-I IFN pathway might be promising targets for insulin resistance and other metabolic diseases [66].

Several groups have reported that male and female mice differ in their metabolic tenors under steady-state conditions and during obesogenic challenges, which may because they have different gonadal VAT Treg cell compartments [6, 22, 67]. The male mice enriched in VAT Treg cells display striking difference in chromatin accessibility, transcriptional landscape, and phenotype as compared to their female counterparts [22, 67]. This is likely because of differences induced by sex hormones. When fed with high-fat diet, Treg cells increased in the adipose tissue of female mice, while the opposite was found in male mice. Moreover, HFD-fed male mice are more likely to develop visceral inflammation, glucose intolerance, hyperinsulinemia, and islet hypertrophy [67]. Female mice seem to be resistant to HFD-induced metabolic changes [67]. Osteoclast differentiation receptor, RANK, and its ligand RANKL play an important role in this gender difference. They are induced by various stimuli, including sex hormone progesterone [68]. RANK could regulate thymic Treg cells, in a female hormone-mediated way, during pregnancy [69]. Studies have found impaired accumulation of VAT Treg cells in thymic-Rank-deleted mice, together with adipocyte size enlargement, tissue inflammation, maternal glucose intolerance, and other metabolic disorders [69]. Besides, IL-33, produced by Gp38$^+$CD73$^+$ stromal cells, is largely restricted to male VAT and is positively associated with androgen [22]. Male mice lacking androgen receptor show fewer CD73$^+$ stromal cells [22]. Accordingly, compared with female mice, male mice exhibit higher level of IL-33 due to the effect of androgen [22].

Cold exposure, adrenergic signals, and high-fat diet are able to increase Foxp3$^+$ Treg frequencies mainly in brown adipose tissue (BAT) [14]. Cold exposure and endogenous controls have the ability to activate ADRB3, which is expressed on human adipocytes and T cells [14]. Firstly, ADRB3-stimulation upregulates regulator-interacting protein C17orf59 (encoded by *Borcs6*), which limits mTORC1 activity and in turn enhances Treg cell induction [14]. These induced Treg cells function as regulators for lipolysis and thermogenesis in BAT by upregulating lipolysis and browning related genes in VAT [14]. Additionally, ADRB3 stimulation activates and upregulates STAT6, which increases Pten expression in CD4$^+$ T cells, thus enhancing Treg cell frequency and Treg cell tolerance as well as regulating Treg cell functions [14].

Although, regulators take part in VAT Treg cells' proliferation, differentiation and function, the detailed functions of these regulators, either alone or in combination with other cytokines, have not been studied thoroughly. Therefore, systematic study of these specific or potential regulators exhibits great academic and clinical significance.

Obesity and AT Treg cells

The prevalence of obesity and type 2 diabetes (T2D) has increased significantly, with similar trends, in recent years [13]. The occurrence and progress of obesity-associated inflammation are closely related to the adjustment of homeostasis in adipose tissue and dysregulated pro-inflammatory immune cells, including M1-macrophages [70]. Besides, several studies have reported reduced proportion of VAT Treg cells, acting as anti-inflammatory regulators, in diet-induced obesity in mice [10, 56, 71]. At the same time, mRNA signatures of VAT Treg cells in obese mice are changed, which means they are no longer bona fide VAT Treg cells [29].

58 Mechanisms behind the reduction of VAT Treg cells have been investigated for several years. Although TCR: MHC interaction plays an important role in maintaining VAT Treg cells, this situation is altered in HFD mice. In HFD mice, elevated leptin increases MHCII expression in adipocyte, enhances IFN-γ expression in adipose resident T cells, and expands T-bet cell number, causing an escalation cycle of inflammation [72]. IFN-γ blocks IL-33 in stimulating Treg cell differentiation, thus preventing their accumulation in obese mice. HFD-fed MHCII$^{-/-}$ mice are reported to be highly resistant to classical HFD-induced decrease of adipose Treg cells [72]. Besides, elevated IL-21 level has been observed in adipose tissues of obese mice and humans, which exerts negative regulation on IRF4 (transcriptional regulator of fasting lipolysis) function and Treg cell activity. Therefore, IL-21 may play a great part in developing and maintaining the inflammatory state within obese adipose tissue and serve as a potential therapeutic target [58]. Additionally, sST2 expression is upregulated in the context of adiposity. It binds to IL-33 and blocks the interaction between IL-33 and ST2 on Treg cells, thus diminishing Treg cells and leading to more severe metabolic disorders in the long-term DIO model [52]. However, in a 2-month HFD treatment, there is an increase in ST2^{hi} Treg cells. This increase owes to the transition from CD73^{hi} Treg cells, which is launched by the rise of insulin levels in adiposity. However, with the decrease of CD73^{hi} Treg cells and the increase of sST2, ST2^{hi} Treg cells also decline in the long run [23]. Moreover, several other regulators such as STAT3 [60] and KLF10 [73] also contribute to the variation of Treg cell subsets in adiposity.

As mentioned previously, loss and dysfunction of VAT Treg cells will aggravate obesity. Thus, modulation of VAT Treg cell function might serve as a credible way to treat obesity-associated diseases. Kathrin et al. have reported that CD4$^+$Foxp3$^+$ Treg cells transfer is able to improve insulin resistance and diabetic nephropathy through tipping the balance between Treg cells and pro-inflammatory cells in favor of Treg cells and inhibiting CD8$^+$ effector T cells [71]. There are two main strategies of Treg cell transfer: using freshly isolated Treg cells or making expansion

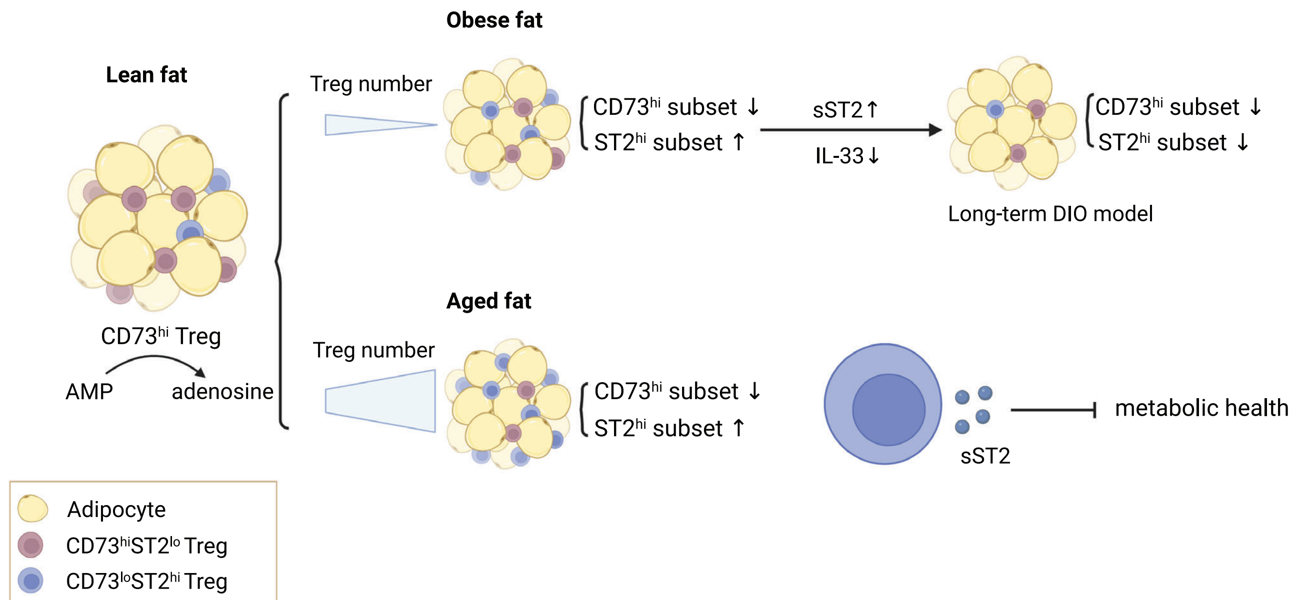

Figure 3. Disparate roles of AT Treg subsets in obesity and aging. Two subsets of Treg cells are present in adipose tissue, the CD73^{hi} subset and the ST2^{hi} subset, in which CD73^{hi} subset is able to generate adenosine to suppress inflammation. The number of Treg cells in obese adipose tissue is lower than that in lean adipose tissue. A two-month High Fat Diet (HFD) treatment increases the percentage of ST2^{hi} adipose Treg cells, while decreases the proportion of CD73^{hi} subset. In long-term DIO (Diet Induced Obesity) model, the proportions of both CD73^{hi} and ST2^{hi} Treg cells are reduced, owing to sST2 functioning as a decoy receptor, which diminishes IL-33 signaling. However, in aged adipose tissue, Treg cell number increases compared to lean adipose tissue. In this case, CD73^{hi} subset decreases while ST2^{hi} subset increases. Meanwhile, the rise of ST2^{hi} subset may lead to increase of sST2 secretion and disruption of metabolic health.

of autologous Treg cells before infusion (reviewed in [74]). In addition to Treg cell transfer, it is also feasible to induce them *in vivo* by oral administration of anti-CD3 antibody with the addition of β-glucosylceramide [75] or injection of a complex consisting of recombinant IL-2 and a particular-IL-2-specific monoclonal antibody [10]. Administration of intestinal Bacteroides uniformis CECT 7771 has also been reported to amplify Treg cells through the stimulation of TSLP production and the increase of IL-33 concentration in EAT (Epididymal Adipose Tissue) [76]. In addition, there is an extensive remodeling of mesenteric lymphatic vessels in human with obesity and mice fed with HFD, which allows the leakage of HFD-modified lymph-containing immune cells into VAT. This process is regulated by the activation of COX-2-PGE2 and VEGF-C-VEGFR3 signaling in lymphatic endothelial cells [77]. Thus, lymph-targeted inhibition of COX-2 could reverse mesenteric lymphatic dysfunction, which indirectly reduces the leakage of HFD-modified immune cells to adipose tissue, blocks weight gain, restores glycemic control and reduces hyperinsulinemia in obese mice [77]. Regulation of the interactions between Treg cells and other cells in adipose tissue serves as another possible treatment for obesity. GRIM19-transgenic HFD mice have been reported to exhibit the ability to maintain the balance between Th17 and Treg cells, disturbed by insulin resistance in the wild-type HFD counterpart, by downregulating STAT3 while upregulating STAT5 expression [78]. In addition, treatment of anti-CD3 antibody or its F(ab')$_2$ fragment reduces the predominance of Th1 cells over Foxp3$^+$ cells in ob/ob mice, leading to reduced insulin resistance for several months [53].

Aging and AT Treg cells

VAT Treg cells start to accumulate from 5 weeks and reach about 50% of CD4$^+$ T cells population between 20–30 weeks in mice [79]. Although significant decrease has been observed at the age of 40 weeks, the number of VAT Treg cells at 44 weeks of age is observably higher than that at 12 weeks [54]. The accumulation of VAT Treg cells may partly attribute to the increase of ST2^{hi} subset, which is ignited by insulin and expanded by IL-33 [11], while insulin levels, IL-33, and IL-33 producers are accrued with aging [11, 33, 80]. Besides, treatment with anti-ST2 antibody is able to deplete 50% percent of VAT Treg cells in 45-week-old mice, which further supports this assumption [79].

Disparate performance of AT Treg cell in obesity and aging

The expansion of AT Treg cells leads to age-related insulin resistance, which is relieved in AT Treg cell knockout mice [54]. While, as mentioned above, obesity-related insulin resistance is accompanied by the reduction of AT Treg cells. These facts indicate that the underlying mechanisms between age-associated IR and obesity-associated IR are different. Obesity-associated IR is a macrophage-centric process as mentioned previously. Newly published research has provided a possible explanation for age-related IR. Aging induces the enrichment of ST2^{hi} adipose Treg cells by increasing insulin, while decreasing the percentage of

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CD73^{hi} Treg cells. CD73^{hi} Treg cells could suppress inflammation through generating adenosine [81], which is able to restrain the capacity of effector T cells and limit the responses of type 1 helper T cell [11]. Thus, the reduction of CD73^{hi} Treg cells potentially aggravates insulin resistance. Although the change trends of AT Treg cell quantities in obesity and aging are different, obese AT Treg cells have recapitulated the phenotype of aged ones, which accounts for the lower insulin sensitivity in obesity as shown in Figure 3 [23].

Human AT Treg cells

Research of AT Treg cells is largely based on animal models. Studies on human AT Treg cells are limited and controversial to some extent. Similar to mouse models, human omental AT Treg cells express a higher level of canonical Treg-defining markers and AT-specific signatures like PPARγ, CCR4, PRDM1, and CXCL2. Although a study has reported that ST2, IL10, and AREG are not notably expressed in human AT Treg cells, which raises doubts about several conclusions obtained in mice [82]. Another study has reported elevated ST2 in VAT Treg cells from human beings [33]. There is another controversial point about human AT Treg cells when it comes to obesity. On the one hand, several studies hold the point that AT Treg cells are negatively correlated with obesity. For example, Foxp3 expression is lower in human VAT in obese situation [83]. Besides, lower omental Treg cell number is associated with higher plasma fasting glucose [84]. On the other hand, two studies have reported positive correlation between AT Treg cells and obesity [85, 86]. These contradictions may partly be attributed to limited number of tissue samples and distinct standards for tissue processing. Further research on human AT Treg cells is urgently needed to elucidate the role of AT Treg cells in humans.

Conclusions

The momentous role of AT Treg cells in modulating immunity and metabolism, through various physiological signals, in adipose tissue microenvironment is drawing greater attention. AT Treg cells, originated from thymus, settle in adipose tissue through a multi-step and environmental cue-boosted process. Physiological signals such as IL-33 and insulin trigger sophisticated transcriptional cascades, which form unique transcriptional modules in adipose Treg subsets. Dysfunction of AT Treg subsets is related to multiple diseases, including obesity and aging-related diseases [87]. Modulating the balances among AT Treg subsets as well as AT Treg cells and other cells in adipose tissue have promising therapeutic potentials against aging or obesity. We believe that a more comprehensive understanding about the function of tissue-resident Treg cells will be accomplished with the advances in innovations and development of new technologies

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Abbreviations: **AT**: adipose tissue · **BAT**: brown adipose tissue · **HFD**: high fat diet · **IR**: insulin resistance · **NLT**: non-lymphoid tissue · **SAT**: subcutaneous adipose tissue · **VAT**: visceral adipose tissue · **WAT**: white adipose tissue

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