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STATE-OF-THE-ART REVIEW

Neuroimmune regulation of white adipose tissues

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The white adipose tissues (WAT) are located in distinct depots throughout the body. They serve as an energy reserve, providing fatty acids for other tissues via lipolysis when needed, and function as an endocrine organ to regulate systemic metabolism. Their activities are coordinated through intercellular communications among adipocytes and other cell types such as residential and infiltrating immune cells, which are collectively under neuronal control. The adipocytes and immune subtypes including macrophages/monocytes, eosinophils, neutrophils, group 2 innate lymphoid cells (ILC2s), T and B cells, dendritic cells (DCs), and natural killer (NK) cells display cellular and functional diversity in response to the energy states and contribute to metabolic homeostasis and pathological conditions. Accumulating evidence reveals that neuronal innervations control

Abbreviations
123I-BMIPP-SPECT/CT, [123I]-β-methyl-p-iodophenyl-pentadecanoic acid-single-photon emission-computed tomography-computed tomography; 18F-FDG-PET/CT, [18F]-fluorodeoxyglucose-positron emission tomography-computed tomography; Adrβ2, adrenergic receptor β2; Adrβ3, adrenergic receptor β3; ALDH1A1, aldehyde dehydrogenase 1a1; Arg1, arginase 1; BDNF, brain-derived neurotrophic factor; BMI, body mass index; CCL2, C-C motif chemokine ligand 2; CCL3, C-C motif chemokine ligand 3; CCR2, C-C motif chemokine receptor 2; CCR4, C-C motif chemokine receptor 4; CCR5, C-C motif chemokine receptor 5; CCR7, C-C motif chemokine receptor 7; CGRP, calcitonin gene-related peptide; ChAT, choline acetyltransferase; Chil3, chitinase-like 3; CHRNA2, cholinergic receptor nicotinic α2 subunit; CINCs, cold-induced neuroimmune cells; CLS, crown-like structures; cPLA2α, cytosolic phospholipase A2; CRP, C-reactive protein; CSF1R, colony-stimulating factor 1 receptor; CXCR1, C-X-C motif chemokine receptor 1; CXCR2, C-X-C motif chemokine receptor 2; DCs, dendritic cells; DRG, dorsal root ganglia; eGFP, enhanced green fluorescent protein; eWAT, epididymal WAT; Ext1, exostosin glycosyltransferase 1; FASN, fatty acid synthase; FFA, free fatty acid; FIP, fibro-inflammatory progenitors; GATA3, GATA-binding protein-3; GCGR, glucagon receptor; HFD, high-fat diet; HSV-1, herpes simplex virus-1; iBAT, interscapular brown adipose tissues; IgG, immunoglobulin G; IL-10, interleukin-10; IL-10Rα, interleukin-10 receptor α; IL-13, interleukin-13; IL-17RC, interleukin-17 receptor C; IL-1RA, interleukin-1 receptor antagonist; IL-1β, interleukin-1β; IL-1β, interleukin-1β; IL-2, interleukin-2; IL-33, interleukin-33; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; IL-6Rα, interleukin-6 receptor α; IL-8, interleukin-8; ILC2s, group 2 innate lymphoid cells; iNKT, invariant natural killer T cells; iNOS, inducible nitric oxide synthase; IRS2, insulin receptor substrate 2; iWAT, inguinal white adipose tissues; LAM, lipid-associated macrophage; LPS, lipopolysaccharide; MAOA, monoamine oxidase A; MCP-1, monocyte chemoattractant protein 1; Met-Enk, methionine-enkephalin; MHCII, class II major histocompatibility complex; MTII, melanotan II; NAMs, nerve- and airway-associated macrophages; NAMs, nerve-associated macrophages; NE, norepinephrine; NETO, norepinephrine turnover; NGF, nerve growth factor; NK cells, natural killer cells; nNOS, neuronal nitric oxide synthase; NP1, neuropilin-1; OCT3, organic cation transporter 3; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1 α; PLZF, promyelocytic leukemia zinc finger protein; PPARγ, peroxisome proliferator-activated receptor-γ; PRV, pseudorabies virus; PSNS, parasympathetic nervous system; RA, retinoic acid; Rald, retinaldehyde; Retnla, resistin-like α, also called Fizz1 (found in inflammatory zone 1); RTX, resiniferatoxin; rWAT, retroperitoneal white adipose tissues; SAMs, sympathetic neuron-associated macrophages; scRNA-seq, single-cell RNA sequencing; Sema3A, semaphorin 3A; SLC6A2, solute carrier family 6 member 2; SNS, sympathetic nervous system; SP, substance P; ST2, suppression of tumorigenicity 2 protein; STAT3, signal transducer and activator of transcription 3; SVF, stromal/vascular fraction; T2D, type 2 diabetes; TCR, T-cell receptor; TGF-β1, transforming growth factor-β1; Th17, T helper 17; Th2, T helper 2; TNF-α, tumor necrosis factor-α; T_{reg}, regulatory T cells; Trem2, triggering receptor expressed on myeloid cells 2; TrkA, receptor tyrosine kinase for nerve growth factor; TRPV1, transient receptor potential cation channel subfamily V member 1; UCP1, uncoupling protein 1; VAChT, vesicular acetylcholine transporter; VAMs, vasculature-associated adipose tissue macrophages; VIP, vasoactive intestinal peptide; WAT, white adipose tissues.

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lipid deposition and mobilization via regulating lipolysis, adipocyte size, and cellularity. Vice versa, the neuronal innervations and activity are influenced by cellular factors in the WAT. Though the literature describing adipose tissue cells is too extensive to cover in detail, we strive to highlight a selected list of neuronal and immune components in this review. The cell-to-cell communications and the perspective of neuroimmune regulation are emphasized to enlighten the potential therapeutic opportunities for treating metabolic disorders.

Introduction

The WAT serve as a critical reserve of energy storage and hormone production [1–3]. Lipodystrophy with the deficiency in WAT formation leads to severe defects in glucose sensitivity, and obesity with excessive fat accumulation is the most common cause of insulin resistance, a key feature of type 2 diabetes (T2D) [4]. The rising prevalence of obesity and comorbidities such as cardiovascular diseases, various cancers, and diabetes has driven continued interest in understanding the WAT biology along with the therapeutic potential [5–7].

A variety of cell types are present in the WAT, including adipocytes, immune cells, endothelial cells, stromal cells, and peripheral nerves which orchestrate the functions of lipid storage, lipid hydrolysis, and oxidation (Fig. 1). The white adipocytes are the major energy storage cells, with a unilocular lipid droplet occupying the majority of the cytoplasmic space. The triglyceride stored in the lipid droplet undergoes lipolysis to provide fatty acids to other tissues as an energy source in demand [8]. The white adipocytes also serve as important endocrine cells, secreting adipokines including leptin and adiponectin to regulate metabolic activities such as food intake and insulin sensitivity [1,2]. Beige adipocytes become discernable in some WAT post-treatments such as cold acclimation and β-adrenergic agonists that are multilocular and express thermogenic gene uncoupling protein 1 (UCP1) [9–16].

The induction of beige adipocytes has been intensively characterized in some fat depots such as the inguinal white adipose tissues (iWAT) (Fig. 2) [12,17–19]. The beige adipocytes share certain morphological similarity with brown adipocytes in the interscapular brown adipose tissues (iBAT), such as high content of mitochondria, but are also different in development, bioenergetics, and functions [16,20,21]. For instance, the metabolic activities of glucose and fatty acid uptake and distribution, assessed by [^{18}F]-fluorodeoxyglucose-positron emission tomography-computed tomography (^{18}F-FDG-PET/CT) and [^{123}I]-β-methyl-p-iodophenyl-pentadecanoic acid-single-photon emission computed tomography-computed tomography (^{123}I-BMIPP-SPECT/CT), respectively, reveal that the 'beige-like' fat tissues retain fatty acid tracers more preferentially when compared to BAT [18], indicating a rather distinct capacity for fuel utilization. The epididymal WAT (eWAT) is less prone to undergo beiging, and they do not display significant ^{18}F-FDG-PET/CT or ^{123}I-BMIPP-SPECT/CT-based signals [18]. The heterogeneous features of various fat pads suggest that a comprehensive characterization of the fat depots would be of great importance, revealing how the heterogeneity is determined developmentally and adapted through adulthood, and how it may contribute to the energy balance in an integrative manner.

A large body of literature has demonstrated that the various cell types in addition to adipocytes affect adipose metabolic activities. The immune cells dictate both the tissue microenvironment and the systemic inflammation, therefore contributing to metabolic health [22–24]. Transient inflammatory response is initiated as part of the healthy adipose expansion, which could influence the remodeling of the extracellular matrix [25]. However, the chronically dysregulated adipose immune profiles precipitate the pathological conditions, contributing to progression of metabolic disorders (Fig. 3) [4,26,27]. A plethora of immune cells including macrophages/monocytes, eosinophils, neutrophils, ILC2s, T and B cells, DCs, and NK cells play diverse roles in regulating immune homeostasis and inflammation [22].

The progress in whole-mount immunostaining and volume fluorescence imaging has aided the illustration of the tissue-wide distribution of peripheral nerves in the WAT [28,29]. Whole-tissue studies show that a dense sympathetic neural network is distributed throughout the tissues with the majority of adipocytes receiving proximal neural input in mouse iWAT housed at ambient temperature [28]. The cell-to-cell

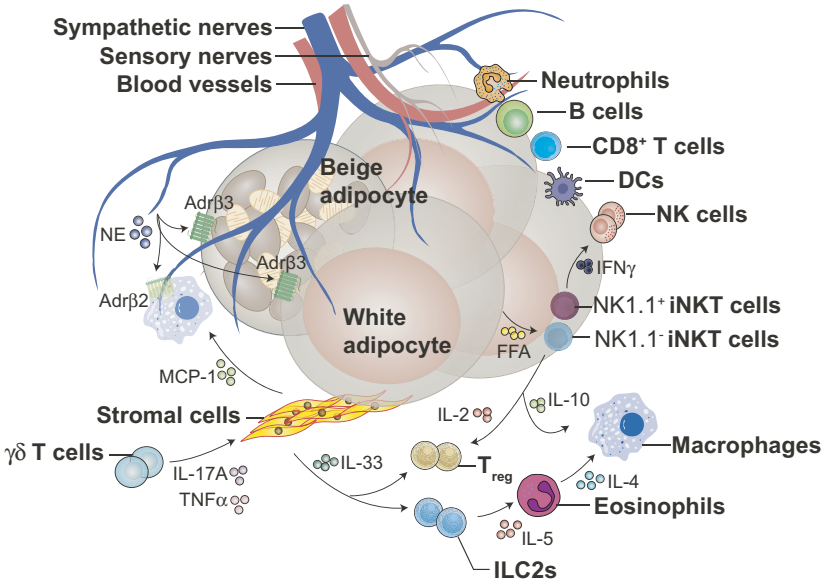

Fig. 1. The white adipose tissues and cellular components. Sympathetic and sensory nerves are detected in the white adipose tissues. Sympathetic nerves release neurotransmitter NE, which signals to Adrβ3 on white and beige adipocytes. Macrophages express the NE receptor Adrβ2. The stromal cell types express IL-33 which could signal to ILC2s and T_{reg}. IL-5 released by ILC2s sustains eosinophils, which further regulate macrophages via IL-4. γδ T cells release IL-17A and TNF-α to promote IL-33 production from stromal cells. NK1.1$^+$ iNKT cells produce IFNγ, and NK1.1$^-$iNKT cells respond to FFA and predominantly release IL-10 and IL-2, which further regulates macrophages and T_{reg}, respectively. Adrβ2, adrenergic receptor β2; Adrβ3, adrenergic receptor β3; DCs, dendritic cells; FFA, free fatty acid; IL-17A, interleukin-17A; IL-2, interleukin-2; IL-33, interleukin-33; IL-4, interleukin-4; IL-5, interleukin-5; ILC2s, group 2 innate lymphoid cells; iNKT cells, invariant natural killer T cells; MCP-1, monocyte chemoattractant protein-1; NE, norepinephrine; NK cells, natural killer cells; TNF-α, tumor necrosis factor-α; T_{reg}, regulatory T cells.

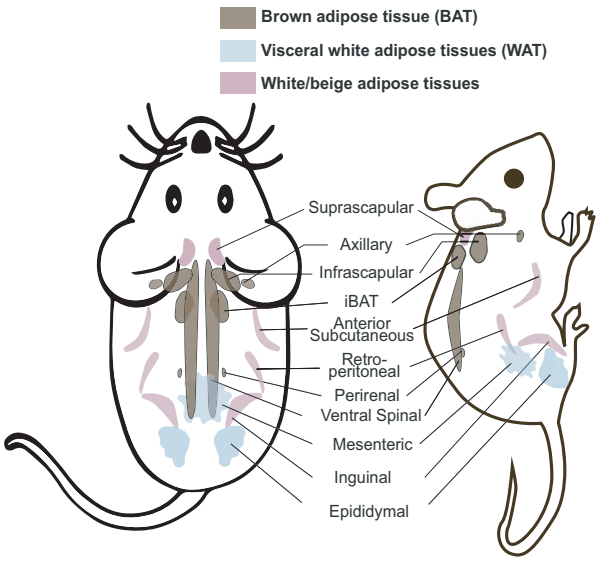

Fig. 2. The distributions of adipose tissues in mice. The adipose tissues are broadly categorized into brown, white, or beige, according to the composition of brown, white, or beige adipocytes. BAT, brown adipose tissues; WAT, white adipose tissues.

commutations are thus extended extensively at the layer of neural regulation, which potentially reaches each cell type within the tissues including the diverse populations of immune cells.

Sympathetic nerves

Sympathetic innervation, axonal plasticity, and fat pad-specific drive

The sympathetic innervation is observed in various WAT, with a significant correlation between the density of the fibers and the number of beige adipocytes [12,28–30]. Sympathetic neurons originate from neural crest cells and form the sympathetic ganglia early in development. The axon growth, dendrite formation, and target innervation occur subsequently during embryonic and postnatal stages [31]. Sympathetic nerves grow into WAT parenchyma between postnatal day 6 and day 28 in mice, overlapping with early beige adipogenesis [32]. Developing axons are guided by both attractive and repulsive cues. The expression of

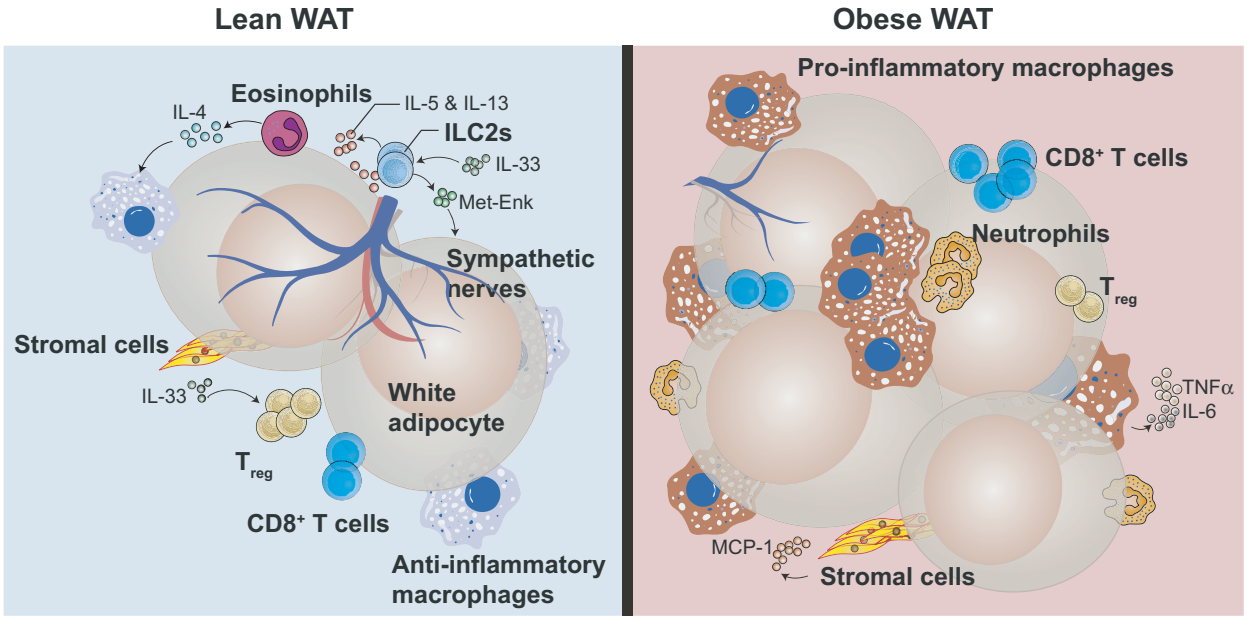

Fig. 3. Neural and immune phenotypes in the lean and obese mouse white adipose tissues. The lean white adipose tissues predominantly contain immune cells at noninflammatory states, including macrophages, eosinophils, ILC2s, $CD4^+$ T_{reg}, and $CD8^+$ T cells. ILC2s maintained by IL-33 from stromal cells produce IL-5 and IL-13, which subsequently regulate eosinophils and macrophages. ILC2s also secrete Met-Enk, which promotes the beiging process. Eosinophils are a major cell source of IL-4 which regulates noninflammatory macrophage phenotype. In obesity, the cellular composition changes with neutrophil infiltration in short-term followed by increased proportion of $CD8^+$ T cells and macrophages. The immune profile shifts to a proinflammatory state. Proinflammatory macrophages increase expression of TNF-α and IL-6. The reduction of sympathetic nerve density and vascularization occurs in obesity. IL-13, interleukin-13; IL-33, interleukin-33; IL-4, interleukin-4; IL-5, interleukin-5; IL-6, interleukin-6; ILC2s, group 2 innate lymphoid cells; MCP-1, monocyte chemoattractant protein 1; Met-Enk, methionine-enkephalin; TNF-α, tumor necrosis factor-α; T_{reg}, regulatory T cells.

Sema3A and NP1 is detected in the rat retroperitoneal WAT (rWAT) and eWAT [33]. Semaphorin 3A (Sema3A) binds to neuropilin-1 (NP1) and activates the transmembrane Plexin to transduce a repulsive axon guidance signal [34]. Sema3A is produced in smooth muscle cells of arteries and white adipocytes, and NP1 is found on perivascular and parenchymal nerves, consistent with a role for secreted Sema3A in the growth and plasticity of the WAT nerves [33]. Besides, preadipocytes and adipocytes produce unknown secretory molecules which could regulate axon growth [35]. Genetic deletion of aldehyde dehydrogenase 1a1 (ALDH1A1), a key enzyme for the production of vitamin A metabolite of retinoic acid (RA) from the precursor retinaldehyde (Rald), leads to increased sympathetic innervation in WAT [35]. Mechanistic analysis indicates that Rald and RA differentially regulate the expression of axon guidance molecules to influence the nerve density [35].

Both environmental stimuli and energy balance affect the intra-adipose neuronal innervation. Metabolic stress such as high-fat diet (HFD)-induced obesity, genetic obese, and diabetic mice homozygous for the diabetes mutations (*Lepr*^{db}, referred to as *db*/*db*) or for the obese spontaneous mutation (*Lep*^{ob}, referred to as *ob*/*ob*) could drive sympathetic axonal degeneration within the adipose tissues [28,36,37]. Further, aging is also associated with adipose neuropathy, which results in loss of innervation around the tissue vasculature [37]. Conversely, cold exposure or exercise leads to increased nerve density [12,37–39] visualized within the whole tissue [39]. The innervation is potentially influenced by the neurotrophic factors including nerve growth factor (NGF) [39] and brain-derived neurotrophic factor (BDNF) [40]. Blockage of NGF through neutralization antibody or pharmacological inhibition of the receptor tyrosine kinase (RTK) for NGF (TrkA) impairs the axonal outgrowth process [39]. BDNF functions in distinct cell types located in the central nervous system and the periphery [36,40]: Deletion of BDNF in the paraventricular nucleus of the hypothalamus blunts the leptin-induced sympathetic re-innervation in the *ob*/*ob* mice [36]; deletion of BDNF from $LyzM^+$ myeloid cells results in a decrease in total innervation of the iWAT [40].

The sympathetic drive to the WAT displays fat pad-specific patterns in response to different lipolytic stimuli [41–43]. For instance, the norepinephrine (NE) turnover (NETO), indicative of sympathetic activity, shows that the eWAT NETO is unaffected by glucoprivation via administration of 2-deoxy-D-glucose but increases with cold exposure or food deprivation; however, iWAT NETO significantly increases across all stimuli in Siberian hamsters [41]. NETO also shows a proportionately higher rate and greater extent of lipolysis in eWAT compared with iWAT when exposed to short winter-like days in Siberian hamsters [42], which could potentially result in energy dissipation in specific fat depots. Prolonged fasting results in increased NETO in rWAT and eWAT, but no change in interscapular BAT (iBAT) in rats [44]. Centrally administered melanotan II (MTII), a synthetic melanocortin 3/4-receptor agonist, leads to increased NETO in iWAT and dorsal subcutaneous WAT, but not in eWAT or rWAT [45]. The underlying mechanism mediating the differential sympathetic outflow remains largely unknown, which probably involves the centrally controlled neural circuitry via selective activation of sympathetic neurons.

Sensory nerves

Sensory innervation, adipose cellularity, and sympathetic-sensory loop

The sympathetic innervation of WAT has been widely observed in mammals [42,46]; detected at a much less density, the sensory innervation is mainly studied in laboratory rats and Siberian hamsters [47,48]. Neuroanatomical tracer of 'true blue' labels fluorescent cell bodies in dorsal root ganglia (DRG) after the tracer is injected into iWAT or dorsal subcutaneous WAT of laboratory rats [49]. Immunohistochemical assessment shows the sensory innervations labeled by calcitonin gene-related peptide (CGRP) and substance P (SP) immunoreactivity in the eWAT and iWAT of Siberian hamsters [47,50,51].

The sensory nervous system could potentially transmit the stimuli derived from the adipose tissues and, further, regulate adipose tissue via a sensory-sympathetic nervous system (SNS) loop [52,53]. The sensory denervation achieved via local microinjections of capsaicin shows unchanged fat pad masses but significantly increased average fat cell size in iWAT but not in eWAT [47], indicating a possible function of sensory nerves on adipocyte cell size in a fat pad-specific manner. However, sensory denervation of eWAT with capsaicin leads to increased rWAT and iWAT masses phenocopying the WAT mass increase after lipectomy in Siberian hamster, suggesting a possibility that the sensory nervous system might convey the information of adipose tissue states to the central nervous system, which subsequently engages the SNS and regulates WAT distantly [50]. Further, the expression of leptin receptor is detected in DRG in Siberian hamsters, and intra-iWAT injection of leptin significantly induces c-Fos immunoreactivity in DRG neurons colabeled with fluorogold iWAT injection, hinting a possible paracrine axis to the sensory neurons from adipocytes [54]. In particular, anterograde transneuroal viral tract tracing through injection of the H129 strain of the herpes simplex virus-1 (HSV-1) into iWAT and eWAT in Siberian hamsters shows substantial overlap in the pattern of WAT sensory afferent projections with multiple SNS outflow sites along the neuraxis including the intermediolateral horn, leading to the proposition that the WAT sensory-SNS circuits might exist to regulate WAT sympathetic drive and lipolysis [55]. The neural tracing by injection of Dil into perirenal adipose tissues in rats shows that the labeled sensory neurons in DRG are categorized into three groups, small transient receptor potential cation channel subfamily V member 1 (TRPV1)-negative, small TRPV1-positive, and large TRPV1-negative cells [56], and the injection of resiniferatoxin (RTX), a capsaicin analog, into perirenal adipose tissue leads to reduced labeling by 36.7% which represents the TRPV1-positive cells and is susceptible to RTX denervation [56]. Nontheless, caution should be taken for that capsaicin treatment mainly target the TRPV1-positive neurons in chemical denervation, and the genetic studies on refined sensory neurons would be necessary to clarify the roles of sensory neurons.

Parasympathetic nerves

Presence or no-presence of parasympathetic nerves

The innervations in the WAT by the parasympathetic nervous system (PSNS) have been explored, but controversies exist as to whether the parasympathetic nerves are present in the WAT to any significant extent [57–59]. When intra-abdominal fat pads in rats were sympathetically denervated and then injected with the retrograde transneuronal tracer pseudorabies virus (PRV), PRV labeling was observed in the vagal motor nuclei of the brain stem, indicating that adipose tissue receives vagal input which promotes glucose and fat uptake [60]. However, the separate study of histological examination of parasympathetic nerve markers in

rats, and wild-type and *ob*/*ob* mouse WAT did not show detectable signals [61]. Vesicular acetylcholine transporter (VAChT), vasoactive intestinal peptide (VIP), and neuronal nitric oxide synthase (nNOS) immunoreactivities were absent in multiple WAT examined (retroperitoneal, epididymal, and inguinal) [61]. In addition, when Siberian hamster iWAT were sympathetically denervated, subsequent PRV injection resulted in no central nervous system or sympathetic chain labeling [61]. Nonetheless, PRV tracing did show the occasional labeling in the vagal motor nucleus despite at a drastically less intensity, leaving a possibility of sparse parasympathetic innervation in WAT [59,61,62]. An investigation of the various fat depots in different species with new technical approaches such as whole-mount immunostaining might offer novel insights into the anatomical distribution of the parasympathetic nerves.

Adipocytes

The sympathetic regulation of lipolysis and beiging in the mix of adipocyte-immune cell communications

White adipocytes are lipid-rich cells storing triglycerides for energy sources and producing leptin and adiponectin among others in endocrine function [2,3,63–65]. The adipocytes form a major volume despite composing < 20% of the total cellularity [66]. WAT are highly vascularized [67,68], and adipocytes can quickly respond to hormones such as insulin and glucagon, which regulate the glucose uptake and lipid turnover during feeding or fasting in the adaptation of the metabolic demand [69]. Sympathetic nerves visualized in the whole fat pad display a high density in the iWAT in mice [28,29]. Adipocytes highly express adrenergic receptor β3 (Adrβ3) which renders them responsive to the sympathetic neurotransmitter NE. Engagement of Adrβ3 signal triggers the breakdown of the triglyceride to glycerol and free fatty acids, the process of lipolysis.

The control of fat deposition and mobilization by the SNS has been revealed by approaches of surgical or chemical denervation, genetic perturbations, and optogenetic stimulation [48,70]. The unilaterally denervated lumbar fat shows more tissue mass than the contralateral intact depot after a 48-h fast in rats [71], and similarly, the rWAT loses more weight than the denervated pad after 14 days of treatment with estradiol benzoate in ovariectomized rats [72]. Neuronal activities affect both lipid deposition and mobilization, as unilateral splanchnicectomy leads to reduced lipid mobilization together with decreased lipid deposit to a lesser degree in the perirenal adipose tissues on the ipsilateral side in rabbits, cats, and rats [73]. In addition, an inhibitory role of the SNS in the control of WAT cellularity has been proposed, based on the observation that unilateral surgical denervation or chemical sympathetic denervation of iWAT increases fat cell number in comparison to the contralateral intact side in Siberian hamsters [51,74]. Moreover, the lipolytic effect of leptin is mediated through the action of sympathetic nerves that innervate the WAT: Genetic blockage of adipose sympathetic inputs blocks the lipolytic pathway; local optogenetic stimulation of sympathetic inputs is sufficient to induce the local lipolytic reaction [70].

The sympathetic activity also promotes beiging through the adrenergic signal in response to cold exposure, exemplified by the appearance of multilocular beige adipocytes highly expressing UCP1 and mitochondria biogenesis gene peroxisome proliferator-activated receptor-γ coactivator 1 α (PGC1α) leading to increased thermogenic capacity [28,29,75].

The signaling molecules such as chemokines and cytokines or the receptors are detected in adipocytes which change in expression during differentiation or inflammation, thereby influencing the immune cell infiltration or functions [76,77]. Adipose tissues produce interleukin-6 (IL-6), and the expression could be induced upon stimulation with bacterial lipopolysaccharide (LPS) in primary adipocytes [78,79]. Acute stress-inducible IL-6 is found to be produced from brown adipocytes in an Adrβ3-dependent manner, which mediates hyperglycemia through hepatic gluconeogenesis [80]. The source of IL-6 shows different effects in inflammatory response, as IL-6 secreted by myeloid cells inhibits adipose tissue macrophage accumulation, but IL-6 secreted by adipocytes promotes macrophage accumulation [81]. Macrophage migration inhibitory factor (MIF) is expressed in rat eWAT [82], and produced and released by human adipocytes, with expression levels positively associated with donor body mass index (BMI) [83]. In addition, the receptors of chemokine such as C-X-C motif chemokine receptor 1 and 2 (CXCR1 and CXCR2), and C-C motif chemokine receptors 2, 4, and 5 (CCR2, CCR4, and CCR5) are induced following *in vitro* differentiation of preadipocyte to mature adipocyte [84]. Besides, direct interaction could occur between adipocytes and immune cells, as class II major histocompatibility complex (MHCII) expression increases in adipocytes upon HFD feeding which mediates T-cell activation [85]. More recently, single-nuclei adipocyte RNA sequencing of iWAT reveals that the lymphocytes and a metabolically active

mature adipocyte subtype could interact intercellularly via interleukin-10 (IL-10) and IL-10 receptor α (IL-10Rα) axis, and IL-10 ablation or adipocyte-specific deletion of IL-10Rα increases adipose adrenergic-responsive pathways [86,87]. White adipocytes also express the NE recycling transporter, organic cation transporter 3 (OCT3), which reduces the NE availability and governs the β-adrenergic activity [88]. The wide spectrum of signal molecules and receptors still awaits to be fully characterized, which together endow the adipocytes both as a source and responder to the immune and neuronal components (Fig. 4).

Macrophages/monocytes

A diverse population with complex spatiotemporal functions

Among the immune cells present in the WAT [89–91], macrophages/monocytes are the most intensively scrutinized immune types which display a high degree of heterogeneity [92–96]. Monocytes circulate in the blood during adulthood identified as $CD11b^{+}Ly6c^{+}$ subset, infiltrate into tissues, and differentiate into macrophages for homeostatic maintenance or upon tissue inflammation. Within the WAT, macrophages can be derived from circulating monocytes or proliferate locally to sustain homeostasis. Though largely categorized as proinflammatory or anti-inflammatory subtypes in early studies [97], the emerging studies have revealed that the adipose macrophages are highly heterogeneous and dynamically respond to metabolic and immune states.

Immune cells compose more than half of the total cellularity of stromal/vascular fraction (SVF) of the WAT [98]. Macrophages are among the predominant immune subtypes [99] and accumulate in obese mice and humans [100–102]. The accrual is attributed to both cellular infiltration, retention, and local proliferation [103–112]. Macrophages are primarily located in interstitial space between adipocytes in the adipose tissues in lean individuals but are aggregated preferentially to dead adipocytes, where they display crown-like structures (CLS) in obese humans and mice [113,114].

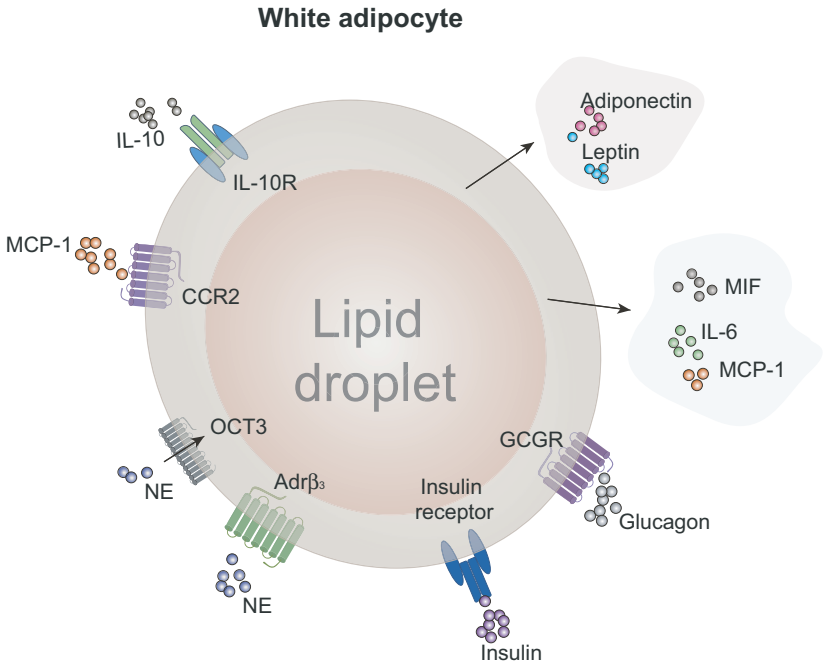

Fig. 4. The white adipocyte with the associated secretory molecules and receptors in metabolic and immune regulation. The white adipocytes express GCGR and insulin receptors to respond to glucagon and insulin, respectively. Adrβ3 mediates the response to the sympathetic neurotransmitter NE, and OCT3 functions as a recyclizing transporter to reduce the availability of NE. White adipocytes secrete leptin and adiponectin to signal to other organs for maintaining energy balance and also produce cytokines and chemokines including IL-6, MIF, and MCP-1 for regulating metabolic homeostasis and tissue inflammation. Adipocytes also express receptors for cytokines ad chemokines such as IL-10R and CCR2 to respond to the immune microenvironment. Adrβ3, adrenergic receptor β3; CCR2, C-C motif chemokine receptor 2; GCGR, glucagon receptor; IL-10R, interleukin-10 receptor; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein 1; MIF, migration inhibitory factor; NE, norepinephrine; OCT3, organic cation transporter 3.

Previous studies have broadly described macrophage phenotype as anti-inflammatory and proinflammatory in lean and obese mice, respectively [97]. Lean mice harbor macrophages expressing Ym1, arginase 1 (Arg1), and IL-10. Cold-induced thermogenic program recruitment is observed together with the accretion of anti-inflammatory macrophages in young mice [115]. HFD-induced obesity induces a phenotypic switch in adipose macrophage activation state which is prone to be inflammatory, expressing tumor necrosis factor-α (TNF-α), IL-6, inducible nitric oxide synthase (iNOS), and CCR2 [116,117]. A large portion of proinflammatory macrophages is derived from circulating monocytes and expresses CD11c on the surface. WAT macrophages comprise $CD11c^{+}CD206^{+}$ cells in CLS and $CD11c^{-}CD206^{+}$ cells at adipocyte junctions [118,119]. The ones outside of CLS are prone to be adipogenic labeled by Ly6C, while $CD9^{+}$ adipose tissue macrophages reside within CLS are lipid-laden [120]. Bulk ablation of either the $CD11c^{+}$ macrophages or $CD206^{+}$ macrophages leads to improved insulin sensitivity in obese animals [118,119].

Extensive characterization has divided the macrophages/monocytes further into distinct subtypes with differential expression profiles. Flow cytometric analysis of the mouse eWAT macrophages distinguishes four subpopulations, including two groups of vasculature-associated adipose tissue macrophages (VAMs) referred to as VAM1 and VAM2, PreVAM, and $CD64^{+}CD11c^{+}$ double-positive macrophages [121]. Among them, VAMs are tightly associated with blood vessels which are the dominant myeloid populations in a steady state [121]. They poorly express Arg1 and chitinase-like 3 (Chil3) but express high levels of CD206, CD301a, CD163, CD209, and resistin-like α (Retnla; also called found in inflammatory zone 1, or Fizz1), displaying a high endocytic capacity [121]. Single-cell RNA sequencing (scRNA-seq) of eWAT in mice distinguishes two subsets of monocytes and three subsets of macrophages (Mac1, Mac2, and Mac3) [122]. Mac1 highly expresses Retnla, CD163, Lyve1, and CD209F, a signature showing overlapping features with the abovementioned VAMs and the perivascular $Lyve1^{hi}MHCII^{lo}CX3CR1^{lo}$ macrophages characterized separately [121,123]. Mac2 and Mac3 expand drastically in obesity, resembling the $CD9^{+}$ cells which accumulate in CLS in obesity [113,120,122], while Mac3 represents a novel subpopulation termed lipid-associated macrophage (LAM) emerging in obesity, which expresses triggering receptor expressed on myeloid cells 2 (Trem2) and prevents adipocyte hypertrophy and loss of systemic lipid homeostasis under obese conditions [122]. A separate scRNA-seq study instead defines seven macrophage subsets among fifteen leukocyte subpopulations in mouse eWAT [124]. Calorie restriction following HFD feeding induces the accumulation of a macrophage subpopulation enriched in genes associated with phagocytosis and endocytosis (termed phagocytic macrophages) [124]. Notably, the majority of the characterization is based on the studies of male eWAT but not the periovarian WAT of female animals. Yet, emerging evidence suggests that a pronounced sexual dimorphism exists in the perigonadal WAT [125]. Future investigation and analysis would help resolve the distinctions between macrophage subtypes across different datasets and genders, which would provide a coherent view of the macrophage dynamics, heterogeneity, and interrelationships.

Many of the factors derived from macrophages influence insulin sensitivity in adipocytes in obesity. The first attestation that adipose inflammation participates in the development of obesity and diabetes is the finding that TNF-α is induced in adipose tissues and interferes with the insulin receptor which links insulin resistance in rodent models of obesity and diabetes such as *db*/*db* and *ob*/*ob* mice [126]. In WAT of obese mice and humans, macrophages are the predominant source of TNF-α and contribute significantly to IL-6 and nitric oxide [100,101,127,128]. Neutralization of TNF-α [126] or obese mice lacking TNF-α demonstrate improved insulin sensitivity [129].

The infiltration of monocytes into the adipose tissues in the HFD-induced obese mice is attributed partly to the increased expression of chemokines. HFD feeding elevates adipose expression of monocyte chemoattractant protein 1 (MCP-1, or C-C motif chemokine ligand 2, CCL2). MCP-1 is produced from SVF and in adipocytes to a lesser extent [130–132]. Overexpression of MCP-1 driven by adipocyte P2 (aP2) promoter in adipose tissues causes macrophage recruitment and insulin resistance [103,105]. On the other hand, insulin resistance, hepatic steatosis, and macrophage accumulation in adipose tissue induced by HFD feeding are reduced in MCP-1 knockout mice compared with wild-type animals [103]. Acute expression of a dominant-negative mutant of MCP-1 ameliorates insulin resistance in *db*/*db* and wild-type mice fed with HFD [103]. As the main receptor for MCP-1, CCR2-deficient mice show susceptibility to HFD-induced obesity in some though not all cases [104,133], but increased MCP-1 level in plasma is detected in CCR2-deficient animals, and normal migration of macrophages occurs in eWAT, suggesting a compensatory response to additional chemokines and signaling redundancy [133]. eWAT show a trend to lower $CD11c^{+}MGL1^{-}$ proinflammatory macrophages and

higher $CD11c^{-}MGL1^{+}$ anti-inflammatory macrophages as a percentage of $CD45^{+}F4/80^{+}CD11b^{+}$ macrophages in CX3CR1 and CCR2 double-deficient mice versus wild-type mice, and single knockout of CCR2 or CX3CR1 does not differ in their adipose macrophage phenotypes [134]. Given the newly defined macrophage subtypes, a more refined characterization would be needed to determine the specific effect of each chemokine and the respective receptor on the accumulation of distinct subpopulations.

The activation states of macrophages are affected by adipocytes revealed by various mechanisms. Adiponectin induces the production of anti-inflammatory mediators IL-10 and interleukin-1 receptor antagonist (IL-1RA) in primary human monocytes and monocyte-derived macrophages [135]. Deficiency of fatty acid synthase (FASN) in adipocytes enhances the appearance of thermogenic beige adipocytes in mouse inguinal WAT, and single-cell transcriptomic analysis of stromal cells reveals increased macrophages displaying gene expression signatures of the alternately activated type, and their depletion abrogates iWAT beiging, suggesting an important role of adipocyte-macrophage axis in regulating adipocyte biology [136]. Moreover, macrophages could acquire mitochondria from adipocytes via a heparan sulfate-dependent process [137]. Deletion of the heparan sulfate biosynthetic gene exostosin glycosyltransferase 1 (Ext1) in myeloid cells decreases mitochondria uptake by WAT macrophages, increases WAT mass, lowers energy expenditure, and exacerbates HFD-induced obesity [137]. The emerging findings indicate that the crosstalk between adipocytes and macrophages could be versatile, involving components not restricted to secreted molecules.

The distinct subsets of macrophages have been characterized in close proximity to the sympathetic nerves which influence sympathetic input (Fig. 5). The $CX3CR1^{+}$ population of macrophages is identified as sympathetic neuron-associated macrophages (SAMs) [138]. SAMs express solute carrier family 6 member 2 (SLC6A2), an NE transporter, and monoamine oxidase A (MAOA), a degradation enzyme, and affect catecholamine levels in WAT by phagocytosing and degrading NE [138]. A population of nerve-associated macrophages (NAMs) that are in close association with sympathetic fibers is independently visualized in visceral WAT, and macrophages play important role in impaired lipolysis in aging by lowering the bioavailability of NE [139]. Consistent with the function in regulating sympathetic drive, mice with deficiency of insulin receptor substrate 2 (IRS2) in $lyzM^{+}$ myeloid cells display increased sympathetic nerve density and catecholamine availability in adipose tissue, and the IRS2-deficient macrophages show alterations in genes involved in scavenging catecholamines and supporting increased sympathetic innervation [140]. A $CX3CR1^{+}$ macrophage subpopulation is also uncovered in iBAT which expresses PlexinA4 and negatively regulates sympathetic innervation via repulsive cue to Sema6A-expressing sympathetic axons [141]. Likely different from the SAMs, cold-induced neuroimmune cells (CINCs), a subset of $Ly6C^{+}$ $CCR2^{+}$ $Cx3CR1^{+}$ monocytes/macrophages interacting with peripheral nerves in the iWAT, are found homing to iWAT upon cold exposure and expressing BDNF [40]. Genetic deletion of BDNF driven by lyzM-Cre in myeloid cells leads to reduced sympathetic nerves in iWAT, supporting the function of CINCs in regulating adipose innervation [40]. scRNA-seq analysis of lung interstitial macrophages identifies two subpopulations exhibiting distinct gene expression profiles and phenotypes, $Lyve1^{lo}MHCII^{hi}CX3CR1^{hi}$ and $Lyve1^{hi}MHCII^{lo}CX3CR1^{lo}$, which are also present in the WAT [123]. The $Lyve1^{lo}MHCII^{hi}CX3CR1^{hi}$ subset resides in close distance with nerves, whereas the $Lyve1^{hi}MHCII^{lo}CX3CR1^{lo}$ subset is located preferentially alongside blood vessels [123]. Though the WAT counterpart subsets remain to be determined, an interstitial subpopulation of $CD169^{+}$ lung-resident macrophages is identified surrounding the airways and is in proximity to the sympathetic nerves in the bronchovascular bundle [142]. These nerve- and airway-associated macrophages (also called NAMs) are tissue-resident which do not require $CCR2^{+}$ monocytes for development or maintenance [142]. Those NAMs highly express immunoregulatory genes and play important roles in dampening excessive production of inflammatory cytokines and innate immune cell infiltration in inflammatory conditions [142]. Overall, functional studies indicate that macrophages could play both stimulatory and inhibitory functions on sympathetic innervations.

The direct role of sympathetic regulation of macrophages has been postulated but remains to be fully illustrated. Macrophages express the adrenergic receptor β2 (Adrβ2); however, deletion of Adrβ2 driven by lyzM-Cre does not alter inflammation in the adipose tissues or change insulin sensitivity fed on chow or HFD; no significant changes on adipose tissue inflammation and function are observed during feeding, fasting, or cold exposure [143].

In human subcutaneous adipose tissues, a positive relationship is found between macrophage transcripts CD68, TNF-α, and plasma IL-6, and an inverse correlation between CD68 and insulin sensitivity [144]. Diabetic patients have significantly increased levels of MCP-1 and RANTES [145], and $CD11c^{+}CD206^{+}$ adipose tissue macrophages are associated with insulin

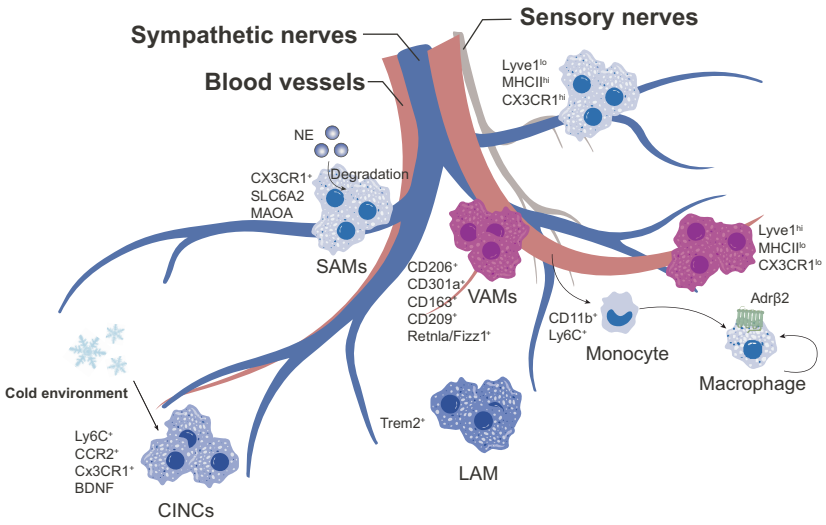

Fig. 5. The emerging macrophage subsets in the white adipose tissues, including the ones associated with sympathetic nerves or vasculatures. The CX3CR1^{+} population expressing SLC6A2 and MAOA is identified as sympathetic neuron-associated macrophages (SAMs). Cold-induced neuroimmune cells (CINCs), a subset of Ly6C^{+} CCR2^{+} Cx3CR1^{+} monocytes/macrophages interacting with peripheral nerves, home to iWAT upon cold exposure and expressing BDNF. Two subpopulations identified in lung are also present in the WAT: The Lyve1^{lo}MHCII^{hi}CX3CR1^{hi} subset resides in close distance with nerves, whereas the Lyve1^{hi}MHCII^{lo}CX3CR1^{lo} subset is located preferentially alongside blood vessels. The groups of vasculature-associated adipose tissue macrophages (VAMs) are tightly associated with blood vessels, and they express high levels of CD206, CD301a, CD163, CD209, and Retnla/Fizz1. Lipid-associated macrophage (LAM) is identified in obesity expressing Trem2. BDNF, brain-derived neurotrophic factor; iWAT, inguinal white adipose tissues; MAOA, monoamine oxidase A; NE, norepinephrine; Retnla/Fizz1, resistin-like α (also called found in inflammatory zone 1, or Fizz1); SLC6A2, solute carrier family 6 member 2; Trem2, triggering receptor expressed on myeloid cells 2.

resistance in human obesity [146]. In obese women, CD11c^{+} adipose macrophages show much higher expression of integrins, antigen presentation molecules, cytokines including interleukin-1β (IL-1β), IL-6, interleukin-8 (IL-8), and IL-10, TNF-α, and C-C motif chemokine ligand 3 (CCL3) than CD11c^{+} macrophages, indicative of a proinflammatory state [146]. Tissue culture medium conditioned by CD11c^{+} adipose macrophages impairs insulin-stimulated glucose uptake by human adipocytes [146]. Dietary or surgery intervention of obesity leads to weight loss and reduced inflammation [130,147]. Weight loss of obese subjects after a very low-calorie diet is accompanied by increased expression of IL-10 and IL-1RA in adipose tissues, predominantly from macrophages [130]. Analysis on subcutaneous WAT of lean and morbidly obese subjects before and three months after bypass surgery shows that the weight loss results in a significant decrease in macrophage number, and genes involved in macrophage attraction such as MCP-1 increase in obesity and decrease after surgery in the SVF [147].

Overall, macrophages could adopt an immune phenotype across a wide spectrum in response to the external stimuli and metabolic states, which renders remarkable functional plasticity in influencing adipose biology.

Eosinophils

Tissue homeostasis and metabolic health

Recent evidence suggests that the functions of eosinophils go beyond the immune reaction in antihelminth infection and allergic response, as demonstrated by their roles in metabolic homeostasis [148,149]. Eosinophils migrate into adipose tissue by an integrin-dependent process [150]. Eosinophils are the major interleukin-4 (IL-4)-expressing cells in WAT which sustains the adipose macrophage [151]. Mice fed with HFD develop obesity, impaired glucose tolerance, and insulin resistance in the absence of eosinophils, while helminth-induced adipose tissue eosinophilia enhances glucose tolerance [151]. Genetic loss of eosinophils or blockage of IL-4 and interleukin-13 (IL-13) signaling impairs cold-induced biogenesis of beige fat [152]. Moreover, a decreased frequency in WAT-resident eosinophils is detected in aging subjects of human participants [153]. Exposure to a young systemic environment could partially restore adipose eosinophil distribution in aged parabionts and reduce adipose tissue inflammation [153]. Eosinophil transfer from youthful donors results in systemic rejuvenation of the aged host, leading to improved physical and immune

fitness partially mediated by eosinophil-derived IL-4 [153].

The eosinophil abundance changes in obesity. When treated with recombinant interleukin-5 (IL-5), eosinophils are increased about threefold in adipose tissues of HFD-fed mice, which is comparable to lean mice [154]. However, no significant improvement in metabolic assays is observed, such as weight gain, body composition, and glucose tolerance [154], indicating unknown mechanism exists in addition to the contribution by the increased quantity of eosinophils in mediating the beneficial role in metabolic health. Alteration of eosinophil abundance is also observed in human participants. A high eosinophil percentage is found to be associated with a reduced risk of T2D [155]. However, the precise functions of eosinophils in different metabolic contexts remain to be resolved. For instance, characterization of eosinophils in human patients of metabolic syndromes without complications from diabetes, atherosclerotic cardiovascular disease, smoking, or inflammatory condition shows that both circulating and eosinophils are increased twofold [156]. An in-depth investigation would help understand whether eosinophils may display heterogeneity and play differential roles at steady state and under pathological conditions.

Neutrophils

Early recruitment and proinflammatory function

Neutrophils are rapidly recruited to WAT within 3 days upon HFD feeding, and this increase remains constant for up to 90 days of HFD [77,157,158]. The short-term HFD feeding causes a significant upregulation of cytosolic phospholipase A2 (cPLA2α) in eWAT which promotes adipose neutrophil infiltration [159]. The accumulation of neutrophils contributes to tissue inflammation and impaired insulin sensitivity via the increased production of neutrophil elastase, reduced neutrophil elastase inhibitor α1-antitrypsin, and increased inflammatory cytokines such as interleukin-1β (IL-1β) in obesity [158,160,161]. Furthermore, severe obesity in human subjects is associated with increased generation of plasmatic neutrophil extracellular traps, suggesting a conserved role of neutrophils in the systemic inflammatory state [162]. Interestingly, social stress which is known to activate the SNS enhances neutrophil accumulation in eWAT and accelerates insulin resistance development upon HFD feeding, and inhibition of neutrophil elastase abrogates the insulin sensitivity impairment of stressed mice [163]. The evidence points to a potential interaction between the sympathetic activity and neutrophil infiltration and activation, which would collectively participate in metabolic deterioration.

ILC2s

Homeostatic maintenance through regulating eosinophil, macrophage, T_{reg}, and adipocytes

ILC2s are resident in adipose tissues and play protective roles against obesity [164]. Interleukin-33 (IL-33) is a cytokine belonging to the IL-1 family associated with type 2 immune response and is important for the maintenance of ILC2s in WAT, limiting adiposity by increasing caloric expenditure in mice [165–168]. The cell source of IL-33 has been refined to the WAT stromal cells. scRNA-seq of visceral WAT stromal cells defines five distinct subtypes, with three subtypes producing IL-33 and two subtypes resembling adipocyte precursors [169]. Independently, the adipose stem and progenitor cells are identified as a source of IL-33 in all WAT depots and mesothelial cells as an additional source in visceral WAT [170]. ILC2s are the major producer of IL-5 and IL-13 in the visceral fat and promote the expansion of eosinophils and macrophages [165]. IL-5 deficiency impairs visceral adipose tissue eosinophil accumulation and results in increased adiposity and insulin resistance when placed on HFD [165]. Further, administration of exogenous IL-33 into HFD-fed mice restores ILC2s as well as the level of tyrosine hydroxylase (TH), a rate-limiting enzyme for catecholamine biosynthesis in sympathetic nerves; conversely, chemical sympathetic denervation reduces the frequency of ILC2s and eosinophils in iWAT, collectively indicating a sympathetic-regulated immune environment [171]. ILC2s also influence energy expenditure in a manner independent of eosinophils, macrophages, or adaptive immune cells, as they produce methionine-enkephalin (Met-Enk) peptides that can act directly on adipocytes to upregulate UCP1 expression and promote beiging process [167].

T cells

Differential functions of $CD4^+$ T subtypes, proinflammatory role of $CD8^+$ T cells, context-dependent effect of iNKT, and homeostatic function of γδ T cells

White adipose tissues is home to both T helper cells and cytotoxic T lymphocytes ($CD4^+$ and $CD8^+$ cells, respectively), and the cellularity and ratio vary among different WAT depots from lean and obese mice [98,172,173]. Mesenteric WAT (mWAT) contain a higher percentage of T cells in the SVF compared with

eWAT and iWAT in lean mice. The CD4 : CD8 ratio is between 1 and 2 detected in iWAT and mWAT, and around 5 in eWAT [98]. T-cell numbers in eWAT and iWAT correlate positively with body weight [98]. In both eWAT and iWAT, the CD4 : CD8 ratio shows a negative correlation with body weight, indicating a relative increase in cytotoxic T cells compared with T helper cells [98]. Analysis of healthy overweight or obese human subjects shows that $CD4^+$ and $CD8^+$ T cells infiltrate both visceral and subcutaneous fat depots, with proinflammatory $CD4^+$ T helper 1 and 17 (Th1 and Th17) cells, and $CD8^+$ T cells [174]. T-cell receptor (TCR) repertoire characterized in visceral WAT shows a restricted pattern which is further compromised in obesity [175].

Among the $CD4^+$ T-cell subsets, the $Foxp3^+$ regulatory T cells (T_{reg}) are highly enriched in the visceral fat of lean mice, but their numbers are reduced in insulin-resistant models of obesity [176]. The maintenance and expansion of T_{reg} cells in visceral WAT depends on IL-33 both at steady state and upon helminth infection [177–179]. IL-33 could activate the signaling event in ILC2s and T_{reg} as they express the receptor complex containing suppression of tumorigenicity 2 protein (ST2). ILC2-intrinsic activation by IL-33 is also crucial for T_{reg} cell accumulation, which occurs in part via ICOSL-ICOS interactions [178]. Besides, IL-33 induces upregulation of OX40L in WAT ILC2s, which promotes T_{reg} expansion as well [180]. Adipose T_{reg} cells show a distinct clonal TCR repertoire, possibly regulated by adipose tissue antigens [177,181]. Depletion and expansion experiments reveal that the T_{reg} cells influence the inflammatory state of adipose tissue and, thus, insulin resistance [176]. Yet, T_{reg} cells show distinct functions in age versus obesity-associated insulin resistance: Mice deficient in adipose T_{reg} cells are protected against age-associated insulin resistance but remain susceptible to obesity-associated insulin resistance and metabolic disease [182]. Moreover, WAT represents a natural memory T-cell reservoir at the steady state [183]. After infection, large numbers of pathogen-specific memory T cells accumulate in WAT that could provide potent and rapid effector memory responses [183].

Upon HFD feeding, a large number of $CD8^+$ effector T cells infiltrate the eWAT, and depletion of $CD8^+$ T cells lowers macrophage infiltration and adipose tissue inflammation and ameliorates systemic insulin resistance [184]. Further, adipose T cells in obese mice and diabetic humans exhibit enrichment of genes characteristic of T-cell exhaustion and decreased capacity for cytokine secretion and cell proliferation, the contribution of which to the tissue inflammation remains unknown [185].

Unconventional T-cell subsets are also enriched in both human and mouse adipose tissues, including invariant natural killer T cells (iNKT) and γδ T cells, which recognize nonpeptide ligands of various types [186–188]. Parabiosis experiments have revealed that iNKT cells and γδ T cells are resident in mouse adipose tissues [186,187]. Studies have indicated the complex roles of iNKT to be fully resolved. Upon short-term HFD feeding, iNKT promotes macrophage polarization to an anti-inflammatory state [189]. iNKT cells produce IL-10 and interleukin-2 (IL-2), which induces an anti-inflammatory phenotype in macrophages and controls the number, proliferation, and suppressor function of adipose T_{reg} cells, respectively [187,190]. Further characterization identifies two distinct populations of adipose tissue iNKT cells as $NK1.1^-$iNKT and $NK1.1^+$iNKT [191]. $NK1.1^-$iNKT cells respond to free fatty acids and produce IL-10, which protects mice from metabolic diseases during obesity [191]. Conversely, $NK1.1^+$iNKT cells predominantly produce IFNγ, which licenses NK cell-mediated killing of adipose tissue macrophages, thus serves to promote metabolic health in the nonobese state [191]. CD1d, a molecule involved in lipid antigen presentation to iNKT cells, is expressed in adipocytes which stimulate iNKT cell activity through physical interaction [192]. Adipocyte-specific deletion of CD1d leads to reduced numbers of adipose iNKT cells; however, the consequence of germline or adipocyte-specific deletion of CD1d on adipose inflammation and insulin resistance in obesity or homeostatic state remains debatable [193–196]. Nevertheless, a declination of CD1d expression and iNKT cell population is observed as adipose tissues expand in obesity [197–199].

γδ T cells are long-lived adipose tissue residential immune cells which function to enhance the thermogenic capacity. Two subpopulations are distinguished based on their expression levels of promyelocytic leukemia zinc finger protein (PLZF) [186]. The innate-like $PLZF^+$ γδ T cells represent the major subpopulation in adipose tissues, which produce interleukin-17A (IL-17A) and TNF-α and signal to stromal cells, potentially inducing the production of IL-33 and promoting the expansion of ILC2s and T_{reg} [186]. Mice lacking γδ T cells or IL-17A exhibited the inability to regulate core body temperature at thermoneutrality and after cold challenge [186]. More recently, γδ T cells are shown to play a crucial role in promoting sympathetic innervation in the thermogenic adipose tissues, partly by driving the expression of transforming growth factor β1 (TGFβ1) in parenchymal cells via the IL-17 receptor C (IL-17RC) [200].

B cells

Proinflammatory role via antibodies and antigen presentation, immunomodulatory role by anti-inflammatory cytokine, and production of neurotransmitter

B-cell accumulation is found in visceral WAT in HFD-fed obese mice [157]. Adoptive transfer of MHCII null but not the wild-type B cells to mice lacking B cells results in increased insulin sensitivity, indicating that B-cell antigen presentation to $CD4^+$ T cells contributes to tissue inflammation and insulin resistance, and pathogenic immunoglobulin G (IgG) antibodies produced by B cells are also shown to promote insulin resistance [157]. In addition, B cells secrete proinflammatory cytokines which enhance proinflammatory T-cell function in obesity, and B-cell-null mice have decreased adipose tissue inflammation and show insulin resistance in obese mice [201]. Further, characterization of B cells reveals distinct functions of different B-cell subtypes in adipose tissues. The B2 cells have a proinflammatory effect, and B1α cells or spleen-supplied innate-like B cells express the anti-inflammatory cytokine IL-10 and prevent the development of adipose tissue inflammation in obesity [202–204]. Deletion of IL-10 in B cells enhances adipose inflammation and insulin resistance in HFD-induced obese mice [205].

Besides, the expression of the cholinergic receptor nicotinic α2 subunit (CHRNA2) is induced in subcutaneous fat during beiging [206]. Though the distribution of cholinergic parasympathetic nerves is sparse in the WAT [28], ChAT-eGFP reporter, which express an enhanced green fluorescent protein (eGFP) under the control of the transcriptional regulatory element of the gene encoding choline acetyltransferase (ChAT) the rate-limiting enzyme in acetylcholine synthesis, labels eGFP-positive cells among immune cells including B lymphocytes, T lymphocytes, and macrophages within the WAT [206]. The immune subtypes could therefore function as a source of acetylcholine to signal to the Chrnα2-expressing beige adipocytes [206].

DCs

Context-dependent role in adipose immune microenvironment

The adipose tissues contain the two major subsets of DCs: conventional dendritic cells (cDCs) and plasmacytoid dendritic cells (pDCs). The conventional DCs are known for antigen presentation and initiation of T-cell response [207,208]. The adipose tissue DCs are predominantly $CD11b^+$ cDCs and make up the bulk of $CD11c^+$ cells in adipose tissue upon HFD exposure [209]. $CD11c^+$ populations are elevated in obesity and contribute to tissue inflammation and insulin resistance in HFD-induced obesity [210]. $CD11c^+CD64^-$ distinguish adipose tissue DCs from macrophages which are marked by $CD64^+$, and cDCs express MHCII and costimulatory receptors, which render them to stimulate $CD4^+$ T-cell proliferation [209]. The recruitment of the cDCs is largely dependent on the chemokine receptor C-C motif chemokine receptor 7 (CCR7), and DC accumulation during obesity is attenuated in *Ccr7*$^{-/-}$ mice and is associated with decreased adipose tissue inflammation and insulin resistance [209]. In obese patients, the presence of $CD11c^+CD1c^+$ DCs is correlated with BMI and an elevation in Th17 cells, and these DCs promote *ex vivo* Th17 differentiation [211], the $CD4^+$ T-cell subset with key proinflammatory function [212]. Though the cDCs contribute to the tissue inflammation in obesity, they acquire a tolerogenic phenotype through upregulation of pathways involved in adipocyte differentiation in visceral WAT to accommodate the tissue expansion at the early stage of obesity [213]. Specifically, the activation of the Wnt/β-catenin and peroxisome proliferator-activated receptor-γ (PPARγ) pathway in the two subpopulations of cDCs, cDC1 ($CD11c^{hi}MHCII^+CD11b^-$), and cDC2 ($CD11c^{hi}MHCII^+CD11b^+$) subsets, respectively, sustains a tolerogenic phenotype to suppress the local inflammation and delay the onset of insulin resistance [213]. Though present at relatively lower frequency in comparison with cDC, pDCs increase in the visceral WAT during prolonged HFD and cause T_{reg} decline via IFN-α production, thereby leading to compromised insulin sensitivity [214].

NK cells

Proinflammatory in adipose immune microenvironment

NK cells contribute to the development of obesity-associated insulin resistance. HFD feeding increases NK cell numbers and the production of proinflammatory cytokine TNF-α in eWAT [215]. Depletion of NK cells leads to decreases in adipose tissue macrophage numbers and tissue inflammation, accompanied by improvement in obesity-induced insulin resistance [215]. A distinct NK subpopulation expressing the IL-6 receptor α (IL-6Rα) and colony-stimulating factor 1 receptor (CSF1R) is identified which expands in obesity [216]. Ablation of the NK subpopulation or

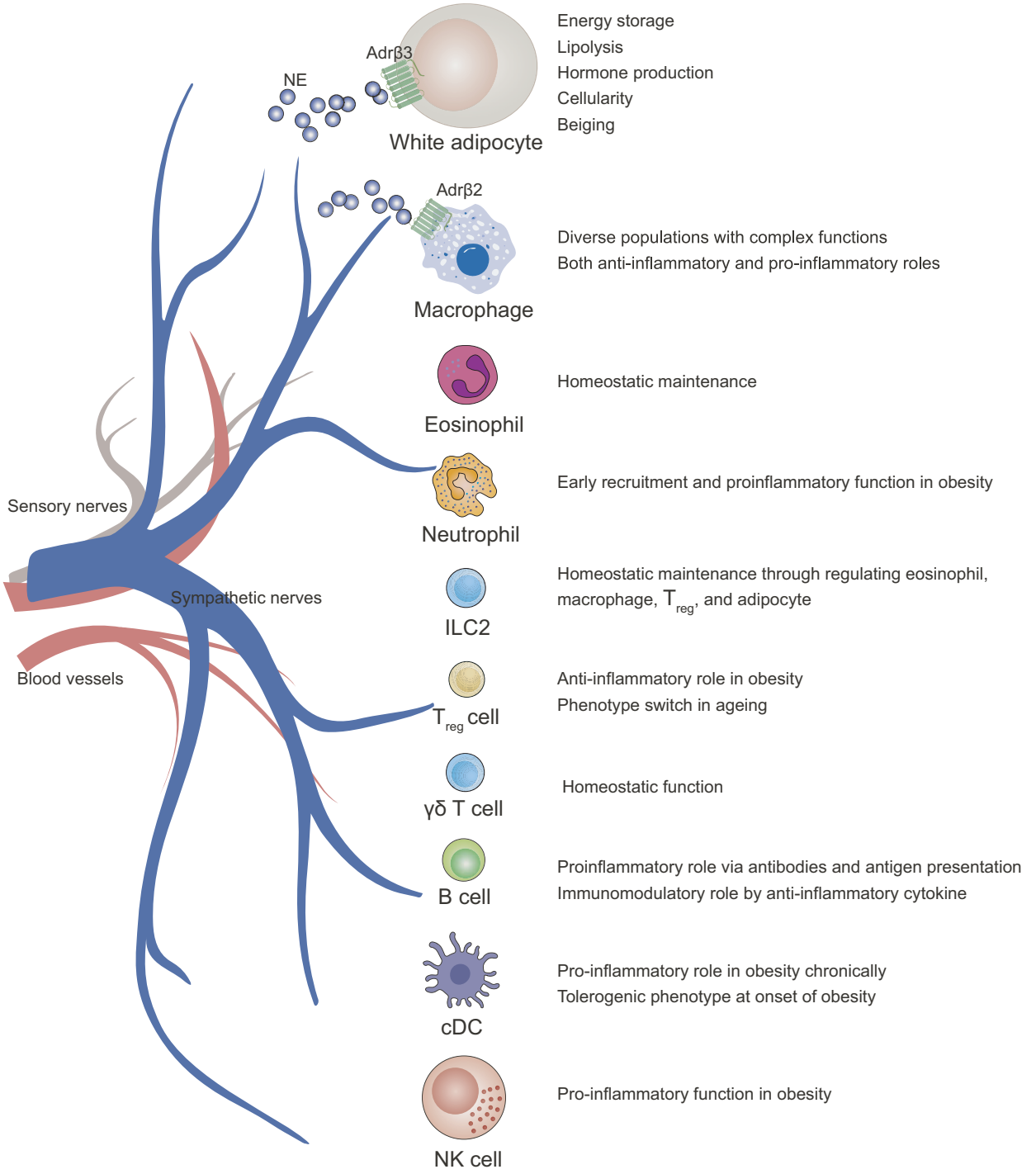

Fig. 6. Summary of the cellular components in the white adipose tissues and their associated functions. The white adipocytes, macrophage, eosinophil, neutrophil, ILC2, T_{reg} cell, γδ T cell, B cell, cDC, and NK cell are illustrated together with the sympathetic nerves, sensory nerves, and blood vessels. cDC, conventional dendritic cell; ILC2, group 2 innate lymphoid cells; NK cell, natural killer cell; T_{reg} cell, regulatory T cell.

conditional inactivation of IL-6Rα or signal transducer and activator of transcription 3 (STAT3) in NK cells prevents obesity and insulin resistance [216].

Conclusions

The WAT represent one key metabolically active reservoir filled with humming intercellular communications among many of the cell types either residential or migratory (Fig. 6). The intricate multidirectional dialogue ensures coordinated responses in the determination of energy storage, consumption, partition, and tissue environment. The highly dynamic nature of the WAT such as in lipolysis and thermogenic capacity alteration endows it as an attractive target in therapeutics, though the predominant function of beige adipocytes in fuel utilization other than thermogenesis and the modifiable potential of the neuroimmune axis in those processes remain to be fully elucidated. With lingering questions just starting to be resolved among the intensively explored cell types, new players are emerging. For instance, a subpopulation of mouse WAT perivascular mesenchymal cells termed fibro-inflammatory progenitors (FIP) is found to activate proinflammatory signaling cascades shortly after the onset of HFD feeding and regulate proinflammatory macrophage accumulation [217]. More recently, the single-cell or single-nuclear sequencing datasets have yielded rich information on the cellular heterogeneity and expression profiles of individual cell types [86,122,124,218–222], and perturbational studies would provide an in-depth understanding of the intercellular signaling axis and network. Further, the role of peripheral innervation on adipose tissue metabolism becomes increasingly recognized as has been reviewed [52,223,224], unraveling a therapeutic opportunity to reshape the energy balance by modulating the neuronal pathways. With the emerging evidence demonstrating the importance of individual cell types, collaborative research efforts on how the neuronal and immune components may interplay will provide invaluable knowledge to understand the systemic metabolism both at the organismal and cellular levels.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

WZ, XQ, XM, and SZ conceived the manuscript and wrote the text, and XQ illustrated the figures.

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