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The immunomodulatory roles of adipocytes

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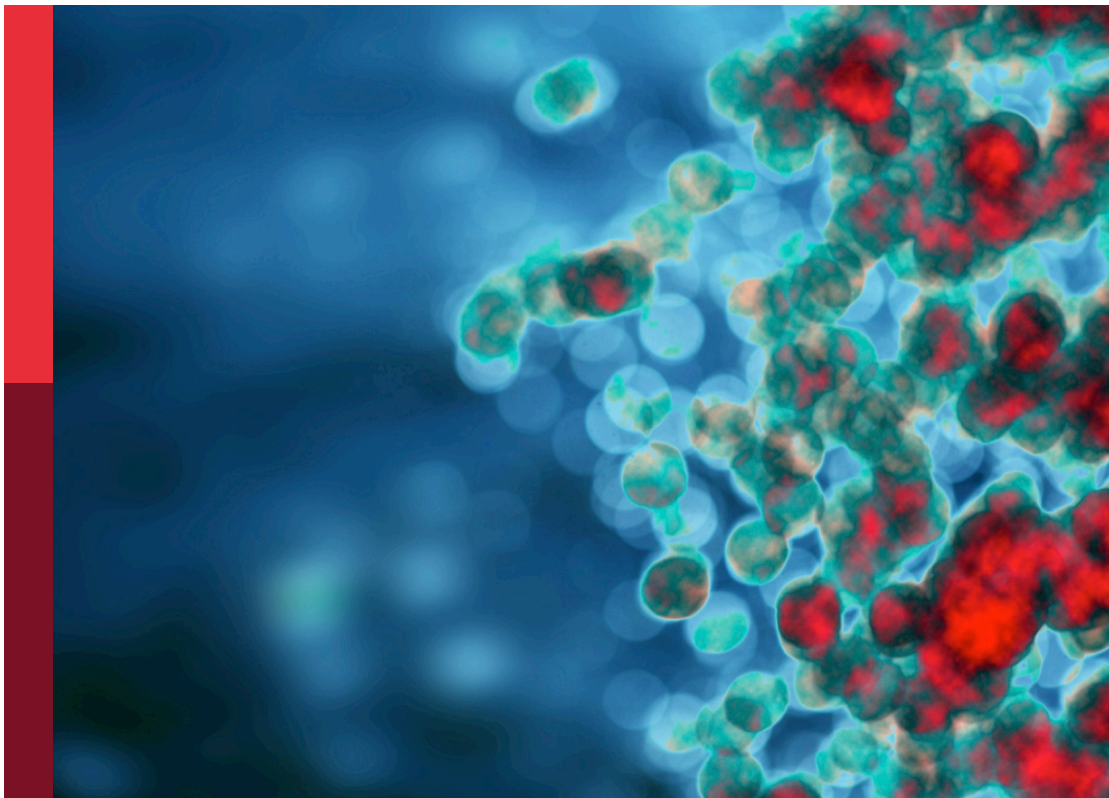

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The immunomodulatory roles of adipocytes

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Editorial: The Immunomodulatory Roles of Adipocytes

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Editorial on the Research Topic

The Immunomodulatory Roles of Adipocytes

Obesity is a global epidemic (1) associated with a state of low-grade, chronic inflammation that enhances the risk of numerous complications, including type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD) and cirrhosis, cardiovascular disease (CVD), cancer, and Alzheimer's Disease, among others (2–7). A major driver of these conditions is the profound inflammatory changes that occur within the adipose tissue (AT) microenvironment, at the heart of which is the adipocyte. Our understanding of the role of the adipocyte in initiating and propagating innate (Blaszczak et al.) and adaptive (Song and Deng) immune responses in lean and obese states has expanded beyond its classical role in energy storage. The adipocyte produces over 600 cytokines and hormones, collectively called adipokines that modulate chronic inflammation, secretes extracellular matrix proteins that impact metabolism (8, 9), and serves as an immunomodulatory and antigen presenting cell to activate or suppress immune responses within AT and systemically (10). Therefore, the current Research Series "*The Immunomodulatory Roles of the Adipocyte*" highlights a wide range of critical factors originating from the adipocyte that mediate immunity and the metabolic syndrome including extracellular vesicle crosstalk (Huang and Xu), lipid metabolites (Park et al.) and ceramides (Chaurasia et al.), adipocyte fatty acid-binding protein (A-FABP) (Lee et al.), TANK-binding kinase 1 (TBK1) (Zhao and Saltiel), the oncostatin M (Sanchez-Infantes and Stephens), clusterin (Wittwer and Bradley), and leptin (Kiernan and MacIver), illustrating the multi-faceted role of the adipocyte (**Figure 1**). Taken together, this series underscores the much underappreciated role of the adipocyte in the instigation and perpetuation of local and systemic inflammation, leading to the multiple inflammatory-induced complications of obesity.

An increasingly recognized means of cell-cell communication is through extracellular vesicles (EVs). Huang and Xu nicely summarize the mechanisms by which AT extracellular vesicles (exosomes, microvesicles, and apoptotic bodies) mediate intercellular communications and inter-organ crosstalk, particularly focusing on adipocyte-derived EVs (ADEVs). Exosomes (30-100nm in diameter) arise from multivesicular bodies and are either degraded by the lysosomal pathway or fuse with the plasma membrane and are released from the cell, while microvescicles (MVs, 100-1000 nm is diameter) are pinched off from the plasma membrane and released. EV cargo consists of microRNAs (miRs), mRNAs, proteins, and lipids and are taken up by cells to influence cell development, metabolism, function, and other activities. ADEV production is markedly increased in human and mouse obesity (11). ADEVs impact local immune cells and have been shown to

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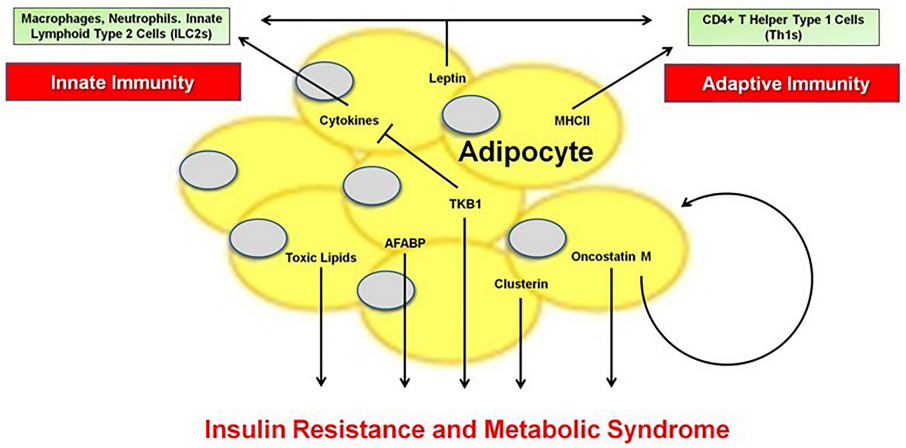

FIGURE 1 | The Innate and Adaptive Immune Functions of the Adipocyte.

activate AT macrophages (11) and promote monocyte to macrophage conversion (12–14). Adipocytes have been suggested to contribute substantially to miRs in circulating EVs, since adipocyte-specific loss of miR production resulted in a 4-fold drop in EV miR cargo (15). However, it remains unclear how many ADEVs enter the bloodstream (11). Figure 2 of the Huang and Xu review illustrates how ADEVs and their cargo act upon distal organs (including liver, skeletal muscle, pancreas, and brain) to influence the immune system as well as systemic metabolism, while Table 1 summarizes specific functions of various cargo. Finally, this review suggests modified EVs can be used as therapy for metabolic and other diseases.

Activation of a pro-inflammatory pathway leads to the secretion of numerous cytokines (16, 17) that enhance adipocyte lipolysis (18–20), leading to toxic fatty acid species (Chaurasia et al.) and impaired insulin sensitivity (21, 22). Chaurasia et al. review the wide-ranging effects of adipocyte-derived lipotoxicity on inflammation and peripheral tissue dysfunction. Specifically, ceramides, which are sphingolipids located in the cell membrane and within the cell cytosol, associate not only with pro-inflammatory cytokines and circulating free fatty acids, but many obesity-related conditions including insulin resistance, T2D, NAFLD, chronic kidney disease, and adverse CV events including mortality. Inhibition of ceramide synthesis specifically within the adipocyte improves insulin resistance and several of these metabolic derangements in mice, underscoring the role of the adipocyte in providing toxic lipids to incite inflammation (23). Inhibiting ceramide synthesis may be a useful therapeutic strategy for metabolic syndrome. Park et al. further discuss an integrated view of how adipocytes communicate with adipose immune cells using lipid metabolites. Invariant natural killer T (iNKT) cells and γ/δ T cells, rapidly respond to changes in lipid metabolism through sensing lipid antigens loaded on antigen presenting cells (APCs). iNKT cells secrete IL-2, IL-4 and IL-10 which support immunosuppressive regulatory T cells (Tregs), while IL-4 and IL-10 promote anti-inflammatory macrophage M2 polarization (24). However, the lipid antigen is unknown and whether lipid-activated iNKT cells are anti- or proinflammatory remains controversial (25, 26). Similarly, γ/δ T cells are abundantly present in AT and actively interact with adipocytes, but their role in inflammation is also unclear. Nevertheless, iNKT cells and γ/δ T cells are models by which adipocytes can present a lipid antigen to activate an immune cell.

Song and Deng further define the adipocyte as a novel APC, substantially contributing to adaptive immunity in AT. The adipocyte major histocompatibility II (MHCII) pathway is markedly enhanced in obesity during which it is stimulated primarily by interferon-γ (IFNγ) (10, 27). Adipocyte antigen presentation to naïve T cells promotes inflammatory Th1 effector cell activation, while further production of IFNγ fosters more adipocyte MHCII production, resulting in an escalating cycle of AT inflammation. Mice with genetic depletion of adipocyte MHCII, gain the same amount of weight as control mice, but are protected from AT inflammation and insulin resistance. Within obese AT, adipocytes also activate innate immune cells including macrophages and neutrophils to promote inflammation, while innate lymphoid cells type 2 may be metabolically protective, as reviewed by Blaszczak et al. They highlight the central role of the adipocyte in linking the innate and adaptive immune systems through the secretion of adipokines and cytokines; exosome release of lipids, hormones, and microRNAs; and contact interaction with other immune cells. During diet-induced obesity, a negative feedback loop involving the non-canonical IKK family member TBK1 regulates both innate immunity and glucose and energy metabolism within the adipocyte, as reviewed by Zhao and Saltiel. Upon activation by inflammatory cytokines and lipids, TBK1 suppresses NFκB signaling and attenuates AMP kinase-mediated metabolic activity. They suggest the potential of a TBK1/IKK inhibitor as a new therapy for metabolic diseases.

Adipocytes secrete a multitude of factors that have either pro- or anti-inflammatory functions that impact systemic metabolism. Leptin, one of the most well-known hormones secreted by adipocytes in obesity, in addition to its metabolic function, has important pro-inflammatory actions as comprehensively summarized by Kiernan and MacIver. They provide data suggesting that nearly every immune cell is activated by leptin. Oncostatin M (OSM) is a proinflammatory cytokine, elevated in human obesity and metabolic disease, which inhibits preadipocyte differentiation and enhances the proinflammatory response of adipocytes in a paracrine manner (Sanchez-Infantes and Stephens). However, loss of this system by genetic ablation of the OSM receptor in adipocytes, in contrast to these findings, also aggravates glucose homeostasis, so Sanchez-Infantes and Stephens argue that some adipocyte inflammation is necessary for normal metabolic function. Lee et al. focus on A-FABP, a lipid chaperone abundantly secreted from adipocytes and macrophages, as a key player mediating adipose-vascular cross-talk. A-FABP, in part *via* its activation of c-Jun NH2-terminal kinase (JNK) and activator protein-1 (AP-1), forms a positive feedback loop to perpetuate inflammatory responses. In mice, selective JNK inactivation in the AT significantly reduced expression of A-FABP and circulating A-FABP levels and alleviated high fat high cholesterol diet-induced atherosclerosis (28). In humans, raised circulating AFABP levels are associated with incident metabolic syndrome, T2D and CVD, as well as nonalcoholic steatohepatitis, diabetic nephropathy and adverse renal outcomes, all conditions closely related to inflammation and enhanced CV mortality (Lee et al.; 29–34). They suggest that A-FABP may be a therapeutic target in obesity-related complications. Finally, various extracellular matrix proteins (ECM) are secreted by adipocytes, which in turn, determines the AT architecture, enhances inflammation, and regulates systemic metabolism. As discussed by Wittwer and Bradley (8), adipocyte ECM production is amplified in obesity, resulting in AT fibrosis and adipocyte hypoxia. Clusterin (apolipoprotein J), an ECM-related protein whose expression and secretion in adipocytes is higher in human obesity, is associated with multiple metabolic syndrome components and CV risk and has key effects centrally to modulate amyloid-beta in Alzheimer's Disease. The insulin antagonizing effects of clusterin appear to be in the liver (8).

In summary, the adipocyte exerts immunomodulatory functions *via* multiple novel mechanisms to regulate inflammation and contribute to obesity-related disease. The original research articles and review papers included in this issue present a range of topics under active investigation. Understanding this function and how it impacts other AT immune cells and obesity-related complications is critical to prevention and treatment. Yet, despite a recognition of the importance of adipocytes in inflammatory dysregulation, the mechanisms underlying the inflammatory regulation of these disorders are not fully understood and should remain a critical focus for future investigation.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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MINI REVIEW
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Adipocyte Ceramides—The Nexus of Inflammation and Metabolic Disease

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Adipose depots are heterogeneous tissues that store and sense fuel levels. Through the secretion of lipids, cytokines, and protein hormones (adipokines), they communicate with other organ systems, informing them of the organism's nutritional status. The adipose tissues include diverse types of adipocytes (white, beige, and brown) distinguished by the number/size of lipid droplets, mitochondrial density, and thermogenic capacity. Moreover, they include a spectrum of immune cells that modulate metabolic activity and tissue remodeling. The unique characteristics and interplay of these cells control the production of ceramides, a class of nutrient signals derived from fat and protein metabolism that modulate adipocyte function to regulate glucose and lipid metabolism. The excessive accumulation of ceramides contributes to the adipose tissue inflammation and dysfunction that underlies cardiometabolic disease. Herein we review findings on this important class of lipid species and discuss their role at the convergence point that links overnutrition/inflammation to key features of the metabolic syndrome.

Keywords: ceramide, inflammation, insulin, diabetes, adipocyte

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INTRODUCTION

Obesity increases one's risk for metabolic diseases such as diabetes, coronary artery disease, non-alcoholic steatohepatitis, and heart failure. The condition promotes (a) the accumulation of deleterious lipid metabolites in non-adipose tissues (i.e., lipotoxicity) and (b) chronic low-grade inflammation, which in turn produces the tissue dysfunction that fuels these disorders. The lipotoxicity is secondary to adipose dysfunction, such that excessive lipids are delivered to peripheral tissues rather than being safely stored as triglycerides within the healthy adipocyte (1–5). The inflammation results from the increased recruitment of pro-inflammatory macrophages into the expanded adipose depots, leading to increased secretion of inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukins (IL), and chemokines (6–8). Together, these lipotoxic and inflammatory pathways account for virtually all of the features of the metabolic syndrome including insulin resistance, dyslipidemia, and hypertension.

Lipids, in addition to being major fuel reservoirs (e.g., triglycerides), have important roles in the regulation of nutrient storage. In particular, sphingolipids such as ceramides are metabolic signals that accumulate in obesity and trigger evolutionarily conserved cellular responses to lipid overload (9). Such mechanisms include inhibiting the uptake of glucose and amino acids, leading to the preferential utilization of free fatty acids (FFAs) for energy; slowing rates of triglyceride lipolysis; and impairing mitochondrial respiration (9). At higher concentrations, ceramides induce apoptosis (9). These sphingolipid actions contribute to the tissue dysfunction that underlies non-alcoholic steatohepatitis, diabetes, and heart disease. Inflammatory cytokines, including TNF-α and IL-1,

reinforce this signal by accelerating ceramide production (10). Ceramides thus function at the nexus of lipid metabolism and inflammation.

Studies in mice reveal that inhibition of ceramide synthesis resolves hepatic steatosis and improves insulin-stimulated glucose disposal to slow the progression of cardiometabolic diseases (11). These ceramide-lowering interventions also alter adipose tissue metabolism and morphology, enhancing glucose utilization, and energy expenditure. These manipulations also decrease adipose tissue inflammation and alter macrophage polarization, converting them from pro-inflammatory M1-macrophages into anti-inflammatory M2-macrophages (12). Herein we will review the synergy between the free fatty acids (FFAs) and ceramides that accumulate in obesity and inflammation that accompanies adipose tissue expansion for the development of cardiometabolic diseases. In addition, we will discuss the potential therapeutic approaches for targeting ceramides to reduce inflammation and improve adipose health.

EXCESS FREE FATTY ACIDS INDUCE METABOLIC DISORDERS

Elevations in circulating FFA resulting from increased nutrient consumption or unchecked lipolysis have been implicated in metabolic disorders including insulin resistance, type 2 diabetes, and cardiovascular disease (13). Emerging studies suggest that these fatty acids fuel production of deleterious lipid metabolites such as ceramides while inducing chronic inflammation (3, 6, 14, 15). To this end, FFA, particularly saturated fatty acids such as palmitate which is a key substrate for ceramide production while also modulating innate immune cells to elicit a proinflammatory response, have important roles at the origin of metabolic disease (16, 17).

PATHWAYS CONTROLLING CERAMIDE SYNTHESIS AND METABOLISM

Ceramides are precursors of complex sphingolipids (e.g., sphingomyelin) that are integral components of cell membranes. The sphingolipid content of the adipose depots is influenced by nutrient availability (e.g., increased levels of sphingolipid precursors such as serine and palmitate), inflammatory signals, adiponectin, and other factors that control global stress responses. Ceramides can thus serve as metabolic messengers that integrate input from a variety of factors associated with obesity and metabolic disease. Their cellular levels are determined by three enzymatic pathways: *de novo* synthesis, sphingomyelin hydrolysis, and the salvage pathway (**Figure 1**) (18, 19).

The *de novo* synthesis pathway comprises four sequential enzymatic steps (20). Serine palmitoyltransferase (SPT) catalyzes the first reaction, condensing palmitoyl-CoA (CoA) and serine to produce 3-ketosphinganine. This transient intermediate doesn't accumulate in cells, as it is rapidly converted to sphinganine by 3-ketosphinganine reductase (*3Ksn*). Ceramide synthases (CERS1-6) then add a fatty acid, ranging in chain length

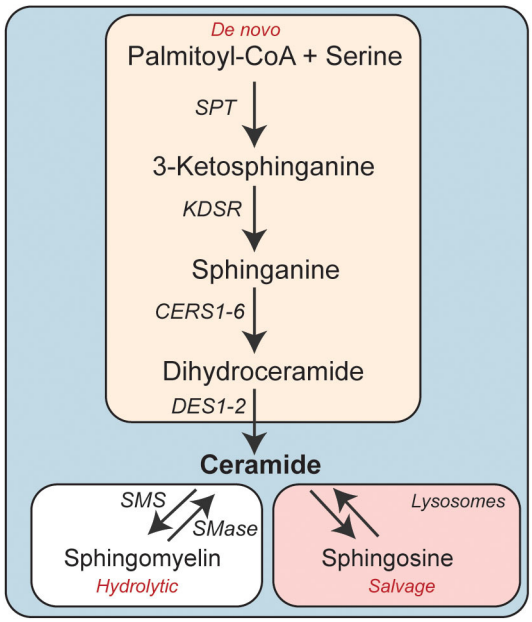

FIGURE 1 | Schematic depicting the enzymatic pathways involved in cellular ceramide synthesis. CERS, Ceramide synthase; DES, Dihydroceramide desaturase; KDSR, 3-ketodihydrosphinganine reductase; SMS, Sphingomyelin synthase; SMase, Sphingomyelinase.

from 14-carbon to 34-carbon atoms, to sphinganine to produce dihydroceramides. The CERS enzymes have variable substrate specificity and unique tissue distributions and account for much of the diversity in sphingolipids (21). In the fourth and final step, dihydroceramide desaturase (*Degs1* and *2*) introduces a critical double-bond into dihydroceramide, generating ceramides (22).

The second pathway involves the hydrolysis of sphingomyelin by neutral or acid sphingomyelinase to produce phosphocholine and re-form ceramide (23).

The third pathway, termed the salvage pathway, allows for the reformation of ceramides from sphingolipids after they are degraded in late endosomes or lysosomes (24). The liberated sphingoid base can be re-acylated by the aforementioned CERS enzymes, re-synthesizing ceramides.

PLASMA AND ADIPOSE CERAMIDES CORRELATE WITH FREE FATTY ACIDS, MARKERS OF INFLAMMATION, AND THE SEVERITY OF CARDIOMETABOLIC DISEASES

Within the last decade, advances in mass-spectrometry have allowed researchers to confidently assess whether plasma and tissue ceramide levels correlate with indices of metabolic diseases. Numerous groups have found that circulating ceramides and FFAs are elevated in subjects with insulin resistance, type 2-diabetes, non-alcoholic fatty

liver diseases, chronic kidney diseases and major adverse cardiovascular events including mortality (25–35). In parallel, researchers have shown that circulating inflammatory cytokines also positively associate with these metabolic outcomes (36, 37). Interestingly, circulating FFAs, ceramides and inflammatory cytokines also correlate with one another in human subjects with coronary artery disease, hepatic steatosis, or insulin resistance (10, 33, 38–40). These studies suggest that they may have interrelated roles in the etiology of metabolic disorders.

Adipose tissue ceramide content and inflammation have also been evaluated in subjects with obesity, insulin resistance and/or diabetes. One such study, by the Yki-Jävinen group, demonstrated that ceramide levels are elevated in the adipose tissues of individuals with insulin resistance, independent of obesity (41). In this study, the tissue also showed increases in inflammatory markers. The Brüning laboratory also found that ceramides, particularly C_{16}-ceramides, were elevated in individuals with obesity (42). They also observed dramatically increased expression of ceramide synthase 6 (CERS6), the enzyme that is responsible for generating C_{16}-ceramides. Additionally, CERS6 expression positively correlated with insulin resistance.

CERAMIDES ARE MODULATED BY SEVERAL INFLAMMATORY AND ANTI-INFLAMMATORY SIGNALING MOLECULES

The oversupply of precursors such as palmitate and serine undoubtedly account for much of the ceramide accumulation that occurs in obesity. Indeed, a small number of dietary studies have shown that dietary fat intake influences ceramide synthesis and accumulation (43, 44). As outlined below, inflammatory modulators also influence the rate of ceramide production.

Tumor Necrosis Factor-Alpha (TNF-α) Produces Ceramides to Contribute to Insulin Resistance

In obesity, the recruitment of macrophages to the expanding adipose depots can induce an inflammatory state characterized by increased expression and secretion of inflammatory cytokines such as TNF-α, IL-6, and IL-1β (6, 15, 45–50). Some of these cytokines have been shown to produce ceramides (51–53). In particular, serum and adipose TNF-α are often elevated in individuals with obesity and/or type 2 diabetes and correlate with the severity of insulin resistance (54–56) and with levels of ceramides (33). In cultured cells, the cytokine stimulates ceramide accumulation by inducing expression of ceramide synthesis genes [e.g., serine palmitoyltransferase (SPT)] and increasing expression and activity of sphingomyelin hydrolyzing enzymes (e.g., sphingomyelinase) (51, 57–62). Similar effects on ceramide synthesis have been demonstrated with certain cytokines such as the TNF-α *in vivo* (63), which antagonize insulin-stimulated glucose disposal in rats and humans (64, 65). In cultured adipocytes and myeloid cells, researchers confirmed that it inhibits insulin signaling and action via receptor-mediated activation of sphingomyelinase (66).

In mice, genetic manipulations to ablate TNF-α or its receptors ameliorate obesity-induced insulin resistance (46, 67). However, clinical trials targeting TNF-α have generally shown little or no beneficial effect on systemic insulin sensitivity (68, 69), indicating that TNF-α lowering is insufficient to combat insulin resistance in humans.

Toll-Like Receptors Induce Ceramide Biosynthesis to Contribute to Insulin Resistance

The lipotoxic environment in obesity increases the supply of saturated fatty acids that either directly or indirectly activate toll-like receptor (TLR)-4 (70–74). These pattern recognition receptors, which are typically involved in innate immune responses, have been implicated in inflammation and insulin resistance that accompanies obesity and underlies metabolic disease. For example, Flier et al. found that mice lacking TLR-4 were protected from lipid or high fat diet-induced insulin resistance (17, 75). They also found that long-chain fatty acids signal via TLR-4 to induce transcription of inflammatory cytokines (e.g., TNF-α and IL-6), thus reinforcing and enhancing the inflammatory state. Using similar approaches with various loss-of-function TLR-4 mouse models, four other laboratories described essential roles for TLR-4 in obesity and/or insulin resistance (76–79). Curiously, Shulman et al. found the opposite result, concluding that TLR-4 was not required for lipid-induced insulin resistance (80).

Activation of toll-like receptor (TLR)-4, via lipopolysaccharides (LPS) or a more specific ligand Kdo(2)-lipid A, induces ceramide accumulation by increasing the expression of several ceramide synthesis enzymes (77, 81–84). In cultured myotubes, nuclear factor kappa B (NFκB) was found to be an obligate intermediate in these TLR-4 mediated effects on ceramide production (77). In contrast, ablation of TLR-4 in mice reduces ceramides, and even prevents their synthesis in models of lipid oversupply (i.e., mice fed a high fat diet or infused with lipid cocktails) (77). These findings indicate that TLR-4 enhances ceramide production and reveal the interplay between TLR-4 and ceramides in the metabolic dysfunction that accompanies obesity.

The mechanisms controlling TLR-4 activation in obesity have been controversial. Though saturated fatty acids were initially speculated to be TLR-4 ligands (70–73), some have argued that fatty acids signal through indirect signaling mechanisms (74). Others have argued that this observation is an artifact, likely due to contamination of the saturated fatty acid preparations with lipopolysaccharide (85). In an elegant study, Lancaster et al. found that saturated fatty acids do not bind directly to the TLR-4 receptors, but rather prime TLR-4 to induce lipid-mediated inflammatory signaling (74). These authors found that activating

TLR-4 led to a marked upregulation of ceramides and ceramide-synthesizing genes (74).

Ceramides Activate the NLRP3 Inflammasome to Increase Cytokine Secretion

Inflammasomes are large, multiprotein complexes that form in response to endogenous stress signals, initiating a wide range of cellular activities that include production of the pro-inflammatory cytokines (e.g., IL-1β). The best characterized inflammasome is termed NLRP3 because of the presence of NOD-, LRR-, and pyrin domain-containing protein 3 within the complex. Other components include the adapter ASC and pro-caspase-1. Saturated FFAs were recently found to induce inflammasome activation in macrophages, prompting speculation that lipotoxic intermediates such as ceramides might drive inflammasome activation (49). In both macrophages and adipocytes, ceramides activate the NLRP3 inflammasome, promoting cleavage of caspase-1 and subsequent stimulation of cytokine secretion (86). Subsequent studies found roles for inflammasomes as a downstream ceramide effector in other cell types (87–89), Within adipocytes, this ceramide interaction with the NLRP3 inflammasome may contribute to the adipose inflammation that contributes to insulin resistance. Interestingly, inhibiting *de novo* ceramide biosynthesis in macrophages did not influence the inflammasome (90), nor did it impact glucose tolerance (11, 12, 90). Moreover, palmitate has been shown to elicit activation of inflammasome by modulating the AMPK-ROS-autophagy pathway, suggesting alternative mechanisms link FFAs to this immune complex (49).

Plasminogen Activator Inhibitor-1 Has a Bidirectional Relationship With Ceramides

Plasminogen activator inhibitor-1 (PAI-1) is a glycoprotein that is synthesized in endothelial cells, liver, adipose tissue, and other tissue types. It inhibits the serine proteases that covert plasminogen into the active fibrinolytic enzyme plasmin (91, 92). Plasma PAI-1 concentrations are elevated in obesity and diabetes and correlate with the severity of insulin resistance (93–95). Pharmacological inhibition or genetic ablation of PAI-1 in mice protects them from both obesity and insulin resistance while improving adipocyte health and decreasing adipose inflammation (96–99). PAI-1 ablation ensures this protection, at least in part, by reducing accumulation of ceramides in adipocytes, which it accomplishes by decreasing expression of ceramide synthesis genes (96). Conversely, ceramides were reported to induce PAI-1 expression in adipocytes (100), revealing bidirectional interplay between PAI-1 and ceramides that modulates adipose tissue inflammation and function.

Adiponectin Receptors Are Ligand Activated Ceramidases

The adipokine adiponectin attenuates many features of diabetes and heart disease, including insulin resistance, dyslipidemia, inflammation and cardiomyocyte, endothelial cell and beta-cell apoptosis (101–106). Holland, Scherer et al. were intrigued by the fact that adiponectin and ceramides have such oppositional roles in biology. Moreover, they observed a sequence similarity between adiponectin receptors (AdipoRs) and a family of ceramidases. They thus tested the provocative idea that adiponectin elicited its broad spectrum of actions by reducing (via diacylation) ceramides. They confirmed that the receptor had ceramidase activity that is activated by ligand binding (105). In mice, the cardioprotective and anti-diabetic actions of adiponectin were accompanied by reductions in ceramides (105). Moreover, they identified key residues in AdipoRs that were required for ceramidase activity and for all of adiponectin's downstream actions (105). These findings were then validated by Vasiliauskaite-Brooks et al. who crystalized the AdipoRs in presence of short-chain ceramide analogs, discovering it bound to the liberated sphingoid base (107, 108). They also confirmed that the purified receptors possess ceramidase activity (107, 108). These studies suggest yet another key regulatory mechanism that controls cellular ceramides in order to modulate inflammation and other features of the metabolic syndrome.

CONVERGENCE OF ADIPOSE CERAMIDES AND INFLAMMATION TO CONTROL INSULIN RESISTANCE

Insulin resistance is a defining attribute of the metabolic syndrome that increases one's risk for diabetes and heart disease. As noted above, numerous studies have described correlational relationships between insulin resistance, circulating cytokines, and ceramides in clinical populations (9, 21, 22). Studies in rodents further indicate that ceramides play causative roles in insulin resistance, often linking inflammatory agonists to their deleterious effects on glucose uptake and utilization.

The earliest studies evaluating the role of ceramides in insulin resistance analyzed their effects in 3T3-L1 adipocytes, a murine cell line that shows many of the hallmark metabolic attributes of human adipose tissue. Those studies revealed that ceramides inhibit glucose uptake by inhibiting activation of Akt/PKB (109), a serine/threonine kinase that is an obligate intermediate in insulin-stimulated glucose transporter GLUT4 translocation, as well as glycogen and protein synthesis and protection from apoptosis. Curiously, ceramides did not inhibit the signaling events that precede Akt/PKB activation, such as the activation of PI3-kinase or generation of its product, 3'-polyphosphoinositides (110). Moreover, they blocked activation of the enzyme by numerous other stimuli, including those that don't utilize the signaling scaffold insulin receptor substrate-1 (110), which had recently been identified as a putative site of insulin resistance (111). This observation prompted a flurry of studies seeking to elucidate the signaling mechanisms that linked elevations in ceramides to the inhibition of this important enzyme. These studies revealed that ceramides inhibit Akt/PKB by two known mechanisms, which impact different portions of the enzyme (112). Ceramides dephosphorylate key activating residues through protein phosphatase 2A(PP2A) (112), which is an established ceramide effector (113). Through an alternate mechanism, ceramide blocks the translocation of Akt/PKB to

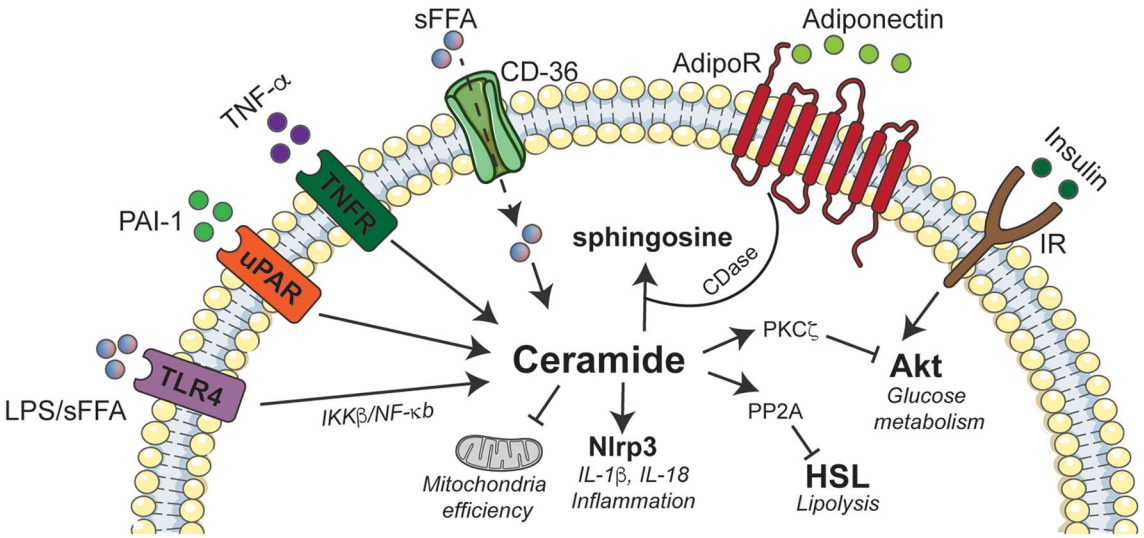

FIGURE 2 | Schematic depicting interactions between ceramides and inflammatory agonists in adipose tissue. Ceramide accumulation elicits deleterious effects on adipose tissue function by activating Nlrp3 inflammasome that induces inflammation, inhibition of Akt via PKCζ to abrogate insulin signaling, and promoting excessive lipid storage by inhibiting HSL. The immunomodulatory adiponectin exhibits some of its beneficial effects by stimulating ceramidase activity that converts ceramides to sphingosine. Akt, Protein Kinase B; CD-36, cluster of differentiation 36; AdipoR, Adiponectin receptor; CDase, Ceramidase; IKK, Ikappa kinase; IL, interleukin; IR, insulin receptor; LPS, lipopolysaccharide; NF-κB, Nuclear factor kappa-light-chain-enhancer of activated B cells; Nlrp3, NLR family, pyrin domain containing 3; PAI-1, Plasminogen activator inhibitor 1; PKC, protein kinase C; PP2A, Protein phosphatase 2A; sFFA, Saturated fatty acids; TLR4, Toll like receptor-4; TNF-α, Tumor necrosis factor alpha; TNFR, Tumor necrosis factor alpha receptor; uPAR, Urokinase-type plasminogen activator receptor.

the plasma membrane (112). Studies by the Hundal laboratory subsequently revealed that the translocation effect was due to ceramide actions on atypical protein kinase C (PKCζ), which phosphorylates a key residue in the pleckstrin homology domain of Akt/PKB to block its recruitment to the plasma membrane (114–117). These disparate ceramide mechanisms are clearly separable, as they impact different protein domains and are responsive to distinct inhibitors (112). They also vary by cell type, seeming to be contingent on the relative quantity of caveolar membranes. Adipocytes that have a high abundance of caveolae favor the PKCζ-Akt/PKB axis rather than the PP2A-Akt/PKB axis (118) (**Figure 2**).

These studies suggested that ceramides, induced by either the oversupply of fatty acid substrates or the inflammation-induced upregulation or activation of ceramide-producing enzymes, might drive insulin resistance *in vivo*. Data in rodents support this hypothesis. For example, a pharmacological inhibitor of SPT (i.e., myriocin) prevents and/or reverses insulin resistance in high fat diet fed mice (12, 77, 119–121), lipid-infused rats (121), fructose-fed hamsters (122), and leptin-deficient mice and rats (i.e., Zucker *fa/fa* rats and *ob/ob* mice) (121). It also resolves steatosis, decreases adipocyte size, and enhances recruitment of M2 macrophages into subcutaneous adipose tissue (12). Similar findings were obtained with pharmacological (i.e., fenretinide) or genetic (i.e., gene knockout) inhibition of DES1 (11, 123, 124). Many of these actions could be explained by ceramide actions within the adipocyte. Adipocyte-specific depletion of SPTLC2, a critical subunit within the SPT complex, or DES1 improved insulin sensitivity, resolved hepatic steatosis, and decreased inflammation of the adipose beds (12). A comparable spectrum of effects was obtained using adipose-specific over-expression of acid ceramidase (125).

While the mechanisms that allow ceramide to modulate lipid and inflammation-induced insulin resistance are fairly clear, the means by which adipocyte ceramides induce the recruitment of macrophages are not. Of note, most of the protective actions of ceramide depletion are unlikely to be driven by ceramides within the macrophage, as depleting SPTLC2 or DES1 from myeloid cells did not influence glucose homeostasis (11, 12, 90).

CONVERGENCE OF ADIPOSE TISSUE CERAMIDES AND INFLAMMATION TO CONTROL ENERGY EXPENDITURE

In mice, myriocin also increases energy expenditure via a mechanism that involves changes to the adipose depot. The SPT inhibitor increased the allotment of adipocytes that express uncoupling protein 1 (UCP1) (12), a mitochondrial protein that dissipates the proton gradient generated by the electron transport chain. This uncoupling reduces mitochondrial membrane potential and leads to high rates of substrate oxidation, heat production and energy expenditure (126). Similar observations were obtained following the adipocyte-specific depletion of the SPTLC2 subunit (12).

Myriocin also caused a shift in macrophage polarization from M1 to M2, which has been shown to induce adipose "browning" characterized by the upregulation of UCP1. Given these data,

we profiled macrophage content in adipose tissue following a myriocin intervention. This revealed a recruitment of M2-macrophages in the adipose tissue that was associated with a reduction in expression of key pro-inflammatory cytokines (e.g., IL-6, MCP-1, and TNF-α) and an induction of a crucial anti-inflammatory cytokine IL-10 (11). To resolve whether these improvements were due to cell-autonomous ceramide actions within the adipocytes or macrophages, we depleted the *Sptlc2* gene from both adipocytes and macrophages. Adipocyte-specific depletion recapitulated the effects of myriocin and increased the recruitment of M2-macrophages and expression of thermogenic genes (e.g., *Ucp1*, *Pgc1a,* and *Prdm16*). These data indicated that adipocyte sphingolipids likely drove the cellular responses that increased energy expenditure. By comparison, depleting *Sptlc2* from macrophages failed to impact energy expenditure. Moreover, ectopic ceramides were also shown to inhibit mitochondrial respiration and block activation of hormone-sensitive lipase by β-adrenergic agonists. The effects on lipolysis were mediated by the aforementioned ceramide effector PP2A (**Figure 2**).

Beyond the effects on UCP1 and HSL, ceramides seem to slow energy expenditure by inhibiting mitochondrial respiration. Indeed, addition of ceramides to cells is sufficient to inhibit mitochondrial activity (12). Hammerschmidt et al. (127) elucidated one mechanism that underlies this effect, determining that ceramides bind to mitochondrial fission factor (MFF) to alter mitochondrial morphology and reduce respiratory capacity (127). This effect is specific for the C_{16}-ceramides produced by CERS6 (127).

Two other studies have evaluated the effects of reducing ceramides in white adipocytes. Curiously, while these studies did find that depleting ceramides from adipose tissue influenced glucose and lipid homeostasis, neither intervention induced adipose browning. One was the aforementioned study evaluating the consequence of acid ceramidase expression, while the other was our study involving DES1 depletion (11). While these interventions affected mitochondrial respiration, they did not induce UCP1 expression. We thus conclude that the effect on UCP1 is not due to direct ceramide actions, but rather to another intermediate in the pathway. One attractive hypothesis is that the browning effects are mediated by the CERS enzymes (128, 129), which have been shown to be transcriptional repressors that move to the nucleus and regulate lipase expression following encounters with fatty acids. By comparison, we conclude that the effects on mitochondrial fission and lipolysis (i.e., HSL) are due to direct actions of the sphingolipid analogs on MFF and PP2A, respectively. The effects on mitochondrial morphology/respiration and HSL were observed in all of the interventional studies described.

CONCLUSION

Inflammation has long been known to be a hallmark of obesity, owing to the recruitment of macrophages to adipose depots and the enhancement of TLR-4 signaling by saturated fatty acids. Herein we discussed how the impact of chronic inflammation on host metabolism are linked to ceramide-driven lipotoxicity. Ceramides, which are universally upregulated by inflammatory stimuli, inhibit insulin-stimulated glucose disposal and mitochondrial respiration. They thus provide a convergence point that links overnutrition/dyslipidemia and inflammation to drive many of the key features of the metabolic syndrome. Curiously, manipulating ceramides in adipose tissue also influences the inflammatory state of the organ, suggesting the existence of feedback mechanisms that involve ceramide-dependent, adipocyte autonomous signals that control the immune cell population (e.g., via the NLRP3 inflammasome). Additional research on ceramides and their inflammatory regulators thus holds great promise as a means to combat metabolic disease and improve adipose tissue health.

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AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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MINI REVIEW
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Interaction of Adipocyte Metabolic and Immune Functions Through TBK1

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Adipocytes and adipose tissue play critical roles in the regulation of metabolic homeostasis. In obesity and obesity-associated metabolic diseases, immune cells infiltrate into adipose tissues. Interaction between adipocytes and immune cells re-shapes both metabolic and immune properties of adipose tissue and dramatically changes metabolic set points. Both the expression and activity of the non-canonical IKK family member TBK1 are induced in adipose tissues during diet-induced obesity. TBK1 plays important roles in the regulation of both metabolism and inflammation in adipose tissue and thus affects glucose and energy metabolism. Here we review the regulation and functions of TBK1 and the molecular mechanisms by which TBK1 regulates both metabolism and inflammation in adipose tissue. Finally, we discuss the potential of a TBK1/IKKε inhibitor as a new therapy for metabolic diseases.

Keywords: TBK1, IKK, inflammation, metabolism, obesity, adipose tissue, overnutrition, undernutrition

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INTRODUCTION

Obesity has reached a pandemic (1). The complications of obesity, including type 2 diabetes, cardiovascular diseases, neurodegenerative diseases, non-alcoholic fatty liver diseases, and cancer, have become leading health threats. Obesity is caused by a positive energy balance, leading to excess lipid accumulation in adipose and other tissues (2–5). In addition to being an inert site for energy storage, adipose tissues play essential roles in metabolic homeostasis (6, 7). As the major cell type within adipose tissue, adipocytes are responsible for lipid storage and mobilization in response to insulin and sympathetic activation respectively. However, these cells can also sense their nutrient status, and respond by secreting a series of hormones known as "adipokines" (6–8). Upon food intake, the resulting elevation of nutrients in the circulation stimulates insulin production. Insulin in turn lowers glucose and fatty acid levels in part by instructing fat and muscle tissue to increase glucose uptake and storage, while reducing lipolysis in fat, glycogenolysis in muscle and liver and gluconeogenesis in liver (9, 10). In adipocytes, nutrients are largely stored as triglycerides. Upon reaching a threshold of lipogenesis, adipocytes trigger the production of adipokines such as leptin, to suppress food consumption and activate the sympathetic nervous system, thus closing a loop to ensure energy homeostasis (9, 11–15). Excessive energy intake or low energy expenditure could lead to a sustained positive energy balance and consequently cause increased adiposity in obesity (3–5).

Obesity is associated with low-grade chronic inflammation in adipose tissue, featured by an increased number of macrophages and an elevated ratio of proinflammatory macrophages (16–20). Although the

immediate trigger for obesity-associated inflammation in adipose tissue remains unclear, multiple factors, including hypoxia, mechanical stress, lipotoxicity, adipocyte death, and bacterial toxins may contribute to this process (9, 21–27). Inflammation has been reported to affect several properties of adipocytes. The activation of proinflammatory pathways has been shown to disrupt glucose uptake and insulin responsiveness and alter adipokine production (28–31), suggesting that inflammation plays an essential role in the pathological response to obesity.

The nuclear factor kappa B (NFκB) is a widely expressed transcription factor that mediates inflammatory responses in numerous tissues. The NFκB signaling pathway plays a key role in the development of inflammation and insulin resistance in adipose tissue (32–34). Transcription through NFκB is mainly controlled by the phosphorylation of inhibitor of NFκB (IκB) by the upstream IκB kinases (IKKs). The canonical IKKs, IKKα, and IKKβ, phosphorylate IκB, and other NFκB subunits to induce the expression of NFκB target genes (35). Besides IKKα and β, the IKK family also includes two non-canonical members, IKKϵ and TANK-binding kinase 1 (TBK1). Interestingly, despite their sequence similarity to the canonical IKK isoforms, TBK1 and IKKε do not appear to play important roles in NFκB activation in response to proinflammatory cytokines (36). However, expression of *Ikke* and *Tbk1* mRNAs are induced by NFκB (37). Moreover, IKKϵ and TBK1 are activated by protein phosphorylation in response to proinflammatory cytokines or other substances that bind to Toll-like receptors 3 and 4 (38). It was reported that activities of IKKϵ and TBK1 are significantly increased in adipose tissue of obese mice (37). We review here the functions of the noncanonical IKKs in inflammation and metabolic regulation in adipose tissue, with a major focus on the roles of TBK1 in crosstalk between inflammation and metabolism.

NON-CANONICAL IKKs

NFκB plays a central role in the transcriptional response to proinflammatory stimuli. In the absence of stimuli, IκB binds to NFκB to sequester the transcription factor in the cytoplasm (39). Inflammatory stimuli increase the phosphorylation and activation of IKKs, which in turn phosphorylate IκB and NFκB to activate the expression of NFκB target genes (35, 39, 40). An IKK complex formed by IKKα, IKKβ, and the NFκB essential modifier (NEMO) directly phosphorylates IκB at Ser^{32} and Ser^{36} to induce ubiquitin-associated degradation. Consequently, NFκB is released to activate gene expression. This pathway represents the canonical NFκB signaling pathway (41, 42). Both IKKα and IKKβ possess a kinase domain (KD), a scaffold dimerization domain (SDD), and a NEMO-binding domain (NBD). A ubiquitin-like domain (ULD) is found in IKKβ but not in IKKα. In contrast to the canonical IKKs, IKKϵ and TBK1 have similar SD, ULD, and SDD, but lack the NBD. Human TBK1 shares 49% identity and 65% similarity to IKKϵ, but only 27% identity with IKKα and IKKβ (43–45). Unlike the canonical IKKs, the roles of IKKϵ and TBK1 in the NFκB signaling pathways remain uncertain. Early studies demonstrated that TBK1 phosphorylates IKKβ to increase its activity, while IKKϵ phosphorylates RelA at Ser^{468} to induce its nuclear translocation (44, 46, 47). However, subsequent studies found that TBK1 or IKKϵ deficiency has no effect on LPS, TNFα, interleukin-1β, or poly(I:C)-induced activation of NFκB (38, 48). Thus, it appears that IKKϵ and TBK1 are not required for the activation of NFκB in response to proinflammatory cytokines (36). Instead, studies showed that the expression of *Ikke* and *Tbk1* are induced by NFκB under proinflammatory conditions (37). Interestingly, two separate studies demonstrated that TBK1 and IKKϵ mediate NFκB activation downstream of the cGAS-STING pathway in response to cytosolic DNA or STING ligand (49, 50).

Multiple studies demonstrated that non-canonical IKKs play important roles in metabolic regulation. The expression of *Ikke* was upregulated in the liver, adipocytes, and adipose tissue macrophages during diet-induced obesity (34). Knockout of *Ikke* reduced inflammation and improved insulin sensitivity in adipose tissue and liver. Hepatic steatosis was largely attenuated by IKKϵ deficiency as well. *Ikke* knockout mice gained less weight and were resistant to high fat diet-induced obesity due to the increased energy expenditure and thermogenesis (34). The expression of Uncoupling protein 1 (*Ucp1*), a major uncoupler utilizing the mitochondrial proton gradient to generate heat, was significantly upregulated in white adipose tissue in these mice (34).

Energy expenditure is largely controlled by sympathetic signals. Catecholamines induce *Ucp1* expression and increase thermogenesis in both brown and subcutaneous white fat (51, 52). During high fat diet-induced obesity, adipose tissue becomes resistant to catecholamines, resulting in decreased energy expenditure (9, 53–55). Mowers et al. demonstrated that IKKϵ directly phosphorylates and activates phosphodiesterase 3B (PDE3B) to reduce intracellular cAMP levels and thus represses cAMP-mediated β-adrenergic signaling (55). *Ikke* knockout restored catecholamine sensitivity, leading to an upregulation of *Ucp1* expression and an increase of thermogenesis (34, 55, 56). Therefore, during obesity, the inflammation-induced expression of *Ikke* represses sympathetic signal and further promotes energy storage (**Figure 1**). IKKϵ mediates the interaction between inflammatory and catecholamine signals, representing one example of how inflammation modulates metabolism in adipose tissue.

TBK1

Although the role of TBK1 in NFκB activation remains unclear, its function in the innate immune response has been well-recognized. In response to infection, pattern recognition receptors (PRRs) sense the pathogen-associated molecular patterns (PAMPs) on bacteria or viruses to activate TBK1-mediated signaling pathways (57, 58). Two major types of PRRs participate in this action. Toll-like receptors (TLRs), especially TLR3 and TLR4, are cell surface receptors that utilize adaptor proteins such as TIR-domain-containing adaptor-inducing interferon-β (TRIF) and Myeloid differentiation primary response 88 (MyD88). Ligands of TLRs,

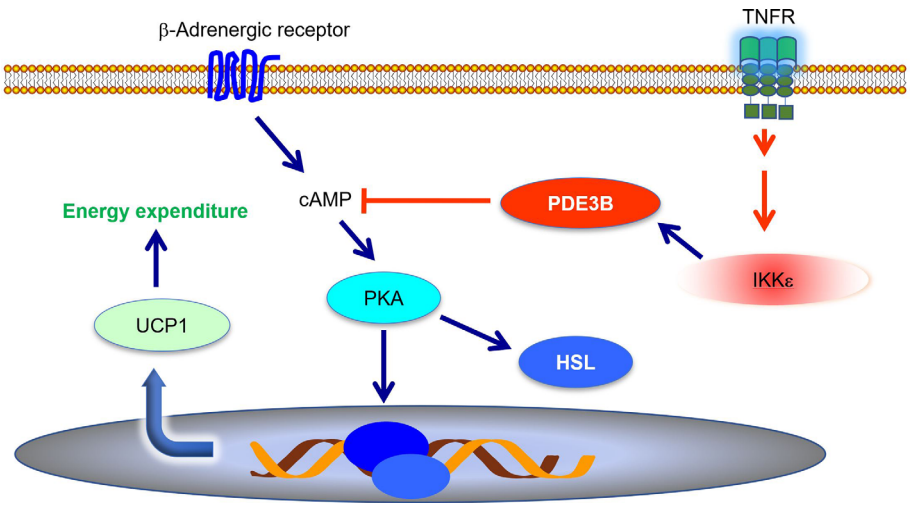

FIGURE 1 | IKKϵ inhibits adrenergic signaling to repress thermogenesis. IKKϵ activity is induced by proinflammatory stimuli. Active IKKϵ directly phosphorylates and activates PDE3B to reduce cAMP levels. Consequently, IKKϵ inhibits cAMP-mediated adrenergic signaling pathway and represses energy expenditure in adipocytes. PDE3B, phosphodiesterase 3B; cAMP, cyclic AMP; PKA, protein kinase A; HSL, hormone sensitive lipase; UCP1, uncoupling protein 1.

such as lipopolysaccharides (LPSs), bind to their receptors to induce the activation of TBK1. Retinoic acid-inducible gene I (RIG-I)-like receptors, NOD-like receptors (NLRs), and cytosolic DNA sensors are the PRRs in the cytoplasm (36, 59, 60). Cyclic-GMP-AMP (cGAMP) synthase (cGAS) is a cytosolic DNA sensor. cGAS utilizes cytosolic DNA to generate cGAMP, which in turn binds to the adaptor protein Stimulator of interferon genes (STING). Consequently, STING interacts with and activates TBK1 (61). Besides pathogen infection, proinflammatory cytokines such as tumor necrosis factor α (TNFα) also produces TBK1 activation (62, 63). Upon activation, TBK1 directly phosphorylates interferon regulatory factor 3 (IRF3) and IRF7 at multiple serine and threonine residues to induce their nuclear translocation (64–67). Consequently, these transcription factors upregulate the expression of type I interferon (*Ifna, Ifnb*) genes in the innate immune response. TBK1 is indispensable for the antiviral immune response (61, 68).

The activity of TBK1 is acutely controlled by phosphorylation on Ser^{172} within the kinase domain (63, 69, 70). However, the molecular mechanism by which this activating phosphorylation occurs is still unclear. Structural studies suggest that TBK1 undergoes multi-order oligomerization. While the kinase usually exists as a homodimer, the kinase domains face outward and are generally not capable of phosphorylation in this configuration (70). However, adapter proteins bring together these homodimers in larger heteromeric complexes, leading to Ser^{172} phosphorylation *via* transautophosphorylation (70). Moreover, recent investigations demonstrated that Unc-51 like autophagy activating kinase 1 (ULK1) can directly phosphorylate Ser^{172} (63). This is consistent with the observations that both ULK1 and TBK1 play essential roles in autophagy (71–75). TBK1 regulates autophagy *via* phosphorylating optineurin on Ser^{177} and SQSTM1/p62 on Ser^{403} to clear pathogen or damaged mitochondria (76, 77). Interestingly, the activation of NFκB also upregulates the expression of *Sqstm1*/p62 to induce mitophagy in response to LPS (78, 79). These studies suggest that NFκB and TBK1 may function synergistically to promote the clearance of damaged mitochondria and pathogens during infection.

Understanding the functions of TBK1 *in vivo* have been hampered by the lethality of global *Tbk1* knockout. Whole-body knockout of *Tbk1* leads to enhanced apoptotic liver degeneration and embryonic lethality at approximately E14.5 (80). In this regard, TBK1 directly phosphorylates receptor-interacting serine/threonine-protein kinase 1 (RIPK1) on Thr^{189} to prevent cell death. TBK1 deficiency substantially increases RIPK1-mediated cell death, resulting in embryonic lethality between embryonic day 13.5 and embryonic day 14.5 (81). In line with this finding, another study found that both TBK1 and IKKε phosphorylate RIPK1 on multiple sites, including Thr^{189}, to prevent TNF-induced cell death (62, 81). To conduct *in vivo* studies on the roles of TBK1 in inflammation, Marchlik et al., generated ($Tbk1^{\Delta/\Delta}$) mice expressing a TBK1 inactive mutant with the deletion of exon 2 (82). $Tbk1^{\Delta/\Delta}$ C57BL/6J mice were still embryonic lethal. However, $Tbk1^{\Delta/\Delta}$ 129S5 mice were fertile and viable, but born at a decreased Mendelian frequency. $Tbk1^{\Delta/\Delta}$ mice had increased mononuclear and granulomatous cell infiltration into multiple tissues, along with elevated circulating monocytes. This is consistent with another study reporting that $Tbk1^{\Delta/\Delta}$ mice die faster and in larger numbers in response to LPS (82).

Regulation of the Crosstalk Between Metabolism and Inflammation by TBK1

Although it was reported that TBK1 expression and activity are induced in adipose tissues during obesity and insulin resistance (34, 37, 63), the role of TBK1 in the pathogenesis of metabolic disease was unclear. A recent study revealed that TBK1 mediates

crosstalk between inflammation and metabolism in adipose tissue (**Figure 2**) (63). During high fat diet-induced obesity, chronic inflammation leads to an increase of proinflammatory cytokines in the adipose tissue (9, 30, 83). Consequently, these cytokines, such as TNFα, produce the activation of TBK1 (63). At the same time, the inflammatory environment also results in enhanced NFκB activity, resulting in an increase in *Tbk1* expression (34, 63). Thus, high fat diet feeding substantially induces TBK1 activity in the adipose tissue through both transcriptional and posttranslational regulation (34, 37, 63). Upon activation, TBK1 attenuates adipose tissue inflammation *via* repressing the atypical NFκB pathway (63). In this pathway, the NFκB-inducing kinase (NIK) phosphorylates Ser^{176} to activate IKKα, which largely resides as a homodimer (84). IKKα in turns phosphorylates the RelB (NFκB2) precursor p100, resulting in the cleavage and maturation of RelB (85). Thus, NIK is responsible for activation of the atypical NFκB pathway, which induces the expression of target genes, such as *Ccl2* (C-C motif chemokine ligand 2), to promote macrophage infiltration and inflammation (86–88). Interestingly, TBK1 directly phosphorylates NIK, leading to its degradation (62, 63). *Tbk1* knockout causes hyperactivation of the atypical NFκB pathway and exacerbates macrophage infiltration and inflammation in adipose tissue of obese mice (63). Moreover, the loss of TBK1 in adipocytes attenuates HFD-induced obesity *via* increasing mitochondrial biogenesis and energy expenditure. TBK1 inhibits AMP-activated protein kinase (AMPK) by catalyzing phosphorylation on inhibitory sites in AMPKα subunit, Ser^{459} and Ser^{476}. *Tbk1* knockout thus ameliorates AMPK repression in adipose tissues of high fat diet-fed mice (63), revealing that TBK1 mediates crosstalk from inflammation to energy metabolism. The inflammation-induced TBK1 activity produced during obesity represses energy expenditure and promotes anabolism, which further enhances obesity through a feedforward loop.

In addition to inflammation-induced TBK1 activation, it has also been reported that TBK1 Ser^{172} phosphorylation is induced in adipocytes during glucose deprivation, which creates an energy shortage condition (63). Thus, TBK1 is activated not only during overnutrition, but also during undernutrition. Mechanistically, energy shortage leads to an increase of AMP/ATP ratio, which in turns activates AMPK. AMPK directly phosphorylates ULK1 at multiple residues to induce its activity (89, 90). ULK1 is able to phosphorylate Ser^{172} to activate TBK1 (63). Similar observations on AMPK-dependent TBK1 activation have been reported in myotubes and Hela cells as well (91). Furthermore, prolonged fasting induced *Tbk1* expression in different depots of white adipose tissues (63). However, the molecular mechanism of this transcriptional regulation is still unknown. Studies on animal models and human subjects reported that fasting or undernutrition leads to a reduction of basal metabolic rate and energy expenditure (92, 93). Given the effects of TBK1 on energy metabolism, fasting likely activates a TBK1-mediated feedback loop to repress energy expenditure in response to undernutrition. The activation of TBK1 could be a protective mechanism to attenuate the loss of body weight during fasting. Moreover, reduced caloric intake has been demonstrated to attenuate adipose tissue inflammation in obesity (94–97). The anti-inflammatory function of TBK1 at least partially contributes to this effect and mediates crosstalk from undernutrition to inflammation.

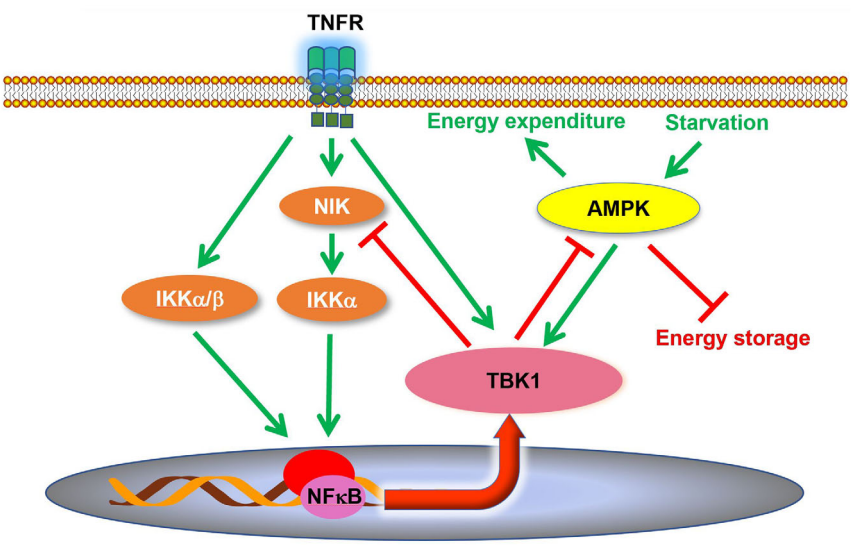

FIGURE 2 | TBK1 regulates inflammation and energy metabolism in adipocytes. TBK1 activity is induced by proinflammatory stimuli and undernutrition. Although TBK1 is not directly involved in TNFα-induced activation of NFκB, active TBK1 phosphorylates NIK to induce its degradation and thus attenuates atypical NFκB pathway in a negative feedback loop. Moreover, TBK1 inhibits AMPK to repress energy expenditure in adipocytes. AMPK, AMP-activated protein kinase; NIK, NFκB inducing kinase.

In summary, TBK1 plays a central role in the regulation of both inflammation and energy metabolism in adipose tissue. It is activated during both overnutrition and undernutrition and mediates a negative feedback loop to repress inflammation and energy expenditure under certain conditions (63). More importantly, TBK1 is responsible for the bidirectional crosstalk between energy metabolism and inflammation. The deficiency of TBK1 in adipocytes leads to the attenuation of high fat diet-induced obesity, but the exaggeration of adipose tissue inflammation (63), indicating a loss of the positive correlation between adiposity and adipose tissue inflammation.

Furthermore, in response to proinflammatory stimuli, TBK1 has been shown to affect metabolic reprogramming in different cell types. Upon the activation of TLRs, active TBK1 was recruited to the myddosome and thus promotes glycolysis in macrophages (98). Another two studies also reported that TBK1 activation mediates TLR ligand-induced glycolytic reprogramming (99, 100). The rapid induction of glycolysis is critical for the production of succinate and inflammatory cytokines in the immune response (99). These findings demonstrate another TBK1-mediated pathway that regulates the crosstalk between inflammation and metabolism. However, further studies are needed to compare the cell type specific roles of TBK1.

Inhibition of TBK1 and IKKϵ in Metabolic Diseases

Insights into the critical roles of the noncanonical IKKs in the pathogenesis of obesity and insulin resistance led to a screen of chemical inhibitors, identifying amlexanox as an inhibitor for both TBK1 and IKKϵ (37). Daily gavage of amlexanox in obese mice prevents genetic and high fat diet-induced obesity. The inhibition of weight gain by amlexanox is reversible after withdrawal of the drug. Amlexanox improved insulin sensitivity, reduced adipose tissue inflammation, increased energy expenditure, and attenuated hepatic steatosis in these obese animal models (37). Considering the phenotypes observed in *Ikke* knockout mice and adipose *Tbk1* knockout mice, the beneficial effects of amlexanox is likely the combined outcomes from the inhibition of both kinases. The inhibition of IKKϵ increases cAMP and catecholamine sensitivity to upregulate thermogenesis and attenuates adipose tissue inflammation (34). On the other hand, loss of TBK1 activity de-represses AMPK to increase mitochondrial biogenesis and other catabolic functions (63). The TBK1 deficiency-induced adipose tissue inflammation is likely compensated by the anti-inflammatory effects of IKKϵ inhibition.

In a proof-of-concept randomized, double-blinded clinical study, 42 obese and diabetic patients received placebo or amlexanox treatment for 12 weeks. Amlexanox significantly reduced hemoglobin A1c levels (101), indicating an improvement of glucose metabolism. Further study found that patients with higher serum C-reactive protein (CRP) levels and higher adipose tissue inflammation were more responsive to the drug. In the responder group, amlexanox improved insulin sensitivity and hepatic steatosis. The expression of thermogenic genes, including *Ucp1*, *Dio2* and *Fgf21*, was upregulated by the treatment as well in these patients. Within the responders, a transient increase of serum Interleukin 6 (IL-6) within 2–4 weeks of amlexanox treatment was reported (101). This observation is consistent with a previous mouse study showing that amlexanox upregulated *Il6* expression and secretion *via* cAMP/Mitogen-activated protein kinase (MAPK) p38 pathway in inguinal white adipose tissue. The increase of circulating IL-6 activates Signal transducer and activator of transcription 3 (STAT3) in the liver to inhibit the expression of the gluconeogenic gene Glucose-6-phosphatase (*G6pc*). As a result, amlexanox represses hepatic glucose output and thus improves glucose tolerance (102).

CONCLUDING REMARKS

Although the causal relationship between inflammation and obesity-associated metabolic disorders remains uncertain, there is little doubt that adipose tissue inflammation correlates well with the occurrence of insulin resistance and type 2 diabetes (16, 17, 19, 20). The crosstalk between inflammation and metabolism in adipose tissue plays a critical role in the pathogenesis of metabolic diseases. Overnutrition causes metabolic stress, which induces the initiation of inflammation to restore the metabolic homeostasis (9). The activation of proinflammatory signaling pathways attenuates insulin responsive signals to prevent further energy storage in adipocytes (103, 104). Both of these effects are the physiological/adaptive responses to overnutrition. However, along the progression of obesity, sustained inflammation causes a shift of homeostatic setpoints, leading to hyperglycemia, hyperinsulinemia, and reduced energy expenditure (9). At this stage, the inflammation causes a pathological/maladaptive response that further exaggerates obesity and obesity-associated metabolic disorders. Therefore, sustained inflammation results in a transition from an adaptive response to a maladaptive response that accelerates the progression of metabolic disorders.

NFκB signals mediate inflammatory responses and interact with metabolic pathways in adipose tissue (33, 105, 106). The activities of non-canonical IKKs, TBK1, and IKKϵ are induced during inflammation (34, 37, 63). TBK1 represses energy expenditure *via* inhibiting AMPK, while IKKϵ desensitizes sympathetic signals (34, 55, 63). The activation of these kinases exacerbates adiposity accumulation and promotes obesity. A recent study reported that escaped mitochondrial DNA activates TBK1 and IKKϵ to repress energy expenditure during metabolic stress (56). Amlexanox, a drug with outstanding safety record, was identified as an inhibitor of TBK1 and IKKϵ. Thus far, multiple studies on both experimental mouse models and human subjects suggest its potential as a new treatment for metabolic diseases (37, 101).

In addition to modulating metabolic pathways in adipocytes, metabolic and inflammatory signals interact at systemic level in other cell types. Metabolic stress has the potential to increase the production of adipokines, including leptin, adiponection, and others (28–31). It has been reported that leptin induces inflammation, while adiponectin attenuates inflammation

(107–110). Moreover, metabolic status could affect the functions of immune cells. Caloric restriction has exhibited systemic anti-inflammatory effects, along with attenuated terminal differentiation of immune cells (111). Given the energy sensing properties of AMPK, the AMPK–ULK1–TBK1 axis may also function in immune cells to mediate anti-inflammatory effects. Nonetheless, the precise roles of adipose tissue inflammation in the progression of obesity and obesity-associated insulin resistance remains unclear. Indeed, more efforts are needed to understand the systemic interactions between immune and metabolic responses, which are essential for the maintenance of homeostasis.

AUTHOR CONTRIBUTIONS

PZ prepared the manuscript. AS reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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The Adipocyte and Adaptive Immunity

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Not only do Adipocytes have energy storage and endocrine functions, but they also play an immunological role. Adipocytes are involved in adaptive immunity to mediate the pathological processes of a variety of chronic inflammatory diseases and autoimmune syndromes. The adaptive immune response consists of T cell-mediated cellular immunity and B cell-mediated humoral immunity. Obese adipocytes overexpress MHC class II molecules and costimulators to act as antigen-presenting cells (APCs) and promote the activation of $CD4^+$ T cells. In addition, various adipokines secreted by adipocytes regulate the proliferation and differentiation of T cells. Adipokines are also involved in B cell generation, development, activation, and antibody production. Therefore, adipocytes play an important role in B cell-mediated adaptive immunity. This review describes how adipocytes participate in adaptive immunity from the perspective of T cells and B cells, and discusses their role in the pathogenesis of various diseases.

Keywords: adipocyte, adaptive immunity, adipokine, T cell, B cell

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INTRODUCTION

Adaptive immunity is characterized by specificity, immunological memory, and self/nonself recognition (1). The function of the adaptive immune system is to recognize, remember and destroy invading pathogens through their antigens, and relieve pathogen-associated toxicities. There are two main mechanisms in the adaptive immune system—humoral immunity and cellular immunity, which are mediated by antibodies and cells respectively. The T and B cells are the major components of adaptive immunity. T cells play a large role in the cellular immune response, while B cells are intimately involved in the humoral immune response.

Adipocytes are the main constituent cells of adipose tissue. Their main function is to store energy in the form of lipid droplets when there is excess energy and to supply energy when the body demands it. In addition to their main functions, adipocytes have endocrine functions and can secrete a variety of adipokines such as leptin, adiponectin, and resistin (2–4). Recently, an increasing number of studies have shown that adipocytes have immunological functions capable of recruiting and activating immune cells. The adipocyte was reported as an antigen-presenting cell (APC) which expresses CD1d and MHC class I and II molecules. Several studies have shown that adipocytes highly express CD1d, which presents lipid antigens to invariant natural killer T (iNKT) cells and stimulates the activation of iNKT cells (5–7). Moreover, like other nucleated cells, adipocytes express MHC class I molecules. However, there is no clear evidence that adipocytes interact directly

with $CD8^+$ T cells through antigen:MHCI complex. In our recent research, we observed that adipocytes express MHC class II molecules and co-stimulatory molecules CD80/CD86, and that their expression significantly increases in response to high fat diet (HFD) challenges (8). Adipocytes can directly activate $CD4^+$ T cells through antigen:MHCII complex in a contact-dependent manner. Simultaneously, adipocytes secrete various cytokines including leptin, resistin, TNF-α and IL-6 to regulate the differentiation and function of T and B lymphocytes.

Adipocytes can regulate adaptive immunity, which is involved with various metabolic diseases. Since there have been many reports on the regulation of metabolic diseases through adaptive immunity (9–11), we focus on how adipocytes regulate adaptive immunity in this review. First, we introduce adipocytes as APCs to participate in T cell-mediated adaptive immune response. Next, we summarize various cytokines produced by adipocytes that regulate the survival, activation and differentiation of B cells. Adaptive immunity mediates the pathological processes of a variety of chronic inflammatory diseases, autoimmune syndromes and cancers. Thus, we discuss the role of adipocytes in adaptive immunity in the context of inflammatory and autoimmune diseases.

THE ROLE OF ADIPOCYTES IN T CELL-MEDIATED ADAPTIVE IMMUNITY

The activation and differentiation of T cells require three signals: antigen presentation, costimulation, and cytokine stimulation. APCs are required for T cell activation. They can process and present antigens to T cells in the form of antigen peptide:MHC molecular complexes, which are recognized by TCR on T cells to provide the first signal for T cell activation. Moreover, APCs highly express co-stimulatory molecules and pair with the corresponding receptor or ligand molecules on the surface of T cells, constituting the second signal for T cell activation. After T cells are fully activated, the further proliferation and differentiation of T cells depends on a variety of cytokines, including IL-2, IL-4, IL-6, IL-10, IL-12, and IFN-γ. In this section, we will describe how adipocytes act as APCs to provide all three signals for T cells activation and differentiation.

Adipocyte-Mediated Antigen Presentation

Adipocytes express both MHC classes I and II molecules. MHCI molecules are expressed in all nucleated cells and mediate $CD8^+$ T cell activation, while MHCII molecules are restricted to antigen-presenting cells (APCs) and induce $CD4^+$ T cell activation by antigen presentation. APCs are divided into professional APCs and non-professional APCs. The former includes dendritic cells (DC), monocytes/macrophages, and B lymphocytes, and the latter comprises endothelial cells, epithelial cells and fibroblasts (12). In our previous studies, we found that adipocytes also express MHCII molecules, and that their levels are significantly increased in adipocytes of HFD fed mice (8). In contrast, MHCI-related genes in adipocytes remain unchanged during obesity.

Adipocyte MHCII begins to increase at 2 weeks of HFD, and the expression of pro-inflammatory Th1 marker genes Tbx21 and Ifng in adipose tissue resident T cells (ART) increase at 2–3 weeks following HFD, suggesting that adipocyte MHCII may mediate Th1 cell activation and trigger obesity-induced adipose inflammation. *In vitro* adipocyte-T cell co-culture experiments show that the activation of T cells by adipocytes is dependent on direct contact between adipocytes and T cells and the MHCII expression in adipocytes (8). Large adipocytes (diameter >25 μm) express higher levels of MHCII than small adipocytes (diameter <25 μm) in both ND (normal diet)- and HFD-fed mice. In obesity, large adipocytes are accumulated in adipose tissues and they overexpress MHCII molecules. These hypertrophic adipocytes can function as APCs to activate $CD4^+$ ART and instigate adipose tissue inflammation, which could cause many obesity-related medical complications (13). Adipocyte-specific MHCII deficient ($aMHCII^{-/-}$) mice are significantly more sensitive to insulin and glucose tolerant than their wild type (WT) littermates when fed with HFD (14). In addition, adipocytes of HFD-fed $aMHCII^{-/-}$ mice exhibit reduced capacity to activate $CD4^+$ T cells, as manifested by attenuated secretion of IFN-γ, a major Th1 cytokine (14). Furthermore, adipocyte MHCII has an indirect effect on Tregs in visceral adipose tissue (VAT). $aMHCII^{-/-}$ mice show increased Treg abundance in VAT, compared with WT mice under HFD. *In vitro* experiments show that IFN-γ dose-dependently inhibits Treg differentiation (14). Thus, in the HFD-fed $aMHCII^{-/-}$ mouse model, the drop of IFN-γ may explain the increase of Tregs in VAT. Given that VAT Treg is a negative regulator of adipose inflammation and insulin resistance (15–17), the improved adipose inflammation and insulin resistance in HFD-fed $aMHCII^{-/-}$ mice may result from the increase of Tregs in VAT. Indeed, the preserved insulin sensitivity of HFD-fed $aMHCII^{-/-}$ mice is attenuated by ablation of Tregs in adipose tissue (14). These results indicate that adipocyte MHCII can promote adipose inflammation and insulin resistance. Consistently, adrenomedullin 2 improves adipose insulin resistance by inhibiting the adipocyte MHCII expression in the early stage of obesity (18). HFD-fed adipocyte HIF-1α KO mice show decreased expression of MHCII genes, and can protect themselves from obesity-induced adipose inflammation (19). In summary, the adipocyte can function as APCs to induce $CD4^+$ T cell activation and polarization in MHCII and antigen dependent pathway.

Current research on adipocyte MHCII antigen presentation and co-stimulation focuses on obesity and type 2 diabetes (T2D). Therefore, the metabolic diseases we have discussed in this review are obesity and T2D. Since adipocyte-mediated antigen presentation promotes adipose inflammation, which is strongly associated with a variety of metabolic diseases, including nonalcoholic fatty liver disease (NAFLD), atherosclerosis, heart disease, etc., adipocyte-mediated antigen presentation may contribute to these metabolic diseases indirectly.

Co-Stimulatory Molecule in Adipocyte

TCR recognition of antigen peptide/MHCII provides the primary signal for $CD4^+$ T cell activation, while the full

activation of $CD4^+$ T cells requires the costimulation signal. Costimulatory molecules on the surface of T cells and APCs bind to each other in a receptor–ligand pairing manner. Costimulatory molecules expressed by T cells interacts with its ligands or receptors on the membrane of APCs, resulting in the activation of these cells and thus triggering immune response (20).

Recent studies have reported the role of T cell costimulators in HFD-induced obesity (21), but the contribution of adipocytes in T cell costimulation is still unclear. CD40 (22), CD80 (B7-1), CD86 (B7-2) (8, 23) and HVEM (24, 25) are induced in adipocytes of obese human or mice, and may costimulate adipose resident T cells (ARTs) in obesity. However, studies show that both CD40 knockout mice and CD80/CD86 double knockout mice under HFD feeding exhibit exacerbated adipose tissue inflammation and metabolic disorders. To understand these unexpected results, investigators explored the involvement of other factors that can also influence the phenotype of these mice. After binding with CD40L, CD40 triggers the recruitment of adaptor proteins, the TNFR-associated factors (TRAFs), to activate intracellular signaling (26). The cytoplasmic region of CD40 contains a proximal binding site for TRAF6 and a distal binding site for TRAF2/3/5. Mice that are deficient in CD40-TRAF2/3/5 signaling in $MHCII^+$ cells display a similar phenotype as $CD40^{-/-}$ mice under HFD, whereas mice with disrupted CD40-TRAF6 signaling in $MHCII^+$ cells are protected against obesity-induced metabolic dysfunction (27). CD40-TRAF2/3/5 and CD40-TRAF6 signaling have opposite effects in obesity-related metabolic disorders. This may explain the unexpected phenotype of $CD40^{-/-}$ mice. In addition, CD80/CD86 double knockout mice have congenital defects in the development of Tregs, which may explain the aggravated adipose inflammation in these mice. Indeed, using antibodies to block both CD80 and CD86 can alleviate adipose inflammation, insulin resistance and fatty liver of diet-induced obese mice (23, 28). Another costimulatory receptor–ligand pair, HVEM-LIGHT, is also involved in the ART activation of DIO mice. LIGHT is expressed in both activated and resting T cells in mice (29). LIGHT binds to HVEM on adipocyte, and promotes the secretion of pro-inflammatory cytokines and chemokines in adipocytes by activating the NF-kB signaling pathway in human and mice (30, 31), thereby inducing the recruitment of T cells and macrophages in adipose tissue. Both HVEM genetical deletion and treatment of HVEM blocking antibodies in HFD-fed mice ameliorates obesity-induced adipose tissue inflammation and metabolic deterioration (24, 32).

These studies have suggested that T cell costimulatory molecules may be involved in the obesity-induced activation of ART and the development of adipose tissue inflammation. However, it is still uncertain whether adipocytes provide the T cell costimulatory signal to activate ARTs during obesity, because no studies have used adipocyte-specific costimulator knockout mice to confirm the function of adipocytes in T cell costimulation. Moreover, several costimulatory molecules have been linked to obesity-induced adipose inflammation and insulin resistance, but it is still unclear which costimulator plays the central role. Further studies are warranted to address these unanswered questions.

Adipokines That Regulate Activation and Polarization of T Cell

A variety of cytokines secreted by adipocytes can regulate the activation and differentiation of T cells and B cells, and participate in various metabolic and non-metabolic diseases. Since the topic of how adipokines contribute to metabolic diseases has been extensively described in many reviews (33–36), in this review, we focus on non-metabolic diseases.

Leptin

Leptin is basically a pro-inflammatory adipokine that directly or indirectly regulates T cells proliferation and differentiation (**Table 1**). As early as 1998, Lord et al. found that leptin promotes the proliferation of naïve and memory T cells and increases the secretion of Th1 cytokines, but suppresses the production of Th2 cytokines (37). Subsequently, it has been reported that leptin

TABLE 1 | The effects of adipokines on T lymphocytes.

Adipokines	Naïve $CD4^+$ T	Th1	Th2	Th17	Treg	Tfh	$CD8^+$ T
Leptin	Proliferation↑	Differentiation↑ Cytokines secretion ↑	Differentiation↓ Cytokines secretion ↓	Differentiation↑ Cytokines secretion ↑	Differentiation↓	Differentiation↑ Cytokines secretion ↑	Activation↑
Adiponectin	Proliferation↓ Apoptosis↑	Differentiation↑↓ Cytokines secretion ↑↓	Differentiation↑ Cytokines secretion ↑	Differentiation↑↓ Cytokines secretion ↑↓	Differentiation↑	Activation↑	Development↑
IL-6	Proliferation↑ Apoptosis↓	Differentiation↓ Cytokines secretion ↓	Differentiation↑ Cytokines secretion ↑	Differentiation↑ Cytokines secretion ↑	Differentiation↓	Differentiation↑	Differentiation↑ Activation↑
TNF-α	Proliferation↑	Differentiation↑ Cytokines secretion ↑ Migration↑	Differentiation↓ Cytokines secretion ↓	Differentiation↑ Cytokines secretion ↑ Migration↑	Differentiation↓	—	Activation↑ Proliferation↑ Migration↑
Resistin	Migration↑	—	—	—	Differentiation↑	—	—
Visfatin	Activation↑	—	—	—	—	—	—

↑ *Indicates upregulation,* ↓ *indicates downregulation,* ↑↓ *indicates both upregulation and downregulation,* — *indicates unknown.*

constrains the activation and proliferation of Treg cells (38). Mechanism studies have shown that leptin activates the mTOR pathway, thereby exerting a positive effect on $CD4^+$ $CD25^-$ $FOXP3^-$ effector T cells (Teffs), but inhibiting Foxp3 expression and the proliferation of Treg cells (39, 40). Leptin also promotes Th17 responses by inducing the transcription of retinoid-related orphan receptor γt (RORγt), the key transcription factor for Th17 differentiation (41). In addition, leptin has positive effects on the generation, maturation and survival of thymic T cells by reducing their apoptosis (42). Furthermore, leptin increases the secretion of inflammatory cytokines (e.g. IL-6, IL-12 and TNF-α) as well as the expression of chemokine ligands (e.g. CCL3, CCL4 and CCL5) by activating the JAK2–STAT3 pathway in monocytes/macrophages from human or mice (43, 44), thereby indirectly promoting differentiation and adaptive immune response of T cells.

Due to its strong effects on T cells, leptin participates in the pathological processes of a variety of inflammatory and autoimmune diseases. In obesity-induced adipose inflammation, leptin stimulates IFN-γ secretion from ART, which leads to an increase in pro-inflammatory Th1 cells and a decrease in anti-inflammatory Tregs in adipose tissue (8). Leptin gene expression in adipocytes is elevated within 1 week of HFD, suggesting that leptin plays a role in initiating the cascade of adipose inflammation. Moreover, because leptin can promote the proliferation of autoreactive T cells and differentiation of pro-inflammatory Th1 and Th17 cells in human and mice, it has been reported to be involved in the induction and progression of IBD (45, 46), multiple sclerosis (47–49), rheumatoid arthritis (50, 51) and systemic lupus erythematosus (41, 52)

Adiponectin

Adiponectin has dual effects on T cell function. Several studies have shown that adiponectin is a negative regulator of T cell activity. It has been reported that adiponectin inhibits the proliferation and cytokine production of T cells, and promotes their apoptosis (53). Recent data indicates that adiponectin inhibits Th1 and Th17 differentiation through the upregulation of SIRT1 and PPARγ and inhibition of RORγt (54). It also suppresses IL-17 production from γδ-T cells (55). Therefore, adiponectin ameliorates Th17 cell-mediated autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) (54) and psoriasiform skin inflammation (55). In a mouse model of abortion, adiponectin increases Treg cell population *via* enhancing Foxp3 expression, thereby improving the pregnancy rate of this model (56). Furthermore, the immunomodulatory effect of adiponectin on T cells is partially mediated by its ability to suppress the allostimulatory capacity of dendritic cells (DCs) (57). Adiponectin suppresses the expression of MHCII and co-stimulators CD80 and CD86, and induces the expression of co-inhibitor PD-L1 in DCs. Adiponectin-treated DCs show a reduced capacity to promote $CD4^+$ T cell proliferation and an enhanced capacity to induce Treg expansion in DC-T cell cocultures (57).

However, some studies showed opposite results that adiponectin is a pro-inflammatory adipokine. In human polyclonally activated $CD4^+$ T cells, adiponectin treatment results in the increased secretion of IFN-γ and IL-6, phosphorylation of p38 MAPK and STAT4 and expression of T-bet, which indicates a potential function of adiponectin promoting Th1 differentiation (58). Moreover, adiponectin aggravates collagen-induced arthritis (CIA) *via* enhancing Th17 cells and T follicular helper (Tfh) cells response (59). Adiponectin also reduces the apoptosis of lamina propria T lymphocytes (LPL-T) in IBD patients by inducing expression of anti-apoptotic proteins Bcl-xL and Bcl-2, leading to T cell-mediated inflammation (46). Adiponectin also indirectly promotes Th1 and Th17 polarization by activating DCs through PLCγ/JNK/NF-κB signaling pathway (60).

The reason for the discrepancy in effects of adiponectin on T cells is unclear. Adiponectin circulating in plasma has three major forms: trimer, hexamer, and high molecular weight (HMW) multimer (61). Different oligomers activate different intracellular signaling pathways, resulting in significantly different effects (62). It is possible that different oligomers of adiponectin were used in different studies, which results in this discrepancy.

IL-6

IL-6 is a pro-inflammatory cytokine that is secreted by various immune cells. Adipocytes also express IL-6. Although adipocytes are not the main source of IL-6 in adipose tissue, IL-6 has been considered as an adipokine (63). IL-6 has different effects on different $CD4^+$ T cell subsets. It was reported that IL-6 inhibits Th1 differentiation by upregulating the expression of a suppressor of cytokine signaling,(SOCS)-1, a potent inhibitor of IFN-γ signaling (64). IL-6 also inhibits TGF-β-induced Treg cell's differentiation (65, 66). However, IL-6 induces the production of IL-4, resulting in increased Th2 polarization (67). In addition, IL-6 is a crucial cytokine for lineage commitment to Th17 cells. IL-6 promotes Th17 differentiation by activating STAT3, which upregulates the expression of RORγt and RORα (68, 69). Furthermore, IL-6 is a positive regulator of Tfh cells (70), which is supported by the observation that the early differentiation of Tfh cells is severely impaired in IL-6 deficient mice (71).

Although the regulatory effect of IL-6 on $CD4^+$ T cells has been extensively studied, the effect of IL-6 on $CD8^+$ T cells is still poorly understood. IL-6 may positively regulate $CD8^+$ T cell function. IL-6 was found to promote the generation of $CD8^+$ cytotoxic T cells (72). It was reported that IL-6 induces the differentiation of naïve $CD8^+$ T cells into IL-21-producing $CD8^+$ T cells, which improve IgG isotype switching in B cells during influenza virus infection (73). This is a new function for IL-6 in the prevention of viral infection. Furthermore, IL-6 promotes the differentiation of IL-22-producing $CD8^+$ T cells, a $CD8^+$ T cell subset with antitumor function (74).

Other Adipokines

In addition to the adipokines mentioned above, some other secreting factors of adipocytes, including resistin, visfatin, and TNF-α, also regulate T cell function. Resistin induces activation of Src and PI3K in human $CD4^+$ T lymphocytes and serves as a

chemokine for these cells (75). Moreover, resistin indirectly enhances Treg expansion through the regulation of DCs, in which interferon regulatory factor (IRF)-1 pathway is suppressed by resistin (76). Visfatin is an adipokine that upregulates the activation of T cells. It promotes the production of IL-1β, IL-1Ra, IL-6, IL-10, and TNF-α and the expression of costimulatory molecules CD80, CD40 and ICAM-1 (CD54) in monocytes, thereby stimulating the activation of T cells (77). It is worthy to note that although visfatin is expressed in adipose tissue, its expression is higher in bone marrow, the liver and muscles (78). Additionally, in the adipose tissue, visfatin is not only expressed in adipocytes. Studies have found that visfatin is mainly produced and released by macrophages in white adipose tissues (79). Therefore, adipocytes may not be the major source of visfatin expression. TNF-α is an important immunomodulatory cytokine, which plays a critical role in regulating the proliferation, differentiation, and apoptosis of T cells, the generation of memory T cells, and maintenance of immune tolerance (80). It has been reported that TNF-α is secreted by adipocytes and other immune cells (81, 82). However, whether adipocytes produce TNF-α is still controversial.

THE ROLE OF ADIPOCYTES IN B CELL-MEDIATED ADAPTIVE IMMUNITY

Similar to T cell activation and differentiation, B cell activation and differentiation also requires three signals. But unlike T cells that recognize antigens presented by APCs, B cells recognize free antigens through B cell receptor (BCR). B cells specifically recognize antigens through BCR, generating the first signal for B cell activation. B cells are per se professional APCs. B cells internalize the antigen bound by BCR and process the antigen to form an antigen peptide–MHCII complex, which is presented to antigen-specific Th cells. After Th cells are activated, they express high levels of co-stimulatory molecules and combine with matched ligands or receptors on the surface of B cells, which provides the second signal for B cell activation. Activated B cells express multiple cytokine receptors, and proliferate and differentiate into antibody-forming cells under the action of cytokines that are secreted by activated T cells or other cells. For B cell activation, the role that adipocyte plays on the two key signals, antigen recognition and costimulation has not yet been reported, but some studies have reported that several adipokines play a role in the development and differentiation of B cells. Here we discuss the role that adipocytes play in regulating B cell-mediated adaptive immune responses through secreted cytokines (**Table 2**).

Leptin

In addition to regulating T lymphocytes mediated immune responses, leptin plays an important role in the regulation of B cell development and function. Deficiency of leptin signaling in ob/ob and db/db mice leads to the decrease of B cells in bone marrow and peripheral blood, while intraperitoneal injection of leptin in ob/ob mice restores the number of bone marrow B cells (83), suggesting that leptin plays a critical role in supporting B cell development. Fasted mice, characterized by low serum leptin levels, show decreased pro-B and immature B cells and increased mature B cells in bone marrow (84). Leptin receptor is expressed on B cells, suggesting a direct effect of leptin on B cells (85). However, the fasting-induced atrophy of bone marrow B cells is reversed by intracerebroventricular leptin injection, indicating that leptin may indirectly regulate B cell development through the central nervous system (86). In addition to its effects on the regulation of B cell development, leptin suppresses apoptosis and induces cell cycle entry of B cells by upregulating the expression of Bcl-2 and Cyclin D1 (87). Moreover, leptin stimulates human B cells to secrete proinflammatory (TNF-α and IL-6) and anti-inflammatory (IL-10) cytokines, *via* activation of JAK2/STAT3 and p38MAPK/ERK1/2 signaling pathway (88). Interestingly, leptin-induced production of TNF-α, IL-6 and IL-10 in B cells from aged individuals are significantly higher than that in B cells from young individuals (89). Furthermore, leptin promotes immunosenescence of human B cells. Leptin treatment results in declined immunoglobulin class switch and influenza vaccine-specific IgG production in human B cells (90).

Adiponectin

Adiponectin has two receptors, ADIPOR1 and ADIPOR2. Both are abundantly expressed on the surface of circulating B cells (91).

TABLE 2 | The effects of adipokines on B lymphocytes.

Adipokines	Pro-B	Pre-B	Immature B	Mature B	Plasma B
Leptin	Development↑	Development↑	Development↑	Development↓ Cytokines secretion↑	Antibody production↑
Adiponectin	Development↓	Development↓	—	Cytokines secretion↑	—
IL-6	—	—	—	Differentiation↑ Proliferation↑	Antibody production↑
Visfatin	—	Colony formation↑	—	Activation↑ Migration↑	—
BAFF	—	—	—	Survival↑ Maturation↑ Proliferation↑	Antibody production↑
Other soluble factors	Development↓	Development↓	—	—	—

↑ *Indicates upregulation,* ↓ *indicates downregulation,* — *indicates unknown.*

However, the immunomodulatory effects of adiponectin on B lymphocytes are not very clear. It has been reported that adiponectin inhibits B lymphopoiesis in long-term bone marrow cultures. This effect is highly dependent on the presence of both stromal cells and early B lineage precursors in the cultures (92). Adiponectin deficient mice treated with dextran sulfate sodium (DSS) present more significant B cells infiltration in colons and appear more severe colitis than WT littermates, indicating that adiponectin may suppress B cell-mediated inflammatory response in DSS-induced colitis (93). Moreover, adiponectin stimulates B cells to secret a peptide, PEPITEM, which specifically inhibits the migration of $CD4^+$ and $CD8^+$ memory T cells (94). Further studies are guaranteed to address the detailed role of adiponectin in regulating B lymphocytes function.

Other Adipokines

Leptin and adiponectin are exclusively expressed in adipocyte. Some other adipokines that are secreted by both adipocytes and other types of cells also have regulatory effects on B cells. These adipokines include visfatin, B cell activation factor (BAFF), and IL-6. Visfatin was previously called 'pre-B cell colony-enhancing factor (PBEF)', since it enhances pre-B-cell colony formation in the presence of both IL-7 and SCF (78). Visfatin is a potent chemotactic factor for B cells and promotes B cell migration *in vitro* cell culture (77). BAFF, also known as 'B lymphocyte stimulator (BlyS)', promotes B cell proliferation, survival, maturation and immunoglobulin secretion (95, 96). The production of BAFF is upregulated in obese human adipocyte, and it may activate B cells in adipose tissue during obesity (97). IL-6 was originally named 'B-cell stimulatory factor 2 (BSF-2)'. This name reflects its function to induce differentiation of activated B cells into antibody (Ab)-producing cells (98). IL-6 is abundantly secreted by adipocytes during obesity, and aggravates obesity-induced insulin resistance (99). In addition, some unidentified soluble factors secreted by adipocytes inhibit B lymphopoiesis (100, 101). These factors may mediate the decline of B lymphopoiesis in aged and obese individuals, and both conditions are characterized by increased fat accumulation in bone marrow.

CONCLUSION AND FUTURE DIRECTIONS

Recently, the immunological function of adipocytes has received increasing attention. Mounting evidence indicates that adipocytes play an important role in adaptive immunity (**Figure 1**). Adipocytes can serve as APCs to regulate T cell-mediated adaptive immunity. The MHCII molecules are expressed in adipocytes and their expressions are upregulated during obesity, providing the first signal for $CD4^+$ T cell activation. Simultaneously, adipocytes of obese mice and humans overexpress several costimulatory molecules, including CD40, CD80 (B7-1), CD86 (B7-2) and HVEM. Those constimulators are associated with obesity-induced adipose inflammation and metabolic disorders. However, studies exhibited conflicting results and did not provide convincing data from adipocyte-specific knockout mouse models. Therefore, it is too early to draw a conclusion that adipocytes provide the key costimulatory signal for ART activation. In addition, adipocytes secrete various cytokines, such as leptin, adiponectin, IL-6, resistin, visfatin and TNF-α, which regulate the proliferation and differentiation of T cells and are involved in many chronic inflammatory and autoimmune diseases. In B cell-mediated humoral immunity, adipocytes regulate B cell development, proliferation, differentiation, activation and antibody production through secreted adipokines.

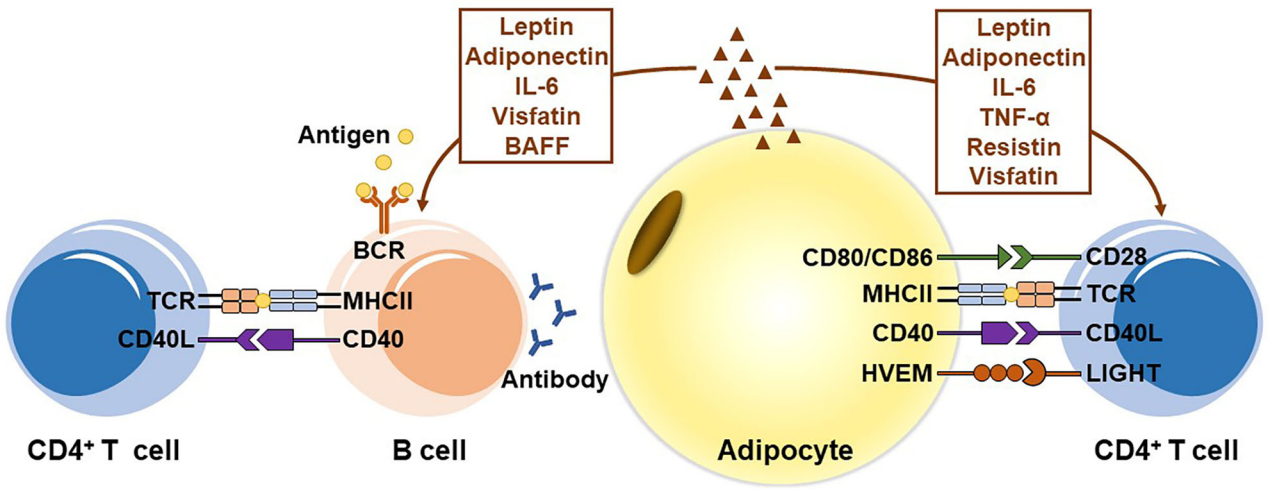

FIGURE 1 | The role of adipocytes in adaptive immunity. Adipocytes express MHC class II molecules and several T cell costimulators to act as antigen-presenting cells (APCs), and induce the activation of $CD4^+$ T cells in visceral adipose tissue during obesity. In addition, adipocytes secrete various adipokines, including leptin, adiponectin, IL-6, TNF-α, resistin, and visfatin, to regulate the proliferation and differentiation of T cells. In B cell-mediated humoral immunity, adipocytes modulate B cell generation, development, aging, activation and antibody production mainly by secreting adipokines, including leptin, adiponectin, IL-6, visfatin, and BAFF.

In the past few years, although great progress has been made in understanding the mechanism and function of adipocytes in adaptive immunity, there are still many imperative questions remaining to be answered in this emerging field. Many studies have implied the existence of specific antigens to activate T cells in adipose tissue, but up until now, no any adipose antigen has been reported. In addition, although many T cell costimulators have been linked to obesity-induced adipose inflammation and insulin resistance, the key co-stimulator(s) in obesity-induced ART activation are not known. Identifying of antigen(s) which are recognized by ART in obesity and the key co-stimulatory signaling in ART activation may provide new targets for specifically block obesity-induced adipose inflammation. We found that obesity induces MHCII expression in adipocytes and causes adipocytes to become APCs. But it is still unclear whether all adipocytes or just a subset of adipocytes are converted to APC in obesity. If the latter is true, further studies are warranted to investigate the origin and features of this special adipocyte subpopulation. Finally, compared with the number of studies which concern adipocytes regulating the function of T cells, there are far fewer studies on adipocytes regulating the function of B cells. Except for adipokines, we know little about how adipocytes regulate the B cell-mediated adaptive immune response. Future studies on the mechanisms by which adipocytes regulate B cell function will help us better understand the physiological and pathological functions of adipocytes in B cell-mediated humoral immunity.

AUTHOR CONTRIBUTIONS

JS and TD wrote and revised the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Adipocytes Are the Control Tower That Manages Adipose Tissue Immunity by Regulating Lipid Metabolism

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Accumulating evidence reveals that adipose tissue is an immunologically active organ that exerts multiple impacts on the regulation of systemic energy metabolism. Adipose tissue immunity is modulated by the interactions between adipocytes and various immune cells. Nevertheless, the underlying mechanisms that control inter-cellular interactions between adipocytes and immune cells in adipose tissue have not been thoroughly elucidated. Recently, it has been demonstrated that adipocytes utilize lipid metabolites as a key mediator to initiate and mediate diverse adipose tissue immune responses. Adipocytes present lipid antigens and secrete lipid metabolites to determine adipose immune tones. In addition, the interactions between adipocytes and adipose immune cells are engaged in the control of adipocyte fate and functions upon metabolic stimuli. In this review, we discuss an integrated view of how adipocytes communicate with adipose immune cells using lipid metabolites. Also, we briefly discuss the newly discovered roles of adipose stem cells in the regulation of adipose tissue immunity.

Keywords: adipocytes, lipid metabolite, invariant natural killer cell, adipose tissue remodeling, adipose tissue inflammation

Abbreviation: α-GC, Alpha-galactosylceramide; APC, Antigen presenting cell; ASC, Adipose stem cell; ATM, Adipose tissue macrophage; CD1dAKO, Adipocyte-specific CD1d depletion; ChREBP, Carbohydrate response element binding protein; ER, Endoplasmic reticulum; FALC, Fat-associated lymphoid cluster; FFA, Free fatty acid; GABA, Gamma-aminobutyric acid; HFD, High-fat diet; HIV, Human immunodeficiency virus; IFN, Interferon; IKKβ, IkB kinase; IL, Interleukin; ILC, Innate lymphoid cell; iNKT, Invariant natural killer T; KD, Ketogenic diet; KO, Knock out; LD, Lipid droplet; MAOA, Monoamine oxidase; NLRP3, NLR family pyrin domain containing 3; NO, Nitric oxide; PAHSA, Palmitic acid esters of hydroxy stearic acid; PGE2, Prostaglandin E2; Plin, Perilipin; scRNA-seq, Single cell RNA-sequencing; SREBP1c, Sterol regulatory element-binding protein 1c; SVC, Stromal vascular cell; sWAT, Subcutaneous white adipose tissue; TCR, T cell receptor; TNF, Tumor necrosis factor; Treg, Regulatory T cell; VLDLR, Very-low-density-lipoprotein receptor; vWAT, Visceral white adipose tissue; WAT, White adipose tissue.

INTRODUCTION

Adipose tissue is a specific type of loose connective tissues present in various anatomical locations. For energy homeostasis and survival, adipose tissue contributes to numerous physiological roles: it provides structural support and protective padding for major organs, it serves as an insulating layer that prevents cutaneous heat loss, it stores extra energy source for longer periods of fasting, and it is a dynamic endocrine system crucial in the regulation of energy homeostasis (1). Among the various cell types residing in adipose tissue, adipocytes are the major cell type that is specialized to synthesize and store large globules of fat (2). When energy level is low, adipocytes break down stored lipid metabolites into fatty acids and glycerol and release them into circulation, which are used for fuels in most organs. This function of adipocytes enables adipose tissue to function as the major energy reservoir. Moreover, adipocytes act as a key component of endocrine activity through secreting a variety of signaling molecules such as adipokines, lipokines, and exosomes (3). These adipocyte-derived factors are involved in the maintenance of systemic energy homeostasis through crosstalk with other tissues such as muscle, liver, and brain (2).

Adipose tissue harbors diverse innate and adaptive immune cells. Dynamic interactions between these innate and adaptive immune cells are closely associated with alterations of adipose tissue function and integrity upon metabolic changes (4–6). For example, adipose tissue immunity shifts toward pro-inflammatory state in response to chronic energy surplus such as obesity, leading to dysregulation of adipose tissue homeostasis (7–10). Among various adipose immune cells, adipose tissue macrophages (ATMs) occupy about 50% and are largely classified into pro-inflammatory M1-type and anti-inflammatory M2-macrophages (11, 12). In obesity, M1-type macrophages are abundantly accumulated and secrete pro-inflammatory molecules such as tumor necrosis factor (TNF)-α, nitric oxide (NO), and interleukin (IL)-6 (13–15). In addition, neutrophil, Th1, Th17, CD8 T cells, and group 1 innate lymphoid cell (ILC1) secrete pro-inflammatory cytokines including interferon (IFN)-γ, IL-6, and IL-17 (16, 17). These pro-inflammatory molecules suppress insulin action in adipocytes by inhibiting phosphorylation of insulin receptor and insulin receptor substrate 1, which provokes insulin resistance. On the other hand, there are numerous anti-inflammatory immune cells that downregulate pro-inflammatory responses, improving insulin sensitivity in adipose tissue. Eosinophil, regulatory T cell (Treg), invariant natural killer T (iNKT), and group 2 innate lymphoid cell (ILC2) stimulate to polarize macrophages towards anti-inflammatory M2-type macrophages through secretion of Th2 type cytokines, including IL-4, IL-5, IL-10, and IL-13, attenuating adipose inflammatory responses and improving insulin sensitivity (11).

Recently, emerging evidence indicates that adipocyte-derived lipid metabolites would function as a crucial regulator of adipose tissue immunity (18–21). In obese adipocytes, aberrant lipid metabolism promotes lipid spillover, which activates NF-κB pathways in ATMs and consequently induces TNF-α secretion (22). Also, dysregulation of lipokines and lipid antigens is manifested in dysfunctional adipocytes, which has been linked to changes in characteristics of adaptive immune cells in adipose tissue. It has been recently shown that adipocyte-derived lipid antigens could alter inter-cellular interactions between innate and adaptive immune cells, followed by alterations of function and fate of adipocytes (23). Despite the close association of lipid metabolism in adipocytes with adipose tissue immunity has been reported for over a decade, the molecular mediators and mechanisms linking adipocyte-derived lipid metabolites to adipose tissue immunity remain poorly understood. In previous reviews, the importance of the crosstalk between innate and adaptive immune cells in adipose tissue on energy metabolism has been well addressed (1, 11, 12). Thus, in this review, we cover the processes by which adipocytes communicate with adipose immune cells using lipid metabolites. Furthermore, we discuss the new concept that adipocytes cooperate with adipose immune cells to protect adipose tissue integrity from metabolic stresses. In addition, we briefly propose the novel roles of adipocyte stem cells in the regulation of adipose tissue immunity.

IMMUNOMODULATORY ROLES OF ADIPOCYTES USING LIPID ANTIGENS

There are distinct types of immune cells that recognize lipid antigens. These immune cells, such as iNKT cells and γδ T cells, rapidly respond to changes of lipid metabolism through sensing lipid antigens loaded on antigen presenting cells (APCs). It has been reported that iNKT cells and γδ T cells are abundantly present in adipose tissue and actively interact with adipocytes, contributing to the regulation of systemic energy metabolism (24–27). For example, in obesity, adipose iNKT cells are activated by adipocyte-derived lipid antigens and modulate the interaction between innate and adaptive immune cells (24, 28, 29). Moreover, activation of iNKT cells by hypertrophic adipocyte-derived lipid antigens stimulates adipocyte turnover in obesity, contributing to adipose tissue remodeling (23). Similarly, γδ T cells regulate adipose tissue immune responses and adipocyte functions (26, 27, 30). Given that γδ T cells recognize CD1-loaded lipid antigens, it has been suggested that adipocytes would control γδ T cell activity (31, 32). In this section, we discuss detailed mechanisms by which adipocytes regulate adipose tissue immune cells *via* lipid antigen presentation.

Lipid Antigen Presentation

In adipose tissue, there are several APCs such as dendritic cells, macrophages, B cells, and adipocytes (24, 25, 33). It has been demonstrated that adipocytes highly express MHC-I like protein, CD1d, and present lipid antigens (24, 34). CD1d belongs to the CD1 family with isoforms such as CD1a, CD1b, CD1c, and CD1e (35). CD1d is a transmembrane protein with two alpha-helices forming an antigen-presenting pocket above and a hydrophobic pocket below (28). This structure encapsulates hydrophobic portion of lipid antigens into the CD1d binding groove, and

the polar portion of the antigen is exposed outside APCs to be recognized by T cell receptor (TCR) (28).

With an antigen-presenting molecule CD1d, adipocytes express high levels of lipid antigen loading and presentation-associated genes (28). There are two major pathways involved in antigen loading and presentation. The first one is endoplasmic reticulum (ER) and Golgi pathway, and the second one is endosomal and lysosomal pathway. In ER and Golgi pathway, the newly synthesized CD1d binds to β2-microglobulin in ER, and lipid antigens are loaded onto CD1d in Golgi by chaperone proteins, including microsomal triglyceride transfer protein (36, 37). Then, CD1d enters the transport step and fuses with the membrane to be exposed to cell surface of APCs. In endosomal and lysosomal pathway, CD1d is internalized in the form of endosome from plasma membrane. Chaperone protein and lipid transport protein replace low-affinity lipid antigens with high affinity lipid antigens (36, 37).

Although the clue for lipid antigen source has been suggested in several studies (38–41), the identity of endogenous lipid antigens in adipocytes has not been clearly elucidated. In the blood, circulating lipid metabolites are potentially subjected to behave as lipid antigens through scavenger receptor and very-low-density-lipoprotein receptor (VLDLR) (42). In VLDL-associated apoprotein APOE-deficient mice, the number of iNKT cells is altered (40). Also, fatty acid amide hydrolase enhances the presentation of lipid antigens by facilitating transport of serum lipids into APCs (41).

Anti-Inflammatory Roles of Adipocytes *via* Lipid Antigen Presentation

The roles of CD1d in adipocytes have been investigated in genetically or diet-induced obesity models. Studies using adipocyte-specific CD1d knockout ($CD1d^{AKO}$) mice have shown that adipocytes are crucial for the regulation of adipose iNKT cell activity (**Figure 1A**) (34, 43). In $CD1d^{AKO}$ mice, the number of iNKT cells is decreased. Moreover, the levels of IL-4 secretion and FasL expression are downregulated in iNKT cells of $CD1d^{AKO}$ mice compared to wild type (WT) mice, leading to aggravation in adipose tissue inflammation and insulin resistance (23, 34). The interaction between adipocytes and iNKT cells has been also examined in Jα18 knockout (KO) mice and CD1d KO mice in which iNKT cells are deficient in whole body (24, 25). In the case of the above animal models lacking iNKT cells, body weight gain and adipocyte size are increased, and pro-inflammatory ATMs are more accumulated in obesity. Stimulation of iNKT cell activity by alpha-galactosylceramide (α-GC), a synthetic lipid antigen for iNKT cell and supplementation of iNKT cells into obese mice downregulate body weight gain and adipocyte size and upregulate secretion of anti-inflammatory adipokines. These metabolic changes are accompanied with restoration of insulin sensitivity (23, 25).

One of the major regulatory mechanisms for adipose tissue inflammation by adipose iNKT cell is through diverse cytokine secretion. For instance, adipose iNKT cells secrete IL-4 and IL-10 which promote M2 macrophage polarization (44). In obese mice, inhibition of IL-4/IL-10 signaling diminishes iNKT cell-dependent glucose homeostasis (25). Also, short-term HFD feeding induces the expression of arginase 1, one of the M2 marker genes, in adipose tissue of WT mice, but not in CD1d KO and IL-4 KO mice, indicating that adipose iNKT cells rapidly respond to HFD and produce IL-4 to suppress inflammatory responses *via* induction of M2 macrophages (45). Moreover, it has been shown that IL-2 secreted by adipose iNKT cells is involved in immunosuppressive function of Treg cells through promoting IL-10 production of Treg cells in adipose tissue (29). Upon short term HFD feeding, the number of adipose Treg cells is elevated in WT mice, but not in $CD1d^{AKO}$ mice, underscoring the crucial roles of adipocyte CD1d in the regulation of the anti-inflammatory responses (33). Furthermore, it has been very recently reported that IFNγ produced by adipose iNKT cells in lean adipose tissue can serve to limit the expansion of ATMs by killing pro-inflammatory macrophages *via* NK cell stimulation (46).

These findings propose that activity control of iNKT cells by adipocytes and lipid antigens appears to be the key for adipose tissue immune balance (**Figure 1A**). In contrast, Satoh et al. has reported that adipose iNKT cells would exhibit pro-inflammatory characteristics by secreting IFN-γ because $CD1d^{AKO}$ mice show adipose tissue inflammation and insulin resistance in obesity (43). Although there is no clear answer to explain opposite phenotypes in $CD1d^{AKO}$ mice above, it has been suggested that these differences are probably due to different types of control mice ($CD1d^{flox/+}$ *vs* $CD1d^{flox/flox}$) and differences in high-fat diet (HFD) composition (tallow and safflower oil of high oleic type *vs* lard) (33). Moreover, it has been shown that adipose iNKT cells can be classified into several subpopulations that reveal either pro-inflammatory responses or anti-inflammatory responses (46), implying that characteristics of adipose iNKT cells might be affected by multilateral relationships between lipid antigen species and iNKT cell subtypes. Thus, it seems that veiled traits of adipose iNKT cells could be further uncovered when lipid antigens loaded on adipocytes and subtypes of adipose iNKT cells are identified in future studies.

Adipocyte Turnover Control by Lipid Antigen(s)

Yearly, 10% of human adipocytes are dead and replaced with new adipocytes (47). Patients with cachexia, human immunodeficiency virus (HIV) or lipodystrophy syndrome show drastic loss of adipocytes (48–51). In obese mice, dead adipocytes are frequently found in epididymal adipose tissue (23, 52). Although adipocyte death is associated with adipose tissue inflammation in obesity, the causal factors that would induce adipocyte death have not been fully elucidated. Recently, it has been reported that, in hypertrophic adipocytes, the expression of Fas (CD95) is upregulated and is positively correlated with the degree of adipocyte death (**Figure 1B**) (23). Apoptotic pathway is induced in Fas-positive cells when Fas is bound to FasL (53). In obese adipose tissue, the portion of FasL-positive iNKT cells is significantly elevated, but not in CD4 and CD8 T cells, indicating that iNKT cells would be a major killer cell type to induce hypertrophic adipocyte death in obesity (23). Through *in vitro*

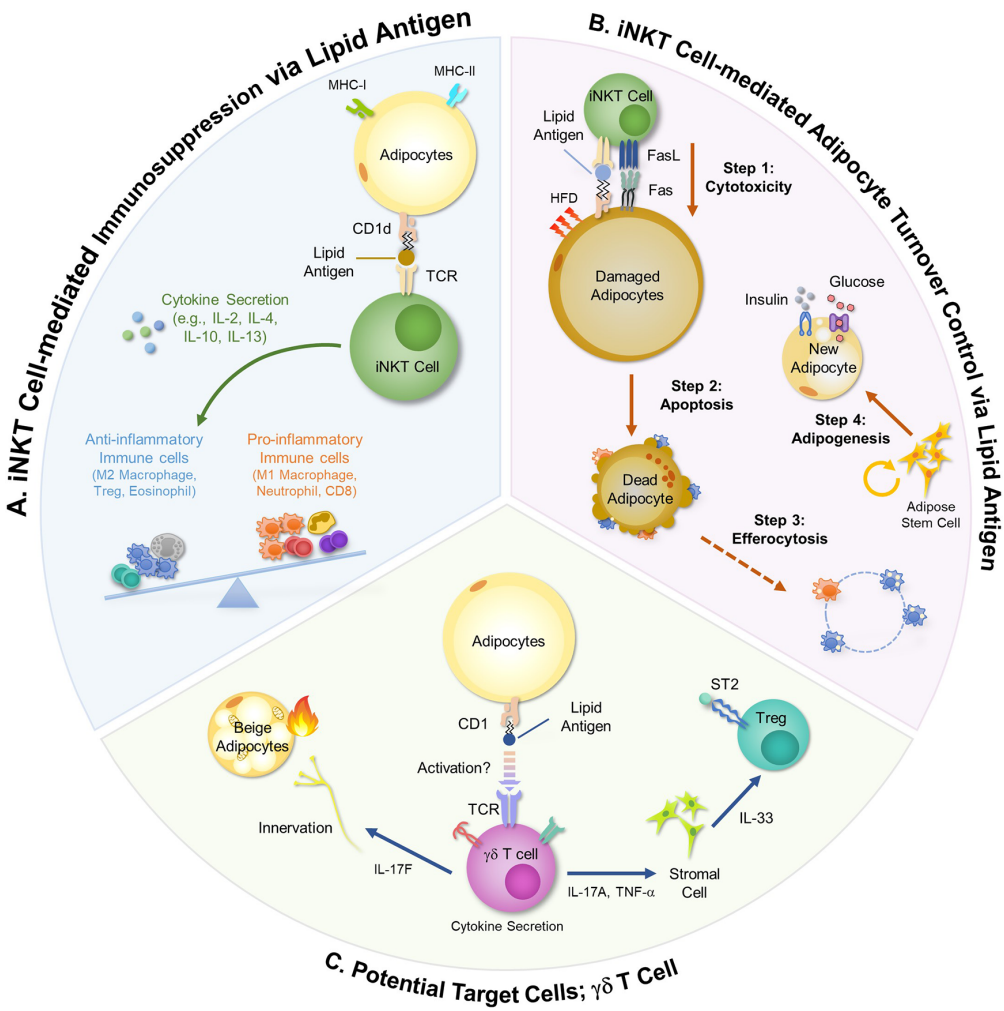

FIGURE 1 | Immunomodulatory Roles of Adipocytes using Lipid Antigens. Adipocytes modulate activities of adipose immune cells *via* lipid antigen presentation. iNKT cells and γδ T cells are activated by lipid antigens and involve in the regulation of adipose tissue immunity and adipocyte functions. **(A)** In obesity, adipose iNKT cells activated by adipocyte-derived lipid antigens secret large amounts of anti-inflammatory cytokines such as IL-2, IL-4, IL-10, and IL-13. These cytokines stimulate Treg cells and polarize monocytes into anti-inflammatory M2 macrophages, thereby ameliorating pro-inflammatory responses in obese adipose tissue. **(B)** Adipose iNKT cells mediate hypertrophic and pro-inflammatory adipocyte death in obesity. Long-term HFD (over 8 weeks) upregulates CD95L (FasL) and CD95 (Fas) in adipose iNKT cells and damaged adipocytes, respectively. Interaction between CD95L and CD95 selectively stimulates damaged adipocyte death. After macrophage-mediated efferocytosis, adipose stem cells proliferate and *de novo* adipogenesis is promoted, leading to the generation of insulin-sensitive new adipocytes. **(C)** Given that γδ T cells recognize CD1-loaded lipid antigens, it has been suggested that adipocytes might regulate γδ T cell activity. γδ T cells secrete several cytokines such as IL-17 and TNF-α, controlling beige adipocyte formation and innervation. In addition, γδ T cells activate stromal cells to secrete IL-33, resulting in Treg cell recruitment.

and *in vivo* experiments, it has been shown that hypertrophic adipocytes with pro-inflammatory characteristics stimulate iNKT cells by lipid antigen presentation *via* CD1d (23). Then, the activated iNKT cells selectively kill hypertrophic and pro-inflammatory adipocytes (23). iNKT cell-mediated hypertrophic adipocyte death is consistently observed in both diet-induced obese mice and genetically obese *db/db* mice (23). After iNKT cell-mediated adipocyte death, adipocyte stem cells proliferate and differentiate into new and small adipocytes exhibiting elevated insulin sensitivity (**Figure 1B**) (23, 54). Together, it has been suggested that, in obesity, activity control of iNKT cells by adipocytes is crucial for adipocyte turnover, contributing to the improvement of insulin sensitivity.

Adipocyte Death and Adipose Tissue Inflammation

Although adipocyte death and ATMs surrounding dead adipocytes are frequently observed in obesity, the relationship between adipocyte death and inflammation remains elusive. Activation of iNKT cells by α-GC administration into HFD-fed obese mice induces apoptosis of hypertrophic adipocytes, accompanied by the increase in the portion of M2 macrophages

compared to that of M1 macrophages (23). Similarly, the number of CD206 and CD301-positive M2-macrophages increases when adipocyte-specific apoptosis is induced in FAT-ATTACK mice (55). It seems that transient induction of apoptosis in adipocytes would upregulate anti-inflammatory responses. On the other hand, continuous adipocyte death resulted from chronic inflammation or deficiency of key enzymes involved in sphingolipid synthesis and mevalonate pathway often causes systemic pro-inflammatory responses (56, 57). Furthermore, if apoptotic cells are not rapidly and properly cleared by efferocytosis, the membrane of apoptotic cells is ruptured and transformed into necrosis-like cells, provoking inflammation. Thus, it is likely that controversial results of adipocyte death on adipose tissue inflammation would be due to several factors: whether types of adipocyte death are apoptotic or necrotic, whether adipocyte death is transient or persistent, and whether debris of dead adipocytes are well cleared.

The clearance of apoptotic cells by professional and non-professional phagocytes is essential for maintenance of tissue homeostasis (58). In response to apoptotic cells, macrophages suppress production of pro-inflammatory cytokines and enhance secretion of molecules that dampen inflammation, and mediate resolution and repair. Thus, defective efferocytosis leads to inflammation and impaired resolution, underlying various chronic inflammatory diseases such as atherosclerosis, obesity, diabetes, cardiovascular diseases, and cancer (58). In obese mice, macrophages appear to exhibit impaired efferocytosis, which is associated with higher number of apoptotic cells and greater expression of pro-inflammatory cytokines within wounds (59, 60). It has been proposed that defects of omega-3 fatty acids, erythropoietin, and MER proto-oncogene tyrosine kinase would suppress efferocytosis of dying/dead cells in atherosclerotic lesions, skin, and heart in obesity (58). However, to date, most studies have not focused on clearance of dead adipocytes, although dead adipocytes and ATMs surrounding them are abundantly observed in obesity. Future studies are required to unravel complex relationships between adipocyte death, efferocytosis, and adipose tissue inflammation.

$\gamma\delta$ T Cells: Potential Target Cells of Adipocytes

$\gamma\delta$ T cell is one of the innate lymphocytes that are not restricted to MHC molecules but recognize CD1 molecules. In adipose tissue, $\gamma\delta$ T cells exhibit resident characteristics and occupy 5–15% of total T cells (26). Upon HFD, the number of $\gamma\delta$ T cells increases and they promote accumulation of pro-inflammatory macrophages, worsening adipose tissue inflammation and insulin resistance (30). In contrast, it has been shown that IL-17A-producing $\gamma\delta$ T cells are involved in the maintenance of adipose Treg population by promoting secretion of IL-33 from stromal cells, contributing to suppression of adipose tissue inflammation (**Figure 1C**) (26). In addition, under short term ketogenic diet (KD) which contains high fat and low carbohydrate, $\gamma\delta$ T cells suppress adipose tissue inflammation and protect metabolic dysregulation through increasing expression of genes related to tissue repair (61). Conversely, long-term KD drastically decrease the number of $\gamma\delta$ T cells and aggravates obesity and glucose intolerance (61). Although it remains to be clarified whether adipose $\gamma\delta$ T cells would upregulate or downregulate inflammatory responses in adipose tissue, it seems that $\gamma\delta$ T cell could play certain roles in inflammatory responses in adipose tissue. In addition to the regulation of adipose tissue inflammation, $\gamma\delta$ T cells modulate adipocyte functions such as lipolysis and thermogenesis (26). In brown and subcutaneous adipose tissue, $\gamma\delta$ T cells boost thermogenic programs by stimulating IL-33 secretion in stromal cells or promoting innervation in adipose tissue (**Figure 1C**) (26, 27). Given that $\gamma\delta$ T cells could recognize lipid antigens loaded on CD1 family, it is plausible to speculate that adipocytes would function as potential APCs in adipose tissue.

RELATIONSHIP BETWEEN LIPID METABOLISM IN ADIPOCYTES AND ADIPOSE TISSUE IMMUNITY

In adipose tissue, lipid metabolism is dynamically regulated upon diverse physiological conditions such as fasting, HFD, and aging. If lipid metabolism is dysregulated in adipocytes due to environmental or genetic factors, adipose tissue immunity and whole body energy metabolism are distorted. It has been suggested that endogenous lipids such as free fatty acids (FFAs) and eicosanoids modulate innate and adaptive immune cells (62). Furthermore, HFD provokes uncontrolled basal lipolysis and promotes unnecessary release of FFAs, causing imbalanced immune responses in adipose tissue. Also, when lipid storage capacity of adipocytes is defective by ablation of lipid droplet (LD) binding proteins such as Perilipin1 (Plin1), the levels of triglyceride and FFAs are elevated in adipose tissue and serum, which is accompanied by adipose tissue inflammation and insulin resistance (63). In this section, we cover how adipocytes regulate adipose immune responses by controlling lipid metabolism.

Regulation of Adipose Immune Responses by Lipid Metabolites

Lipid metabolites are associated with numerous human diseases, including atherosclerosis, rheumatoid arthritis, and other inflammation-linked metabolic diseases (64). While it has been considered for a long time that lipid metabolites are key energy sources, the importance of lipid metabolites as signaling molecules has been accumulated (65–67). Eicosanoids, certain FFAs, and FFA derivatives are able to act as signaling molecules in the regulation of immune responses (64). Among them, several lipid metabolites are produced by adipocytes or adipose tissues (19–21). Palmitoleate (C16:1n7), a long-chain monounsaturated FA, is produced through *de novo* lipogenesis in adipose tissue and downregulates pro-inflammatory gene expressions in macrophages (68–71). Also, in adipocytes, palmitic acid esters of hydroxy stearic acids (PAHSAs)

synthesized by carbohydrate response element binding protein (ChREBP) regulate adipose tissue inflammation. While adipocyte-specific ChREBP knockout ($ChREBP^{AKO}$) mice exhibit decreased PAHSA levels and increased ATMs in adipose tissue, PAHSA administration ameliorates pro-inflammatory responses in adipose tissue of $ChREBP^{AKO}$ mice (72).

In addition to *de novo* lipogenesis, certain lipid metabolites which regulate adipose tissue inflammation are produced by lipolysis. Recently, it has been shown that Plin1 inhibits futile prostaglandin secretion to restrict pro-inflammatory responses in adipose tissue (63). Plin1 deficiency in adipocytes impairs lipid storage into LDs and stimulates lipolysis, causing adipose tissue loss and unnecessary leakage of pro-inflammatory lipid metabolites. In adipose tissue of Plin1 KO mice (**Figure 2**), pro-inflammatory gene expression and M1-type ATM accumulation are increased. Suppression of lipolysis by knockdown or inhibition of lipases attenuates the effects of Plin1-deficient adipocytes on monocyte migration. Moreover, lipidomic analysis and administration of cyclooxygenase inhibitor indicate that enhanced adipose tissue inflammation is mediated by excessive prostaglandin E_2 (PGE_2) secretion in Plin1-deficient adipocytes (62). Thus, it has been proposed that reducing futile lipolysis in adipocytes could downregulate adipose tissue inflammation through the control of pro-inflammatory lipid metabolite secretion (63).

Circulating FFAs are elevated in obesity and lipodystrophy, which is closely related to metabolic disorders including type 2 diabetes and atherosclerosis. FFAs including palmitic acids are able to activate inflammatory responses and also used to produce ceramides. Ceramides are one of important metabolites whose levels are elevated in obesity (73). Increased ceramides contributes to adipose tissue inflammation and dysregulation of energy homeostasis. In macrophages, ceramide initiates p38 MAPK and JNK signaling pathways, polarizing ATMs towards M1 macrophages (74). Moreover, ceramides activate NLR family pyrin domain containing 3 (NLRP3) inflammasome and promote secretion of IL-1β and IL-18 in macrophages, aggravating adipose tissue inflammation and glucose intolerance in obesity (75).

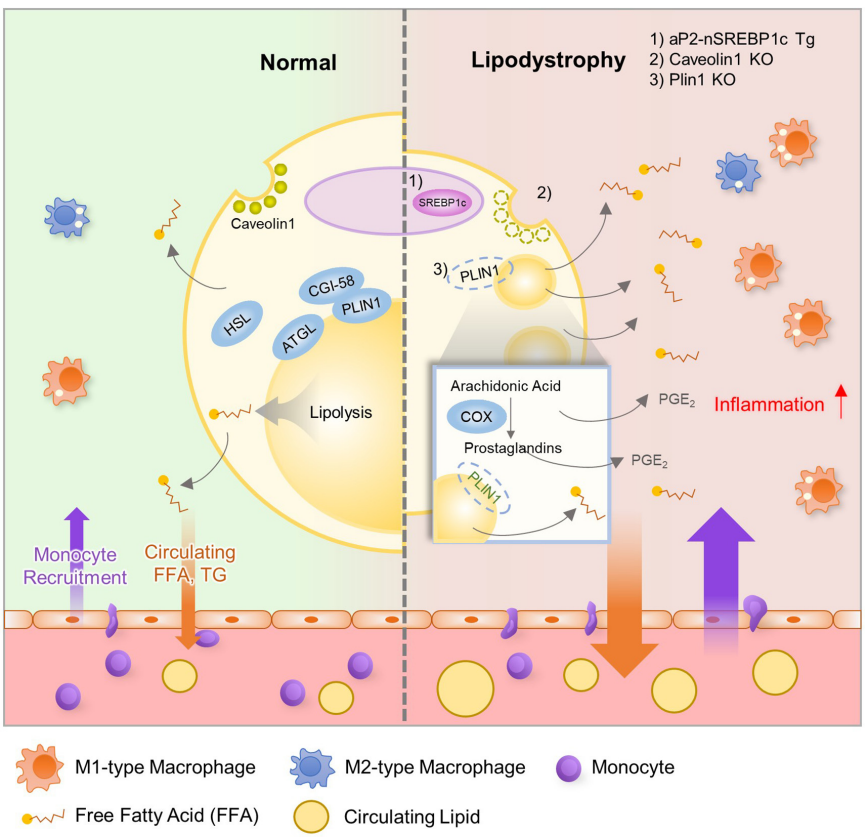

FIGURE 2 | Relationship between Lipodystrophy and Adipose Tissue Inflammation. In adipocytes, lipid metabolism is well balanced by several genes, including Srebp1c, Atgl, Hsl, Cgi-58, Plin1, and Fsp27. However, lean subjects with lipodystrophy show dysregulated lipid metabolism with increased inflammation and insulin resistance. Evidence suggests that dysregulation of lipid metabolism could influence adipose tissue inflammation in lipodystrophy. aP2-nuclear form of SREBP1c transgenic (aP2-nSREBP1c Tg) mice and Caveolin1 KO mice show significantly reduced fat mass and display metabolic dysregulation including insulin resistance and dyslipidemia. In addition, Plin1 deficiency induces partial fat loss, leakage of FFAs, ATM accumulation, dyslipidemia and systemic insulin resistance. In these lipodystrophic models, several lipid metabolites such as FFA and PGE2 recruit monocytes into adipose tissue and worsen adipose tissue inflammation.

Lipodystrophy and Adipose Tissue Inflammation

Although lipodystrophy and adipose tissue expansion such as obesity are somewhat opposite in terms of adipose tissue mass, both pathological states often exhibit similar metabolic dysregulation (76–78). Obesity-induced low-grade and chronic inflammation is one of the major factors to promote insulin resistance (12, 79). Also, severely lean patients with lipodystrophy or cachexia reveal enhanced inflammation with insulin resistance even though underlying mechanisms are not fully uncovered. Nonetheless, it has been suggested that immune responses in adipose tissue could be involved in the development of insulin resistance in lipodystrophy (80, 81). Pro-inflammatory gene expression and ATM accumulation are promoted in adipose tissue of lipodystrophic animal models even with less adipose tissue mass. For instance, aP2-nuclear form of sterol regulatory element-binding protein 1c (SREBP1c) transgenic (aP2-nSREBP1c Tg) mice and Caveolin1 KO mice show significantly reduced fat mass and display metabolic dysregulation including insulin resistance and dyslipidemia (82–84). In these lipodystrophic models, increases in pro-inflammatory cytokine and ATM accumulation are observed in adipose tissue (**Figure 2**) (84). In addition, Plin1 deficiency reveals partial fat loss, ATM accumulation, dyslipidemia and systemic insulin resistance in both mouse and human (63, 85). In aP2-nSREBP1c Tg mice, anti-inflammatory strategies such as salicylate treatment or crossing with myeloid cell-specific IκB kinase (IKKβ) KO mice do not ameliorate insulin resistance (83). On the other hand, in Plin1 KO mice, macrophage depletion by clodronate treatment or inhibition of synthesis of pro-inflammatory lipid metabolites in adipocytes mitigates systemic insulin resistance (63). These results indicate that the precise relationship between adipose tissue inflammation and systemic energy homeostasis remains to be thoroughly elucidated under lipodystrophic conditions.

Aging-Related Decrease in Lipolysis

Aging is a chronic and complex physiological process that gradually deteriorates energy homeostasis (86). Dysfunction of adipose tissue is one of the major factors to provoke aging-related metabolic disorders including type 2 diabetes and cardiovascular diseases. In the elderly, the processes of lipolysis and lipid storage in adipose tissue are not properly controlled. As a result, mobilization of FFAs is dysregulated, causing visceral adiposity, lower exercise capacity, and cold intolerance. These alterations of adipose tissue are closely associated with adipose tissue immunity (87). Adipose macrophages and B cells are involved in age-related reduction of lipolytic activity. In aged mouse model, macrophages degrade catecholamine in a NLRP3 inflammasome-dependent manner in adipose tissue, driving lipolysis resistance in adipocytes (88). When NLRP3 inflammasome is activated in aged macrophages, the expression of monoamine oxidase (MAOA) which is known to degrade noradrenaline is increased by growth differentiation factor-3 (88). Moreover, aging stimulates expansion of adipose B cells in fat-associated lymphoid clusters (FALC), which is mediated by activation of NLRP3 inflammasome and IL-1 signaling (89). It has been shown that inhibition of MAOA in macrophages or depletion of B cell reverses the age-related decline in lipolysis and restore age-associated adipose tissue impairment (89). However, in human adipose tissue, the major cell type expressing MAOA is different from mice. In human adipose tissue, MAOA is mainly expressed in mature adipocytes, unlike mice, contributing to aging-associated reduction in lipolysis (90).

THE NOVEL ROLES OF ADIPOSE STEM CELLS IN THE REGULATION OF ADIPOSE TISSUE IMMUNITY

ASCs are composed of heterogeneous populations and each population has unique characteristics. ASCs are largely divided into adipogenic and non-adipogenic subtypes (91). Adipogenic ASCs preferentially differentiate into adipocytes in response to excess energy, which increases energy storage capacity of adipose tissue. This process, called hyperplasia, mediates healthy adipose tissue expansion and attenuates adipose tissue inflammation in obesity. On the other hand, non-adipogenic ASCs secrete various pro- and anti-inflammatory cytokines, lipokines, and collagens, which could affect activity and recruitment of adipose immune cells. In addition, it appears that non-adipogenic ASCs would be key players for distinct immune responses between subcutaneous white adipose tissue (sWAT) and visceral white adipose tissue (vWAT). As the roles of adipogenic ASCs have been well discussed in previous reviews (92, 93), we cover the novel roles of non-adipogenic ASCs in the regulation of adipose tissue immunity.

Novel Roles of ASCs in the Regulation of Adipose Tissue Immunity

Adipose tissue is divided into adipocyte and stromal vascular cell (SVC) fraction, and SVC fraction is further classified into ASCs ($CD45^-CD31^-$), immune cell ($CD45^+$), endothelial cell ($CD31^+$), and red blood cell. In the last several years, single cell RNA-sequencing (scRNA-seq) has been used to reveal subpopulation and characteristics of ASCs, providing compelling evidence that ASCs would exhibit molecular heterogeneity and functional diversity (94, 95). Interestingly, it has been proposed that ASCs not only have adipogenic potential, but also exhibit anti-adipogenic and immunomodulatory roles (96).

ASCs secrete pro-inflammatory cytokines (*e.g.*, IL-6, IL-8, IL-11, TNF-α), anti-inflammatory cytokines (*e.g.*, TGF-β, IL-10), growth factors, chemokines (Cxcl5), and lipokines (PGE2) (97). Upon HFD, the number of fibro-inflammatory stem cells ($lin^-Pdgfr\beta^+Ly6c^+$ cells, $lin^-Pdgfr\alpha^+Gp38^+CD9^+$) is upregulated and they highly express pro-inflammatory cytokines (*e.g.*, IL-6, Ccl2, Cxcl2, Cxcl10) and extracellular matrix components (*e.g.*, Col1a1, Col3a1), causing adipose tissue inflammation (**Figure 3**) (98–100). In human and mouse, $CXCL1^+$ mesothelial cells ($CD45^-CD31^-Ter119^-CD41^-PDPN^{+/-}$) recruit neutrophils into the

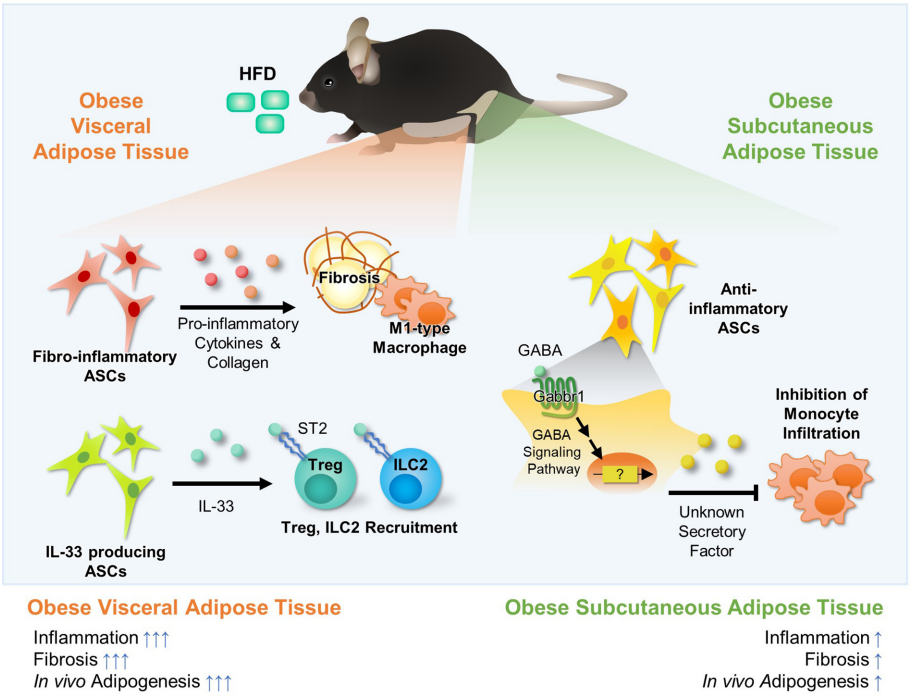

FIGURE 3 | Fat Depot-specific Roles of Adipocyte Stem Cells (ASCs) in the Regulation of Adipose Tissue Immunity. White adipose tissues consist of major two fat depots; visceral adipose tissue and subcutaneous adipose tissue. These two fat depots exhibit several differences in inflammatory responses, fibrosis, and adipogenesis. ASCs are major cell types comprising of adipose tissue, and they are largely divided into adipogenic and non-adipogenic clusters. In visceral adipose tissue, there are fibro-inflammatory ASCs (lin–Pdgfrβ+Ly6c+ cells or lin–Pdgfrα+Gp38+CD9+). The number of fibro-inflammatory ASCs increases in obesity and they secret pro-inflammatory cytokines (*e.g.*, IL-6, Ccl2) and ECM components (*e.g.*, Col1a1, Col3a1), promoting fibrosis. Moreover, it has been reported that IL-33 producing non-adipogenic ASCs (lin–Pdgfrα+PPARγ–) are involved in recruitment of Treg and ILC2 *via* IL-33 secretion, which suppresses inflammation in visceral adipose tissue. Recently, it was reported that, in subcutaneous adipose tissue, ASCs (CD31–CD34+Sca1+) suppress monocyte infiltration, which is potentially regulated by GABA signaling. However, the secretory factors that inhibit monocyte infiltration in subcutaneous adipose tissue have not been elucidated yet.

FALC *via* protein arginine deiminase 4 during peritonitis and promote the aggregation of neutrophils, providing first layer of immunological defense in vWAT (101). On the other hand, another population of ASCs that suppress adipose tissue inflammation has been also reported (102–104). Lin$^-$ Pdgfrα^+Sca1$^+$ population is a major source of IL-33 in vWAT (**Figure 3**) (102). IL-33$^+$ ASCs recruit anti-inflammatory Treg and ILC2 cells in lean subjects, contributing to suppression of adipose tissue inflammation (102).

It has been shown that ASCs would be the key cell type that explains distinct inflammatory patterns between sWAT and vWAT in obesity (**Figure 3**) (100, 103, 104). In obese mice, vWAT shows the higher number of infiltrated macrophages and crown-like structures, whereas sWAT is less prone to inflammation. However, it is still unknown which factors make the differences in inflammatory responses between the two major fat depots in obesity. Very recently, it has been demonstrated that SVCs of sWAT secrete certain factors to repress monocyte recruitment, and that transplantation of ASCs derived from sWAT into vWAT suppresses ATM infiltration in vWAT (103, 104). Interestingly, gamma-aminobutyric acid (GABA) signaling is one of the most differentially expressed pathways between sWAT and vWAT in obesity. In HFD-induced obese mice, GABA treatment inhibits ATM infiltration in sWAT-selective manner, but not in vWAT (102). Thus, it has been proposed that GABA signaling in ASCs might be one of the potential pathways that could selectively suppresses inflammatory responses in sWAT (103).

Given that ASCs have high proliferation rate, adipogenic potential, and immunomodulatory roles, they have been considered therapeutic target for recovery of adipose tissue homeostasis. Recently developed scRNA-seq analysis dissects ASCs into three or more subpopulations with their own distinct functions. Proliferative and stem cell-like ASCs can be used in tissue repair and regenerative processes. Adipogenic and anti-adipogenic subpopulations of ASC can increase or decrease buffering capacity of adipose tissue, respectively. In addition, ASCs that exhibit immunomodulatory properties can be used to control inflammatory responses of adipose tissues. Although complicated networks between ASCs and adipose tissue constituent cells need to be further investigated, recent approaches equipped with high techs would provide new therapeutic targets against adipose tissue dysfunction, particularly, in obesity.

LIMITATIONS AND FUTURE DIRECTIONS

There are several points to be solved in future studies. First, it remains elusive which kinds of endogenous lipid antigens would be presented by adipocyte CD1d in obesity. Even though α-GC has been used as an activator for iNKT cells, α-GC is an exogenous and quite potent activator, which might be different from patho-physiologic conditions. Second, it is required to identify antigen presenting cells and lipid antigens that regulate the activity of γδ T cells in adipose tissue. Third, the mechanisms of ATM recruitment by lipid metabolites such as PGE_2 should be elucidated in future studies. Lastly, while recent technical advances (e.g., scRNA-seq) have proposed novel subpopulations of adipocytes and discovered new relationships between adipocyte subpopulations and immune cells, it remains to be validated with proper *in vivo* models (105–108). Also, there are still huge technical obstacles in the analysis of lipid profiles from each adipocyte subpopulations as well as immune cells.

CONCLUSION

Lipids are key energy sources and primary building blocks for plasma membranes and intracellular organelles. Moreover, lipid metabolites participate in numerous signal transduction and regulate multiple cellular functions. Recently, it has been suggested that lipid metabolites are crucial bioactive molecules in immune system (18–20). Here, we have discussed the immunomodulatory roles of lipid metabolites of adipocytes upon metabolic stimuli. In response to altered metabolic environments, adipocytes sensitively and dynamically control lipid metabolism and present or secrete lipid metabolites to modulate characteristics of adipose immune cells. Thus, it is plausible to speculate that adipocytes not only use lipid metabolites to maintain their structures and functions, but also actively utilize lipid metabolites as key messengers to communicate with adipose immune cells. The interplay between adipocytes and adipose immune cells leads to fine-tuning adipose tissue immunity and adipose tissue remodeling, which eventually contributes to maintenance of systemic energy metabolism. Nonetheless, there are remaining issues to be solved in future studies. For instance, the lipid antigen presented by adipocytes and lipid metabolites secreted by adipocytes are not fully identified. There have been technical difficulties such as extraction of lipids, identification of specific lipid species, and quantitation of the vast array of lipids. Thus, solving these issues will enhance our insights about the mechanisms by which adipocytes govern adipose tissue immunity, and further suggest new therapeutic approaches on metabolic complications caused by adipose tissue inflammation.

AUTHOR CONTRIBUTIONS

JP, JHS, SMH, YJP, JYH, SSC, and JBK contributed to the writing of the manuscript under JK's supervision. All authors contributed to the article and approved the submitted version.

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REVIEW
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The Role of the Adipokine Leptin in Immune Cell Function in Health and Disease

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Leptin is a critical mediator of the immune response to changes in overall nutrition. Leptin is produced by adipocytes in proportion to adipose tissue mass and is therefore increased in obesity. Despite having a well-described role in regulating systemic metabolism and appetite, leptin displays pleiotropic actions, and it is now clear that leptin has a key role in influencing immune cell function. Indeed, many immune cells have been shown to respond to leptin directly *via* the leptin receptor, resulting in a largely pro-inflammatory phenotype. Understanding the role of adipose-tissue derived mediators in inflammation is critical to determining the pathophysiology of multiple obesity-associated diseases, such as type 2 diabetes, autoimmune disease, and infection. This review, therefore, focuses on the latest data regarding the role of leptin in modulating inflammation.

Keywords: leptin, obesity, inflammation, adaptive immunity, adipose tissue

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INTRODUCTION

Obesity is associated with a chronic, low-grade systemic inflammation that has been shown to promote the development of multiple disorders of health including type 2 diabetes, autoimmunity, nonalcoholic fatty liver disease, asthma, and cardiovascular disease (1, 2). This obesity-associated inflammation is characterized by increased circulating inflammatory cytokines such as tumor necrosis factor (TNF)and interleukin 6 (IL-6) as well as an increase in pro-inflammatory immune cells, particularly macrophages and lymphocytes (3–9).

The etiology of obesity-associated inflammation is complex. While many tissues demonstrate obesity-associated inflammation, adipose tissue is considered to be the central or key site of inflammation, responsible for driving systemic inflammation and disease (10, 11). Adipose tissue is altered in obesity, leading to increased adipocyte volume and lipid content. These alterations are associated with changes in adipose tissue-resident immune cells, characterized by an increase in immune cell number, particularly pro-inflammatory macrophages and lymphocytes (12–20). Inflammatory immune cells found within adipose tissue in obesity in turn promote adipocyte production of inflammatory molecules (21). Adipose tissue production of the pro-inflammatory hormone leptin, and the role of leptin in mediating obesity-associated inflammatory disease, is the subject of this review.

Leptin can be produced by multiple cells in the body, including immune cells, but is primarily produced by adipocytes in proportion to adipocyte mass, such that increasing adiposity leads to

increased systemic concentrations of leptin (22, 23). Although leptin is produced in a diurnal manner (24), it is not a fast-acting signal or cytokine, but rather communicates stable nutritional status to the body as a whole. Leptin has a well-defined role as a metabolic mediator and communicator of nutritional status at the level of the hypothalamus where leptin receptors are highly expressed. Increased leptin signaling at the hypothalamus regulates appetite and leads to decreased nutrient intake and increased energy expenditure. Studies of leptin deficiency and fasting have demonstrated that leptin signaling is also required for normal reproductive hormone production, as well as thyroid hormone. Therefore, leptin plays a critical role in controlling energy homeostasis, metabolism, and neuroendocrine function. These functions of leptin have been thoroughly reviewed (25–27).

Over the last two decades, it has become apparent that leptin also has a critical role as an immune modulator. This was initially observed in individuals with rare mutations in leptin or the leptin receptor, who are obese from lack of leptin signaling at the hypothalamus, but were also found to have an increased risk of intracellular infections secondary to immune cell deficiencies (28). Leptin has subsequently been shown to act on several different immune cell types and can affect both immune cell development and function. Through that mechanism, increased systemic leptin levels in diet-induced obesity directly promote obesity-associated inflammation.

Leptin receptor is expressed by most cells of the immune system and many immune cells have been shown to be leptin responsive to varying degrees. In general, leptin receptor expression is important for hematopoietic cell development, immune cell proliferation and survival, and pro-inflammatory function (29, 30). In this review, we will characterize the effects of leptin on innate and adaptive immune cells, with a particular focus on $CD4^+$ T cells, which are known to be highly leptin responsive, as summarized in **Table 1**. We will explore the mechanisms by which leptin is proposed to act on these cells, both through traditional signaling pathways and through altering cellular metabolism, much of which has been discovered in the mouse model. Finally, we will review the effects of leptin in human studies and identify the clinical relevance of this adipokine in the setting of both health and disease. Although leptin may have a role as a nutritional regulator of immunity in the setting of both under- and overnutrition, we will focus here on the effects of leptin on the immune system in the context of obesity.

TABLE 1 | Distinct effects of leptin across immune cell types.

Immune cell	Leptin Effect
$CD4^+$ T cells	Required for T cell development in the thymus (31–34)
	Increases proliferation of naïve T cells (35, 36)
	Promotes Th1 cytokine production (35)
	Promotes Th17 differentiation and cytokine production (34)
	Promotes increased glycolytic metabolism (31, 34)
B cells	Reduces apoptosis (37)
	Promotes cell cycle entry (37)
	Increases inflammatory cytokine production (38)
	Reduces class switching and IgG production (38)
Macrophages	Promotes bacterial clearance and phagocytosis (39, 40)
Monocytes	Increases TLR2 expression (41)
	Promotes inflammatory cytokine production (42)
Mast Cells	Promotes mast cell phenotype that drives inflammatory M1-like macrophage cell phenotype (43)
Dendritic cells	Reduces apoptosis by increasing expression of Bcl-2 and Bcl-xL (44)
	Promotes DC maturation and function (45)
	Increases inflammatory cytokine production (44)
Neutrophils	Inhibits apoptosis (46)
	Acts as chemoattractant (22, 47)
	Increases oxidative species production (48)
Basophils	Inhibits apoptosis (49, 50)
	Acts as chemoattractant, promotes trafficking toward other chemo attractants such as eotaxin (49)
	Increases IL-4 and IL-13 production (49)
Eosinophils	Inhibits apoptosis (49, 50)
	Acts as chemoattractant, promotes trafficking toward other chemo attractants such as eotaxin (51)
NK cells	Brief exposure promotes increased cytotoxicity (52)
	18-h exposure increases IFN-γ and perforin production (52, 53)
	72-h exposure inhibits IFN-γ and cytotoxicity (52)
ILCs	Promotes type-2 cytokine production (54)

ADAPTIVE IMMUNE CELLS

The effect of leptin on immune cells has been best studied in the context of adaptive immunity, particularly its effects on $CD4^+$ T cells. Leptin has been shown to have a role in modulating T cell development, as well as T cell function and metabolism. Moreover, distinct functional $CD4^+$ T cell subsets respond to leptin in different ways that reflect their function. $CD8^+$ T cell and B cell responses to leptin have also been studied, but to a lesser extent.

T Cells

Leptin plays an important role in T cell development. Leptin deficiency has been shown to result in thymic atrophy and decreased circulating T cell numbers (31, 33, 34). Interestingly, leptin receptor has been found to be expressed on double negative, double positive and CD4 single positive thymocyte subsets, but not on CD8 single positive thymocytes (32). Moreover, leptin treatment rescued $CD4^+$ T cell development in leptin mutant (*ob/ob*) mice, but did not rescue $CD8^+$ T cell development (32). Together this suggests that leptin is required for early T cell development and for later development of $CD4^+$ T cells, but not $CD8^+$ T cells.

$CD4^+$ T cells express high levels of the long isoform of the leptin receptor (Ob-Rb), which is significant because it is the only isoform that can signal through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway (55), as shown in **Figure 1**. Leptin receptor signaling in T cells has been shown to promote survival, proliferation, cytokine production, and differentiation. *In vivo*, leptin treatment of wildtype (WT) mice was shown to inhibit steroid-induced apoptosis of lymphocytes (59). In response to leptin treatment, naïve $CD4^+$ T cells, but not memory T cells, showed an increase in proliferation in a mixed lymphocyte reaction (35). In an older study of human cells, monocyte-depleted peripheral blood mononuclear cells (PBMCs) stimulated with phytohemagglutinin (PHA) and Concanavalin A (ConA) and treated with leptin had

increased proliferation compared to untreated cells (60). More recent studies have demonstrated that $CD4^+$ T cells from leptin receptor mutant (*db/db*) mice have reduced proliferation when compared to WT $CD4^+$ T cells, suggesting that leptin signaling on $CD4^+$ T cells is required for proliferation (31).

JAK-STAT signaling is downstream of many lymphocyte receptors that promote the production of various cytokines. Thus, as one would predict, leptin treatment of bulk, non-differentiated T cells influenced cytokine production by these cells. Leptin treatment of $CD4^+$ T cells increased pro-inflammatory cytokine production, namely T helper 1 (Th1) cytokines interferon gamma (IFN-γ)and IL-2, while decreasing production of the T helper 2 (Th2) cytokine IL-4 (35). Moreover, activated $CD4^+$ T cells generated from T cell specific leptin receptor conditional knockout mice were found to produce less IFN-γ than WT $CD4^+$ T cells (31). Together, these data suggest that leptin promotes pro-inflammatory cytokine production in $CD4^+$ T cells.

Leptin has also been shown to play a role in the differentiation of T cells into functional subsets. Hypoleptinemia induced by fasting has been shown to suppress the number of effector T cells, but not regulatory T cells (Treg cells) in mice. In fact, the same study found that while Treg proportions were increased in fasting, absolute numbers of Treg cells were unchanged, suggesting that leptin promotes the differentiation of effector T cells, but not Treg cells, and that any change in Treg cell proportions were indirect (34). In contrast, $CD4^+$ T cells isolated from fasted hypoleptinemic mice had decreased differentiation into T helper 17 (Th17) cells *in vitro* compared to $CD4^+$ T cells isolated from *ad lib* fed mice. When the fasted mice were given leptin injections twice daily, Th17 differentiation was restored, suggesting that leptin is critical for differentiation into Th17 cells (34). In support of this, Th17 differentiation *in vitro* was decreased in $CD4^+$ T cells isolated from mice with T cell specific knockout of leptin receptor compared to WT controls (34). Furthermore, T cell specific leptin receptor knockout mice had decreased frequency of Th17 cells and increased frequency of Treg cells in the lamina propria (61).

The mechanism by which leptin promotes Th17 differentiation has been investigated. Leptin signaling promotes transcription of RAR-related orphan receptor gamma (RORγt), which is the critical transcription factor for Th17 fate. When RORγt-deficient $CD4^+$ T cells were retrovirally transfected with a plasmid containing the *Rorc* gene, which encodes for RORγt, leptin treatment was shown to increase transcription of RORγt in these cells (62). This mechanism could also explain the inhibition

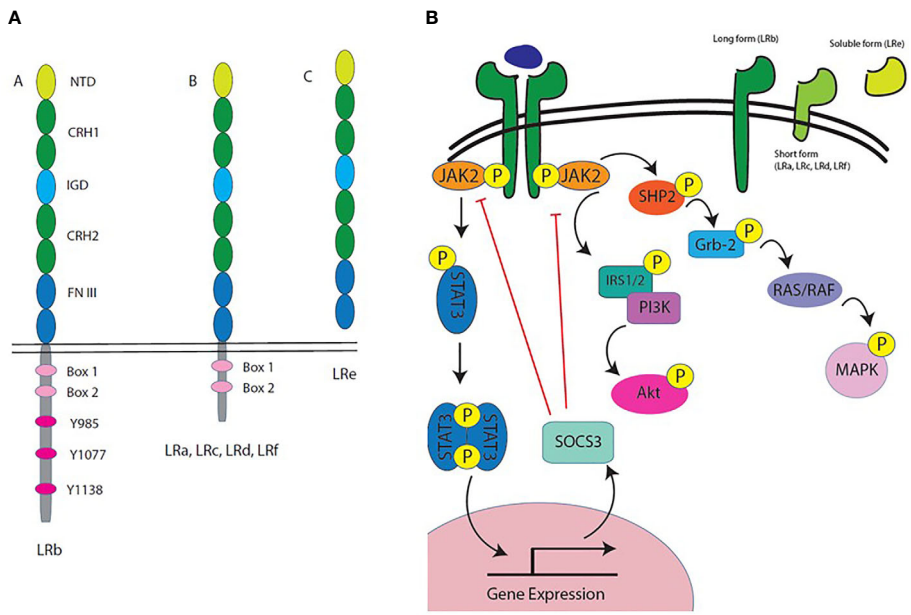

FIGURE 1 | Leptin receptor isoforms and intracellular signaling. **(A)** Leptin receptor is composed of an extracellular domain, a transmembrane domain, and a cytoplasmic domain. All variants of the leptin receptor include the extracellular domain. The extracellular domain is composed of several protein motifs: the N terminal domain (NTD), two cytokine receptor homology (CRH) domains that make up the leptin binding site, an immunoglobulin-like domain (IGD), and two fibronectin type 3 (FN III) domains. The cytoplasmic domain of leptin receptor varies between isoforms. LRb, the long form receptor, includes two box domains and several tyrosine residues important for leptin receptor signaling. The other leptin receptor variants are labeled LRa, LRc, LRd, LRf and they all have the complete extracellular binding domain, but their intracellular tails differ; however, they all contain the two box domains. There is also a soluble form of leptin receptor in both humans and mice called LRe. In mice, LRe is directly secreted, while in humans, LRe is generated by ectodomain shedding (metalloproteases cut the receptor off the surface). **(B)** Leptin receptor isoforms are generated by alternative splicing or processing at the cell membrane. The long form of leptin receptor, also known as LRb, is the only known receptor variant that is capable of signaling through the JAK-STAT pathway. LRb has a long intracellular tail that includes several tyrosine residues that are phosphorylated for signal transduction by JAK2. LRb signaling primarily occurs through the JAK2/STAT3 pathway, with STAT3 translocating to the nucleus to modify gene expression. LRb also signals through the PI3K/Akt pathway and the MAPK pathway. These pathways in immune cells have been shown to lead to metabolic and functional changes, which could account for the pleiotropic effects of leptin on different immune cell types (56–58).

of Treg differentiation by leptin, because Th17 and Treg cells have an antagonistic developmental program, where expression of the Th17 transcriptional program inhibits Treg development and vice versa, so that leptin promotion of Th17 fate by increasing RORγt transcription also directly inhibits Treg differentiation (63, 64). Given the pro-inflammatory effect of leptin on T cells, leptin is being investigated for use in cancer treatment to enhance the tumor-fighting action of T cells (65).

Interestingly, Treg cells express high amounts of leptin receptor, and have been shown to be capable of secreting leptin (66, 67). However, Treg cells are decreased in diet-induced obesity, which is consistent with the role of leptin in inhibiting Treg cell proportions, given that leptin levels are elevated in this setting (68). Treg cell proportions are also specifically decreased in the adipose tissue in diet-induced obesity, where leptin levels are expected to be highest (69). On the other hand, leptin mutant *ob/ob* mice were shown to have increased peripheral Foxp3+ $CD4^+$ Treg cells compared to WT mice, further supporting the role of leptin, and not obesity alone, in decreasing Treg cell proportions (67). Leptin has also been shown to inhibit Treg cell proliferation in primary human cells, and blockade of leptin binding to Treg cells using anti-leptin antibodies led to increased Treg cell proliferation (67).

B Cells

Leptin has been shown in both *ob/ob* mice and in fasting hypoleptinemic mice to be critical for normal B cell development in the bone marrow (70). Fasted mice and *ob/ob* mice both exhibited reduced proportions of pre-B, pro-B and immature B cells in bone marrow, which could be rescued by either intraperitoneal or intracerebroventricular injections of leptin (70). These findings demonstrate a possible central (neurological) mechanism as well as a peripheral mechanism by which leptin may promote B cell development (70).

Additionally, leptin has been shown to promote B cell homeostasis by inhibiting apoptosis and promoting cell cycle entry. B cells from *db/db* mice showed increased apoptosis compared to B cells from WT mice (37). Moreover, leptin treatment of WT B cells *in vitro* reduced apoptosis when B cells were treated with anti-IgM, CD40L, or LPS (37). Bcl-2 expression was upregulated upon leptin treatment, while anti-apoptotic members of the Bcl-2 family such as Bax, Bim and Bad were decreased, suggesting a possible mechanism for leptin's effect on B cell survival (37). Leptin also promoted cell cycle entry by increasing the transcription of genes that regulate cell cycle, particularly in the presence of co-stimulation (37).

Human B cells stimulated with leptin *in vitro* were shown to exhibit a more pro-inflammatory phenotype characterized by increased expression of inflammatory cytokines IL-6 and TNF, as well as toll-like receptor 4 (TLR4), a pattern recognition receptor that recognizes lipopolysaccharide (LPS) found on gram-negative bacteria (71). These B cells also showed reduced class switching and IgG production in response to leptin, suggesting that while they may be more inflammatory, they do not necessarily have increased function (71). These findings are supported by another study that showed human peripheral blood B cells have increased IL-6, TNF, and IL-10 production when treated with leptin *in vitro* (72). This study further demonstrated that leptin signaling in B cells activated JAK2, STAT3, ERK1/2, and p38 MAPK pathways (72). Inhibiting these signaling molecules decreased IL-6, TNF, and IL-10 production following leptin treatment, demonstrating that signaling through JAK2, STAT3, ERK1/2 and p38 MAPK is required to increase cytokine production in response to leptin (72). Similar findings were described in B cells from obese patients, suggesting that the phenotype of inflammatory B cells in obesity may be mediated, at least in part, by leptin signaling (38, 73).

INNATE IMMUNE CELLS

Leptin has been shown to have a generally pro-inflammatory effect on innate immune cells, but with distinct effects on each innate immune cell type, as discussed below.

Macrophages and Monocytes

Macrophages are key regulators of adipose tissue inflammation in obesity and, therefore, the effects of leptin on macrophages is highly relevant in the setting of diet-induced obesity. Bone marrow derived macrophages from leptin receptor mutant *db/db* mice showed decreased phagocytosis and decreased inflammatory cytokine production in response to LPS treatment *in vitro* (39). In leptin mutant *ob/ob* mice, bone marrow derived macrophages were shown to have decreased phagocytic ability *in vitro*, and *ob/ob* mice failed to clear infections such as *Escherichia Coli* and *Klebsiella pneumonia in vivo* (39, 74). Obese Zucker (*fa/fa*) rats with a leptin receptor mutation, had reduced ability to clear the fungal infection *Candida albicans in vivo*, as measured by colony-forming units in lung, liver, spleen, heart, and kidney (75). Furthermore, mice with macrophage-specific deletion of the leptin receptor had impaired clearance of *Streptococcus pneumoniae* in the lungs and spleen (40). The same macrophage specific leptin receptor knockout mice also had elevated pulmonary IL-13 and TNF compared to WT mice 48 h after infection with *S. pneumoniae* (40). Complementary *in vitro* studies of alveolar macrophages from macrophage specific leptin receptor knockout mice likewise showed decreased macrophage killing and phagocytosis (40). Thus, leptin acts specifically on macrophages *via* the leptin receptor to promote both phagocytosis and cytokine production (40).

Monocytes are innate immune cells that can differentiate into tissue-specific macrophages and myeloid-derived dendritic cells. Primary human monocytes from PBMCs and THP-1 monocytes, a human monocyte cell line, have been shown to increase toll-like receptor 2 (TLR2) expression in response to leptin treatment *in vitro* (41). TLR2 is a pattern recognition receptor that allows innate immune cells to recognize pathogens. By promoting TLR2 expression on monocytes, leptin is able to promote the innate immune response to pathogens such as *E. coli*. In human studies, leptin treatment of monocytes isolated from PBMCs increased the production of type 1 cytokines, including IL-1β, IL-6, and TNF, and resistin (42). Like in T cells, leptin appears to promote an inflammatory phenotype in monocytes.

Mast Cells

Another innate immune cell that has been shown to respond to leptin is the mast cell. Mast cells are best known for their roles in allergic response and protecting against helminth infection. Leptin mutant *ob/ob* mice showed decreased percentage of mast cells in inguinal adipose tissue, but did not show mast cell deficiencies in other tissues (76). Several studies have proposed a role for mast cells in polarization of macrophages by secretion of cytokines (77). For example, IL-33 treatment of mast cells causes production of IL-6 and IL-13, which are cytokines known to promote alternatively activated macrophages that suppresses T cell inflammation (77). One group has investigated the role of leptin in mast cell function and the subsequent effect on macrophages in the context of obesity (43). In this study, mast cells derived from WT bone marrow (BMMCs) were co-cultured with bone marrow-derived macrophages (BMDMs) from leptin receptor mutant *db/db* mice, in the presence or absence of leptin. Leptin treatment of the mast cells led to increased macrophage production of IFN-γ (43). In the same study, leptin inhibited the anti-inflammatory M2-like macrophage phenotype by decreasing arginase-1 and IL-10 expression (43). Mast cells from leptin mutant *ob/ob* mice, on the other hand, promoted maturation of WT macrophages to an M2-like anti-inflammatory phenotype when they were co-cultured *in vitro*, suggesting that leptin production by mast cells may be important in promoting a pro-inflammatory macrophage phenotype (43). Mast cells are also known to play a role in adipose tissue remodeling in obesity, promoting the inflammatory phenotype of adipose tissue by secreting inflammatory molecules such as TNF and pro-angiogenesis molecules such as chymase (78).

Dendritic Cells

Dendritic cells (DCs) function at the interface of the innate and adaptive immune system by uptaking, processing, and presenting antigens to T cells. DCs were shown to express leptin receptor, both at the protein and mRNA level, which signals through STAT3 upon stimulation (44). Furthermore, leptin was found to have an anti-apoptotic effect on DCs *in vitro* by increasing expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL (44). Mature DCs are more capable of stimulating an appropriate and strong T cell response; at homeostasis, leptin promoted DC maturation and function (45). Leptin treatment of DCs increased production of IL-1β, IL-6, IL-12, TNF, and MIP-1α (44). DCs generated from the bone marrow of leptin mutant *ob/ob* mice (BMDCs) showed reduced expression of MHC-II, CD80, CD86, and CD40 (45). MHC-II and CD80/86, in particular, are critical for activating $CD4^+$ T cells, and $CD4^+$ T cells stimulated in co-culture by BMDCs from *ob/ob* or *db/db* mice produced less IFN-γ and proliferated less than $CD4^+$ T cells stimulated by BMDCs from WT mice (45). Furthermore, BMDCs from *ob/ob* mice produced less IL-6, IL-12, and TNF after two days of maturation (45).

Neutrophils, Basophils, and Eosinophils

Neutrophils are some of the best studied innate immune cells with regard to leptin response. Interestingly, neutrophils only express the short form leptin receptor, which lacks JAK-STAT signaling (79), as shown in **Figure 1**. Leptin has been shown to inhibit neutrophil apoptosis, suggesting that leptin acts as a survival factor for neutrophils (46). Leptin also acts like a chemoattractant for neutrophils in the wildtype setting (47). *In vitro*, WT neutrophils from bone marrow (isolated by density gradient) were shown to exhibit chemotaxis toward leptin, whereas neutrophils from mice with a leptin receptor variant (Q223R) show reduced chemotaxis toward leptin (22, 47). In various infection models, leptin receptor deficiency (*db/db* mice) was shown to reduce neutrophil trafficking to the site of infection (80, 81). In a model of LPS-induced lung injury, neutrophil trafficking to the lungs was impaired in *db/db* mice, as demonstrated by reduced numbers of neutrophils in the airways (BAL), while there was increased neutrophilia in the blood (81). In a model of *Clostridium difficile* colitis, leptin receptor STAT3 mutant mice (S1138) showed decreased neutrophil numbers in the lamina propria following infection (80). Furthermore, leptin administration by oropharyngeal aspiration was shown to promote neutrophil trafficking to the lungs after *E. coli* infection as determined by neutrophil numbers in bronchoalveolar lavage fluid (47). Overall, it appears that leptin primarily acts as a chemoattractant for neutrophils, particularly during infection in the lung. Polymorphonuclear neutrophils (PMNs) isolated from human blood were shown to increase their production of oxidative species after leptin treatment *in vitro*, which the authors propose would promote bacterial clearance (48). This data points to leptin promoting neutrophil function as well as chemotaxis.

Basophils and eosinophils have also been shown to express leptin receptor (49, 50). Leptin has been shown to be a survival factor for both eosinophils and basophils (49, 50). Similar to neutrophils, leptin has also been shown to act as a chemoattractant for both basophils and eosinophils. Basophils and eosinophils isolated from human blood migrated in a dose dependent manner toward leptin *in vitro* in a transwell system or similar experimental setup (49, 51, 82). Additionally, leptin promoted basophil and eosinophil trafficking toward other chemoattractants, such as eotaxin (49, 82). Specifically, human basophils exposed to leptin demonstrated increased migration *in vitro* toward eotaxin (49). Human eosinophils were pre-treated *in vitro* with leptin for 1 h prior to assessing the migration of eosinophils toward eotaxin; more leptin treated eosinophils migrated toward eotaxin than untreated eosinophils (51). Given that leptin promotes type 1 cytokine production in other immune cells, leptin treatment of basophils had a slightly counter-intuitive result in that basophils increased type 2 cytokine production, including IL-4 and IL-13 (49).

NK Cells and ILCs

At the interface between adaptive and innate immunity sit natural killer (NK) cells and innate lymphoid cells (ILCs). These cells are able to respond to pathogens with rapid cytokine production and, in the case of NK cells, killing of infected cells. NK cells and ILCs are part of a complex family of lymphocytes that have phenotypic characteristics that mirror $CD4^+$ and $CD8^+$ T cell families, and are currently under intense

study. In the leptin receptor mutant *db/db* mouse, NK percentage and number were found to be decreased in spleen, liver, lung, and blood (83). This indicates that leptin receptor is required for normal NK cell development. When NK cells from *db/db* mice were activated by poly I:C, fewer NK cells expressed CD69, an early NK cell activation marker. This indicates that leptin receptor is required for rapid activation of NK cells (83). The nuances of NK cell response to leptin treatment appear to be extremely dependent on dose and length of exposure. Brief treatment (20 min) of human NK cells with leptin increased NK cell cytotoxicity as measured by a chromium release assay (52), and 18-h leptin treatment increased human NK cell IFN-γ and perforin production, as well as inflammatory markers, such as TRAIL (52, 53). Long exposure (72 h) to leptin, however, inhibited NK cell production of IFN-γ, as measured by ELISA, and cytotoxicity, as measured by chromium release assay (52).

Leptin was shown to promote ILC2 and Th2 cytokine production in allergic airway disease, demonstrating that increased leptin levels associated with obesity could be driving the increased risk for allergy/asthma that is observed in obesity (54). While a Th2-type phenotype is not considered pro-inflammatory, this is another example of how leptin can license immune cells to perform their functions, even in tissues outside of adipose.

MECHANISMS OF LEPTIN EFFECTS ON IMMUNE CELLS

The downstream effects of leptin receptor signaling have been best studied in $CD4^+$ T cells, where leptin signaling promotes a measurable and direct effect on cellular metabolism.

Leptin Receptor Signaling

The mechanism of leptin's actions on immune cells is complex, in part because leptin receptor has several isoforms generated though alternative splicing, which each have differing signaling capacities (84), as shown in **Figure 1**. For example, T cells express the long form of the leptin receptor, particularly after activation, while neutrophils only express the short form, and NK cells express both the short and long form receptors (85). These isoforms differ primarily in the intracellular domain responsible for downstream signaling. While both the short and long receptor isoforms are capable of transmitting some signals inside the cell, it is believed that only the long form has complete signaling capabilities.

The long form of the receptor contains fully functional JAK2 binding sites, and upon leptin binding, the leptin receptor has been shown to homodimerize, bind to, and phosphorylate JAK2 (84). STAT proteins are then recruited to the receptor complex and phosphorylated, which leads to STAT dimerization, translocation to the nucleus, and binding to promoter sites. The system is highly regulated, as this signaling also leads to transcription of SOCS3, which is a negative regulator of the JAK/STAT signaling cascade. Leptin receptor can also signal through the PI3K/Akt and MAPK pathways through IRS-1/2 and SHP-2 recruitment, respectively (86).

Leptin Effects on Cellular Metabolism

It is now clear that leptin signaling through leptin receptor promotes a metabolic change in $CD4^+$ T cells. Since immune cell metabolism and function are intimately related, recent work has investigated if leptin-induced changes in $CD4^+$ T cell function are mediated by changes in T cell metabolism (87). This was first explored in a fasting model of hypoleptinemia. $CD4^+$ T cells isolated from fasted mice and activated *in vitro* showed decreased glucose uptake and decreased glycolytic rate compared to $CD4^+$ T cells isolated from *ad lib* fed control mice, suggesting that leptin signaling promotes glycolytic metabolism in $CD4^+$ T cells (31). As glycolytic metabolism is strongly associated with inflammatory function, this fits with the previously discussed role of leptin in promoting inflammatory cytokine production in $CD4^+$ T cells (31, 34). $CD4^+$ T cells isolated from leptin receptor mutant *db/db* mice also showed reduced glucose uptake, in part secondary to decreased glucose transporter Glut1 expression, and decreased glycolytic rate compared to WT $CD4^+$ T cells when activated *in vitro*. Additionally, $CD4^+$ T cells from *db/db* mice were less metabolically active with decreased extracellular acidification rate (ECAR), a measure of lactate production downstream of glycolysis, as well as decreased oxygen consumption rate, a measure of mitochondrial oxidation (31). These studies indicate that leptin receptor signaling in T cells leads to changes in cellular metabolism.

The functional subsets of $CD4^+$ T cells have distinct metabolic characteristics, and leptin influences the metabolism of these subsets in different ways. $CD4^+$ T cells were isolated from WT mice that were either fed *ad lib*, fasted for 48 h to promote hypoleptinemia, or fasted while receiving twice daily intraperitoneal leptin injections, and differentiated *in vitro* into Th17 or Treg cells. Th17 cells generated from fasted mice showed decreased ECAR and oxygen consumption rate (OCR), but this was rescued when fasted mice received leptin injections (34). In contrast, Treg cell metabolism was not impacted by fasting (34). To investigate the direct role of leptin signaling on T cell metabolism, $CD4^+$ T cells were isolated from T cell specific leptin receptor conditional knockout mice or WT controls and differentiated into Th17 or Treg cells *in vitro* (34). Th17 cells from leptin receptor knockout mice, but not Treg cells, showed decreased expression of key metabolic genes Glut1 and hexokinase 2 (HK2), which is a rate-limiting enzyme of glycolysis (34). Th17 cells from leptin receptor knockout mice also had decreased glucose uptake and lactate production compared to Th17 cells from WT controls, suggesting that leptin signaling promotes appropriate Th17 cells glycolytic signaling to fuel Th17 cell function (34). Combined, these data suggest that leptin has a T cell intrinsic effect on metabolism that promotes glycolytic and oxidative metabolism necessary for proper T cell function.

ROLE OF LEPTIN IN IMMUNE-MEDIATED DISEASE

Leptin has been implicated in a number of immune-mediated diseases, many of which are also associated with obesity. These

range from type 2 diabetes to autoimmune disease to infection. In this section, we will explore the role that leptin plays in mediating the immune response in obesity-associated disease.

Metabolic Disease: Type 2 Diabetes

The incidence of type 2 diabetes mellitus (T2DM) is increasing in parallel with the prevalence of obesity. Obesity-associated inflammation has been shown to drive insulin resistance, leading to T2DM (56). Methods that eliminate the inflammatory T cell or macrophage response in obesity prevent insulin resistance and progression to T2DM. For example, several immunocompromised mouse models (NOD and SCID mice) have been found to be resistant to the development of obesity and insulin resistance when fed high fat diet (88). Elimination of CD11c+ macrophages in a mouse model of obesity resulted in increased insulin sensitivity (89), and a less specific macrophage deletion strategy using chlodronate liposomes leading to apoptosis of phagocytic cells also resulted in increased insulin sensitivity and improved systemic glucose tolerance (90). T cell-deficient TCR-knockout mice that lack $CD4^+$ and $CD8^+$ T cells had decreased obesity-induced macrophage infiltration and decreased insulin resistance on high fat diet compared to wildtype controls (91), and obese mice that lack IFN-γ had improved insulin sensitivity compared to obese wildtype controls (92). Similarly, knockout of the Th1-associated transcription factor T-bet improved insulin sensitivity in high-fat diet fed mice (93). Based on the pro-inflammatory effect of leptin on immune cells as described above, it is possible that obesity-associated hyperleptinemia is responsible, at least in part, for promoting the obesity-associated inflammation that leads to insulin resistance and diabetes in obesity.

Autoimmunity

In addition to metabolic syndrome and T2DM, obesity predisposes patients to select autoimmune and inflammatory diseases such as multiple sclerosis (MS), rheumatoid arthritis, and systemic lupus erythematosus (1, 2). Leptin deficiency has been shown in mice to protect against experimental autoimmune encephalomyelitis (EAE) (94), colitis (95), T cell mediated hepatitis (96), and glomerulonephritis (97). One key example is the well-studied autoimmune model EAE, a mouse model of MS. Leptin has been shown to play a critical role in EAE progression, and leptin mutant *ob/ob* mice are protected from development of EAE (94). Furthermore, EAE disease scores were reduced when anti-leptin antibodies were administered either before or after the induction of EAE in mice (98).

Since inflammatory Th17 cells play an important role in the pathogenesis of EAE, and leptin is known to promote Th17 cell differentiation, the role of leptin signaling on T cells in EAE was investigated. T cell specific leptin receptor knockout mice were protected from EAE compared to WT mice, with lower disease scores (61). Furthermore, the cytokine profile of mice treated with anti-leptin antibodies was changed to a non-inflammatory Th2/Treg cytokine profile (IL-4, IL-10) instead of the pro-inflammatory Th1/Th17 cytokine profile typically seen in EAE (98). Blocking leptin also decreased proliferation of antigen specific T cells in this autoimmune model (98). These studies indicate a specific role for leptin in promoting inflammatory T cell proliferation and function that promotes EAE disease progression.

In a model of fasting-induced hypoleptinemia, C57BL/6 mice fasted for 48 h had lower disease scores than *ad lib* fed mice following EAE induction, but this effect was reversed by exogenous leptin treatment administered during the fasting period (34). This demonstrates that leptin alone is sufficient to license the development of autoimmunity in undernourished mice that were otherwise protected against disease. In the same study, Th17 cells from fasted mice undergoing EAE induction had decreased expression of the key glycolytic protein HK2 as well as decreased expression of the glycolysis-promoting regulator HIF-1α, and both HK2 and HIF-1α levels were normalized when fasted mice were treated with leptin. In human studies, serum leptin levels were found to be increased prior to onset of clinical symptoms in relapsing-remitting MS, indicating that leptin may both contribute to the pathogenesis of MS and be a useful marker of disease (99, 100).

Infection

The link between leptin and susceptibility to infection has been studied in animal models. Leptin mutant *ob/ob* mice were shown to be more susceptible to death by LPS stimulation, and leptin treatment was shown to partially reverse this effect (101, 102). Interestingly, LPS and other inflammatory signals have been shown to induce leptin production from adipose tissue (103–106). It is possible that this increase in leptin can then stimulate the inflammatory response necessary to fight the infection that LPS is modeling.

Many studies have examined the effect of leptin treatment on various bacterial models of infection in mice. Leptin universally decreased bacterial load and improved survival or immune response to infection with *Mycobacterium tuberculosis*, *Klebsiella pneumonia*, and *Pneumococcal pneumonia* (107). These data indicate that leptin is important for promoting the proper immune response to clear bacterial infections.

Leptin receptor mutant *db/db* mice also had reduced survival and impaired viral clearance when infected with influenza virus, as well as reduced IFN-γ production in the lungs following infection (108). Interestingly, when lung epithelium or alveolar macrophages, specifically, were deficient in leptin receptor, the mice cleared virus better than global leptin receptor knock out mice (108). These data indicate that in influenza infection, the response to leptin of other immune cells, such as T cells, B cells or NK cells, is key to clearing virus.

LEPTIN STUDIES IN HUMANS

Congenital leptin deficiency in humans, while rare, can provide important information regarding the role of leptin. Genetic mutations in both the leptin gene and the gene for leptin receptor have been described, and these genetic variants cause similar phenotypes in terms of immune response. Mutations in leptin or the leptin receptor gene cause early onset extreme obesity, hyperphagia, hypogonadism, and metabolic disorders

(109). Furthermore, these patients develop repeat infections, and humans with leptin deficiency are at increased risk of death due to intracellular infections (28). Leptin replacement therapy has been shown in humans to increase $CD4^+$ T cell numbers and reverse defects in $CD4^+$ T cell proliferation and cytokine production (110). These data clearly underscore the importance of leptin in normal immune function and protection from infection. Consistent with this, fasting reduces leptin levels and leads to reduced lymphocyte counts in the blood (111).

On the other hand, obesity is also associated with increased morbidity and mortality in response to select infections such as bacterial cellulitis (112), influenza (113–117), and coronavirus (118–124), although the role for leptin in this setting has not been determined. While the etiology of obesity is complex, it is possible that increased leptin signaling promotes excessive inflammation and potentially cytokine storm.

CONCLUSION

Leptin is a pleiotropic adipokine with diverse effects on cell types throughout the body. Its role in neuroendocrine signaling, homeostasis, and metabolism has been well studied. More recently, leptin has been identified as an important immune modulator with a wide range of functions, many of which are pro-inflammatory. The complexity of leptin receptor signaling, as well as the several variants of the receptor with unique signaling capabilities likely allows for the diversity of effects that are mediated on distinct immune cells, sometimes located within the same tissues. Overall, it is clear that leptin plays a critical role in obesity-associated inflammation by promoting pro-inflammatory immune phenotypes. While leptin has not been successful in treating obesity as a weight loss drug, it is possible that targeting leptin or leptin signaling could be therapeutic for autoimmune disease or the low-grade, chronic inflammation associated with obesity and metabolic syndrome.

AUTHOR CONTRIBUTIONS

Both KK and NM contributed to the writing and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Adipose Extracellular Vesicles in Intercellular and Inter-Organ Crosstalk in Metabolic Health and Diseases

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Adipose tissue (AT) is a highly heterogeneous and dynamic organ that plays important roles in regulating energy metabolism and insulin sensitivity. In addition to its classical roles in nutrient sensing and energy storage/dissipation, AT secretes a large number of bioactive molecules (termed adipokines) participating in immune responses and metabolic regulation through their paracrine and/or endocrine actions. Adipose-derived extracellular vesicles (ADEVs), including exosomes, microvesicles (MVs), and apoptotic bodies, have recently emerged as a novel class of signal messengers, mediating intercellular communications and inter-organ crosstalk. In AT, ADEVs derived from adipocytes, immune cells, mesenchymal stem cells, endothelial cells are actively involved in modulation of immune microenvironment, adipogenesis, browing of white adipose tissue, adipokine release and tissue remodeling. Furthermore, ADEVs exert their metabolic actions in distal organs (such as liver, skeletal muscle, pancreas and brain) by sending genetic information (mainly in the form of microRNAs) to their target cells for regulation of gene expression. Here, we provide an updated summary on the nature and composition of ADEVs, and their pathophysiological functions in regulating immune responses, whole-body insulin sensitivity and metabolism. Furthermore, we highlight the latest clinical evidence supporting aberrant production and/or function of ADEVs as a contributor to obesity-related chronic inflammation and metabolic complications and discuss the opportunities and challenges in developing novel therapies by targeting ADEVs.

Keywords: exosome, microRNA, adipose tissue macrophage, metabolic homeostasis, inflammation, cell-cell communication

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INTRODUCTION

Obesity, characterized by excessive accumulation of adipose tissue (fat), is a highly complex multifaceted chronic disease and one of the major risk factors for a cluster of cardio-metabolic diseases, including type 2 diabetes (T2D), dyslipidemia, non-alcoholic fatty liver disease (NAFLD), hypertension, coronary heart disease and stroke (1). Furthermore, obesity-related complications are the most commonly reported underlying conditions that predispose individuals with viral infections, including the current coronavirus disease (COVID-19) to severe outcomes (2).

Adipose tissue is a complex metabolic organ with profound effects on the regulation of systemic metabolism and maintenance of energy homeostasis. In addition to its classical role in nutrient handling, including energy storage in the form of triglycerides during feeding and releasing of free fatty acids (FFAs) during fasting, adipose tissue also serves as an active endocrine organ secreting a variety of adipokines, which are bioactive peptides including cytokines, peptide hormones and enzymes acting in an autocrine, paracrine or endocrine manner to regulate energy metabolism, immune responses and cardiovascular homeostasis. Adipokines have been shown to modulate adipogenesis, adipocyte metabolism, and immune cell infiltration locally within adipose tissue. Additionally, they can exert their biological effects in distal organs to maintain systemic energy homeostasis and insulin sensitivity (3, 4). However, during obesity, adipose tissue undergoes unhealthy expansion leading to numerous detrimental consequences, including dysregulated secretion of adipokines, hypoxia, cell death and altered immune microenvironment which give rise to adipose inflammation (5). Unresolved chronic inflammation in adipose tissue is a major contributor to systemic low-grade inflammation and has been reported as a culprit in obesity-related comorbidities. The first evidence for adipose inflammation at the interface between obesity and metabolic dysregulation was provided by studies demonstrating increased production and secretion of the inflammatory cytokine tumor necrosis factor alpha (TNFα) from adipose tissues in obese rodents and human subjects (6, 7). Neutralization of TNFα in obese rats counteracted diet-induced insulin resistance and glucose intolerance (7). Subsequent studies reported that selective inactivation of pro-inflammatory signaling pathways in adipose tissue by inhibition of key signaling molecules, including c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) disrupted the link between obesity and metabolic dysregulation (8–10).

In addition to the classical polypeptide adipokines and cytokines, various types of cells in adipose tissue also produce and release extracellular vesicles (EVs) including exosomes with a diameter of 30–100 nm originated from cytoplasmic multivesicular bodies that fuse with the plasma membrane and microvesicles (MVs) that are 100–1000 nm in diameter and released directly from the plasma membrane into the extracellular space. Both types of such adipose-derived extracellular vesicles (ADEVs) are similar to the original cells in composition, transporting bioactive molecules, including proteins, lipids, and nucleic acids to their target cells within adipose tissue or in distant organs, therefore mediating intercellular and interorgan crosstalk. A growing body of evidence suggest that ADEVs play important roles in the regulation of metabolic inflammation, energy metabolism and insulin sensitivity (11–15). Altered abundance or content of ADEVs may be causally linked to obesity-related metabolic complications.

In this review, we summarize the nature and compositions of ADEVs derived from different cellular origins in adipose tissue and their roles as local and/or distal signaling mediators in regulating metabolic homeostasis. Furthermore, we highlight the latest evidence for the clinical association of aberrant production and/or functions of ADEVs with various obesity-related metabolic disorders, and discuss the therapeutic potentials of targeting ADEVs for the treatment of obesity-related metabolic complications and challenges in this field.

ADIPOSE TISSUE IN HEALTH AND METABOLIC DISEASES

Heterogeneity of Adipose Tissue

Adipose tissue in mammals is categorized into two main types, white adipose tissue (WAT) and brown adipose tissue (BAT). WAT mainly consists of white adipocytes, which contain a single large lipid droplet (referred to as unilocular lipid structure) and few mitochondria, thus is a primary site for energy storage. White adipocytes are highly responsive to hormones such as insulin to take up and store nutrients in the form of triglycerides after food ingestion. They also respond to biogenic amines such as catecholamines to supply energy in the forms of FFAs and glycerol during nutrient deprivation (5). WAT is distributed throughout the body. Main depots include subcutaneous adipose tissue (SAT), which is beneath the skin storing more than 80% of total fat in the body and is mainly located in the abdominal and gluteofemoral regions in humans or between the scapulae and in the inguinal region spreading from the dorsolumbar to the gluteal region in rodents, and visceral adipose tissue (VAT), which stores 5–20% of total body fat and is associated with internal organs mainly in perigonadal, mesenteric, retroperitoneal, epicardial and periadventitial regions in rodents and humans (16, 17). In addition, there are also small adipose depots including epicardial and intermuscular adipose tissue with specialized functions related to cardiovascular system or skeletal muscle (17). While the major function of SAT is to store excess energy in response to energy surplus and is therefore considered as beneficial, VAT is more closely linked to adverse metabolic profile and inflammation in obese subjects (18, 19).

BAT mainly consists of brown adipocytes with multilocular lipid droplets and a large number of highly oxidative, naturally uncoupled mitochondria, and is important for the regulation of body temperature through non-shivering thermogenesis. The thermogenic capacity of brown adipocytes is primarily attributed to the mitochondrial inner membrane protein, uncoupling protein-1 (UCP1), which catalyzes a proton leak across the inner mitochondrial membrane, thus uncouples oxidative phosphorylation from ATP synthesis, and converts chemical energy to heat (20, 21). Brown adipocytes are located in the well-defined anatomical BAT depots such as interscapular, peri-aortic, intercostal and mediastinal regions of rodents. In addition to the classical brown adipocytes, beige adipocytes also contribute to thermogenesis. Although beige adipocytes share similar morphological characteristics and thermogenic capacity with classical brown adipocytes, they arise from different precursor cells (22, 23). In humans, although early studies suggested that BAT is present only in neonates to prevent from hypothermia resulted from high body surface area-to-mass ratio, recent positron emission tomography coupled with computer

tomography (PET/CT)-based approaches have identified the existence of metabolically-active BAT in the supraclavicular, ventral cervical and thoracic regions of adults (24–26). While the predominant form of the interscapular BAT in human neonates is classical brown adipocytes, BAT in human adults share more molecular features with beige adipocytes (23). Furthermore, both amount and activity of BAT in adults is negatively correlated with body weight, T2D and cardiovascular events, but positively correlated with energy expenditure (24, 25, 27).

Cellular Composition of Adipose Tissue

Although adipocytes are the dominant cell type in adipose tissue, there are also non-adipocyte compartment named as stromal vascular fraction (SVF), which include preadipocytes, adipose tissue-derived stem cells (ADSCs), endothelial cells, pericytes, and various immune cells. Preadipocytes can be differentiated into mature adipocytes to regulate adipogenesis and WAT expansion (28). ADSCs undergo self-renewal and are multipotent, with the potential to differentiate into numerous cell types, including adipogenic lineages, endothelial cells, osteoblasts, chondrocytes and myocytes (29). Endothelial cells and pericytes provide vasculature to adipose tissue by forming capillaries (22, 30–33). Presence of immune cells was not realized till discovery of adipose-resident macrophages responsible for producing pro-inflammatory cytokines in obese mice and humans in the early 2000s (34, 35). It is now known that adipose tissue is home to both innate immune cells such as macrophages, neutrophils, eosinophils and dendritic cells and adaptive immune cells, including T cells and B cells, which collaboratively play important roles in clearance of apoptotic cells, maintenance of adipose tissue function and homeostasis (36).

Adipose Tissue Inflammation as a Culprit in Obesity-Related Disorders

A growing body of evidence suggests that chronic inflammation in adipose tissue, characterized by infiltration of pro-inflammatory immune cells and aberrant production of adipokines, is a major contributor to obesity-induced systemic inflammation, insulin resistance and metabolic dysregulation (37, 38). Obesity leads to an expansion of adipose tissue driven by adipocyte hyperplasia and hypertrophy. The lipid-laden adipocytes in obesity undergo necrosis and/or apoptosis, leading to aberrant production of adipokines and altered cellular composition in adipose tissue (39). In obesity, the hypertrophic adipocytes exhibit impaired secretion of anti-inflammatory adipokines such as adiponectin, but augmented secretion of a large number of pro-inflammatory mediators, such as IL-6, C-C motif chemokine ligand 2 (CCL2), IL-1β and resistin that lead to a chronic inflammatory state linking obesity to its cardiometabolic comorbidities including insulin resistance, T2D and cardiovascular events (40).

During the progression of obesity, expansion of adipose tissue also causes infiltration and activation of immune cells involved in both innate and adaptive immunity, which in turn trigger a series of inflammatory responses within the tissue. Among adipose-resident immune cells, macrophages are the most abundant cell type, accounting for 40–50% of total cells of adipose tissue in obese humans (41). In obese adipose tissues, macrophages form crown-like structures (CLSs) surrounding dying or dead adipocytes. The number of adipose tissue–resident macrophages (ATMs) is closely associated with the magnitude of insulin resistance and metabolic perturbance, whereas selective depletion of ATMs by genetic or pharmacological approaches is sufficient to prevent obesity-related insulin resistance and metabolic complications in obese mice (34, 42). Macrophages are highly plastic in nature, exhibiting different phenotypes ranging from the classically activated, pro-inflammatory M1 to alternatively activated, anti-inflammatory M2 in response to changing environment (41). The lean adipose tissue is dominated by M2 macrophages which plays an important role in maintaining the tissue homeostasis through phagocytosis of dead adipocytes, secretion of anti-inflammatory cytokines and other regulatory factors for angiogenesis, adipogenesis, and regulation of adaptive thermogenesis (43). However, obesity causes a striking phenotypic change of ATMs from the anti-inflammatory M2 toward the pro-inflammatory M1, the latter of which produce pro-inflammatory cytokines to exacerbate metabolic inflammation and insulin resistance (41, 43). However, the precise mechanisms whereby adipocyte and various immune cells crosstalk with each other to aggravate obesity-induced adipose inflammation and metabolic dysregulation remain poorly defined.

CELLULAR ORIGIN OF ADEVs AND THEIR ROLES IN CELL-CELL COMMUNICATIONS

EVs are enclosed by a lipid bilayer and classified into three main classes, including exosomes, MVs and apoptotic bodies (44). Exosomes are a homogenous population of EVs at 30–100 nm in diameter. Biogenesis of exosomes begins from endocytosis-mediated invagination of the plasma membrane, resulting in endocytotic vesicles, which are subsequently transported to the early endosomes. Membranes of the endosomes are budded into the lumen to form intraluminal vesicles (ILVs) or multivesicular bodies (MVBs). MVBs can fuse with lysosomes for degradation or with the plasma membrane to release the internal vesicles into extracellular space as exosomes (45). Exosomes show the same orientation with the plasma membrane composed of a lipid bilayer with extracellular domains of proteins exposed at the surface. The lipid bilayer of exosomes encloses a droplet of cytoplasm containing various types of molecules including nucleic acids, proteins and lipids (45). Cells can also produce MVs with heterogenous populations ranging from 100 to 1,000 nm in diameter. In contrast to exosomes derived from the endolysosomal pathway, MVs are formed by direct budding and shedding from the plasma membrane (44). EVs released from cells undergoing apoptosis are referred to as apoptotic bodies with a diameter of 1,000–5,000 nm (44).

Multiple types of cells in adipose tissue, including adipocytes, macrophages, ADSCs and endothelial cells are known to secrete EVs, which in turn, act in a paracrine or endocrine manner to mediate intercellular and inter-organ crosstalk in modulation

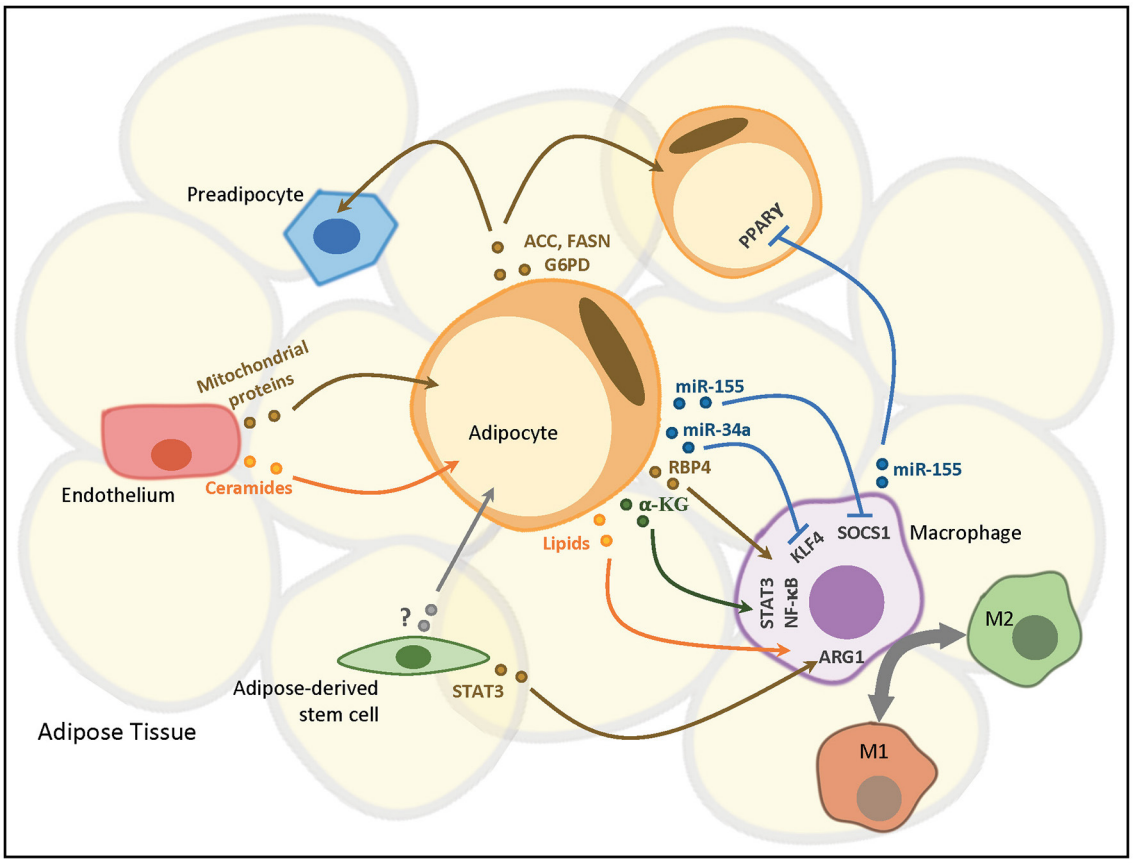

FIGURE 1 | ADEVs-mediated intercellular communication in adipose tissue. Adipocytes mediate the polarization and immunomodulatory responses of adipose-resident macrophages (ATMs) in a paracrine manner via various vesicular components. ATMs reciprocally regulate adipocyte insulin sensitivity by releasing miRNA-containing EVs to adipocytes. Adipocytes also delivery exosomal proteins to neighboring preadipocytes and adipocytes in both a paracrine and an autocrine manner, respectively, to modulate lipogenesis. Adipose-derived stem cells (ADSCs) confer EV-mediated paracrine effects on both adipocytes and ATMs to regulate adipocyte reprogramming and macrophage polarization, respectively. Endothelial cells in adipose tissue transfer EVs containing proteins and lipids capable of modulating cellular signaling pathways to adipocytes. ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; PPARγ, peroxisome proliferator activated receptor gamma; RBP4, retinol binding protein 4; α-KG, α-ketoglutarate; SOCS1, suppressor of cytokine signaling 1; KLF4, krüppel-like factor 4; STAT3, signal transducer and activator of transcription 3; NF-κB, nuclear factor kappa B; ARG1, arginase 1.

of adipose tissue and systemic homeostasis (**Figure 1**), as detailed below.

Adipocytes

The presence of EVs in the culture medium of adipose tissue explants has been demonstrated in both mouse and human studies (46, 47). Further analysis of EVs isolated from *ex vivo* human adipose tissue explant cultures has identified both adiponectin-positive and adiponectin-negative subsets by differential ultracentrifugation combined with immunoblotting analysis (47). Since adiponectin is expressed predominantly in adipocytes, the adiponectin-positive EVs were suggested to be derived from adipocytes. In agreement with this notion, certain portion of exosomes isolated from mouse serum has also been demonstrated to contain adiponectin and low level of resistin (48). However, as detection of adiponectin in EVs was achieved by immunoblotting, whether adiponectin is located on the surface or inside of adipocyte-derived EVs remains unknown. The secretion of EVs from adipocytes has been further confirmed by *in-vitro* cultures of rat primary adipocytes or adipocytes differentiated from mouse 3T3-L1 pre-adipocytes and human Simpson Golabi Behmel Syndrome (SGBS) pre-adipocytes (47, 49). In addition to adiponectin and resistin, there are several other adipocyte-specific proteins have been identified as the markers of adipocyte-derived EVs, including perilipin A and fatty acid binding protein 4 (FABP4) (50, 51).

Although adipocyte-derived exosomes account for a minority of circulating exosomes under normal condition (52), production of ADEVs can vary under different conditions. For example, the circulating level of lipid-filled vesicles derived from adipocytes was increased by approximately 2-folds in obese mice vs. lean animal (52). Similarly, the number of exosomes isolated from VAT was elevated in human patients with insulin resistance (47). Under chronic cold exposure, the number of exosomes released from explants isolated from both interscapular BAT and inguinal WAT

of mice was significantly induced. *In-vitro* studies showed that release of exosomes from beige and brown adipocytes was increased by treatment with cAMP, which is the second messenger induced by cold exposure or beta-adrenergic stimulation (53).

Adipocyte-derived EVs confer in part the paracrine interaction between adipocytes and macrophages. EVs released from human adipocyte culture were able to induce differentiation of monocytes into ATM-like macrophages *in vitro*. Adiponectin-positive EVs from human adipose tissue explants were more potent than the adiponectin-negative subset in promoting monocyte differentiation into ATMs as they induced the expression of mixed pro- and anti-inflammatory markers, which are characteristic of ATMs, in monocytes *in vitro* (47). Furthermore, adipocyte-derived EVs isolated from high-fat diet (HFD)-fed mice drove polarization of macrophages toward the pro-inflammatory M1 phenotypes in bone marrow-derived macrophages (BMDMs) *in vitro* by miR-155, which inhibited the expression of suppressor of cytokine signaling 1 (SOCS1), leading to suppression of signal transducer and activator of transcription 6 (STAT6) (54). In addition to adipose tissue, adipocytes also exist in tumor microenvironment, including melanoma. It is reported by recent studies that adipocyte-secreted exosomes were taken up by tumor cells, resulting in increased melanoma migration and invasion through fatty acid oxidation. Such effects were amplified in obese animals (55, 56).

Macrophages

In addition to adipocytes, ATMs also produce EVs to modulate inflammatory responses and metabolic homeostasis. *In vitro*, secretion of EVs was detected in the culture medium of human THP-1-derived macrophages (14, 57). These EVs were identified as exosomes with a diameter of 30–100 nm by using transmission electron microscopy (14). The macrophage-derived exosomes can be effectively internalized into adipocytes. Exosomes from LPS-activated macrophages promote the expression of inflammation-related genes in adipocytes (57). Interestingly, when THP-1 monocytes-derived macrophages are polarized to M1 or M2 phenotype by LPS plus IFN-γ or IL-4, respectively, exosomes derived from M1 macrophages impair insulin signaling in human adipocytes, while the M2 macrophage-derived exosomes enhance insulin signaling and glucose uptake in adipocytes (14). Likewise, EVs harvested from ATMs isolated from VAT of mice were also found to be exosomes as they are 30–100 nm in size (13). It is further evidenced by detection of exosomal membrane markers in the EVs, including TSG101, syntenin 1, CD63, and CD9. In line with the *in-vitro*-based findings, treatment with ATM-derived exosomes from lean mice ameliorated diet-induced glucose intolerance and insulin resistance in obese mice, whereas administration of exosomes isolated from ATMs of obese mice promoted glucose intolerance and insulin resistance in lean recipients (13). These studies collectively support a critical role of ATM-derived exosomes in the regulation of neighboring adipocytes under physiological and pathological conditions.

Adipose-Derived Stem Cells (ADSCs)

ADSCs have emerged as a potential tool for regenerative therapy due to its multipotency in differentiating into different types of cells (58). Additionally, ADSCs are also a critical player in immune regulation, and have shown potential for treatment of inflammatory and autoimmune diseases, including colitis, autoimmune diabetes and arthritis, as well as to resolve obesity-induced inflammation and metabolic dysregulation by polarization of macrophages toward the anti-inflammatory M2 phenotypes (59–62). These beneficial effects may be attributed at least in part to the paracrine effects of EVs produced from ADSCs. Zhao *et al.* isolated ADSCs from mouse VAT and found that ADSC-derived EVs were approximately 100 nm in diameter and positive for the exosomal markers TGS101, CD9, CD63, HSP90, and ALIX, thus of exosomal origin (15). It has been shown in another study that human primary ADSCs also secreted 40–100 nm particles, which had the typical characteristics of exosomes (63). However, Katsuda et al. reported that human ADSC-derived exosomes had a peak size distribution of 150–200 nm which was larger than that reported by others. However, exosomal markers CD63 and HSP90 were present, suggesting that the size range of exosomes may differ among different cell types (64).

ADSC-derived exosomes isolated from patients with and without cancer show distinct miRNA profiles. Selective enrichment of certain miRNAs, including let-7-a-1, miR-21, and miR-1260b has been identified in ADSC-derived exosomes from cancer patients (65). Treatment of hepatocellular carcinoma cells with ADSC-exosomes containing miR-122 showed increased sensitivity to chemotherapies (66). Human ADSC-derived exosomes promoted migration of breast cancer cell line (63). ADSC-derived exosomes can be internalized into ATMs, and treatment of obese mice with ADSC-derived exosomes isolated from mouse VAT attenuated obesity and insulin resistance by inducing polarization of macrophages toward the M2 phenotypes through transactivation of arginase-1 by exosome-carried active STAT3, thus beiging of WAT (15). In addition to the undifferentiated ADSCs, EVs isolated from human ADSCs during white and beige adipogenic differentiation provided biochemical cues such as miRNAs to induce the differentiation of ADSCs into white and beige adipocytes, thereby promoting adipogenesis and adipose tissue remodeling, respectively (67).

Endothelial Cells

A recent study also identified adipose tissue endothelial cells as a source of ADEVs (68). These ADEVs are enriched with the exosomal markers CD9, CD63, TSG101, and ALIX. Production of EVs from adipose endothelial cells was increased under the fasted condition, mainly through the action of glucagon. As endothelial cells are located at the interface between the circulation and adipose tissue extracellular space, endothelial cell-derived ADEVs can take up proteins and lipids such as mitochondrial components and ceramides from the bloodstream, and subsequently release the components to the adjacent adipocytes through internalization (68). Notably, the changes of EV secretion from adipose endothelial cells in response to fasting and refeeding was absent in dietary or genetic obese mouse

models, implicating the possible involvement of dysregulated adipose endothelial cell-derived EVs in the pathogenesis of obesity and its related metabolic diseases.

MAJOR COMPONENTS OF ADEVs AND THEIR ROLES IN IMMUNE RESPONSES AND METABOLIC REGULATION

EVs exert their biological functions by carrying various types of bioactive cargos including mRNAs, miRNAs, DNA, proteins and lipids to their target cells through phagocytosis or endocytosis (55, 69, 70), which in turn mediate cell-cell communications. Additionally, since EVs have the same transmembrane proteins on their surface as the cell of origin, they can also act as the ligands directly binding and activating the surface receptor of target cells to initiate cellular signaling (71). Likewise, ADEVs modulate immune responses in local adipose tissues through cell-cell communication, and systemic insulin sensitivity, glucose and lipid metabolisms through their distal effects on other major metabolic organs such as liver, skeletal muscle, and brain (**Figure 2**). Such local and distal effects of ADEVs are attributed to their unique vesicular composition, which has been characterized in great details (**Table 1**).

miRNA, mRNA and lncRNA

The exosome-mediated cellular signaling is largely dependent on their composition of miRNAs, which are small non-coding RNA molecules that post-transcriptionally regulate gene expression by binding to the 3′-untranscribed region of target mRNAs, leading to mRNA degradation and repression of translation. miRNAs are critically involved in adipogenesis and regulation of adipose tissue functions (77). Recently, ADEVs have been found as an important source of circulating miRNAs in both mice and humans. It was evidenced by a significant reduction in exosomal miRNAs in serum of adipocyte-specific Dicer knockout (ADicerKO) mice, which have abrogated miRNA processing in adipocytes (12). DICER is a key enzyme that cleaves pre-miRNAs into mature miRNA, thus important for miRNA biogenesis (78). The same study also examined the circulating exosomal miRNA profiles in patients with congenital generalized lipodystrophy and patients with HIV-associated lipodystrophy who have general loss of adipose tissue and reduced expression of adipose Dicer respectively, and found that dominant miRNAs in exosomes were significantly downregulated in the serum of both patient cohorts, suggesting that circulating miRNAs in humans also originate mainly from ADEVs (12). Defects in ADEV-derived miRNA production resulted in reduced WAT, whitening of BAT, insulin resistance and dyslipidaemia in the ADicerKO mice, demonstrating the importance of adipose tissue-specific exosomal miRNAs in the physiological regulation of systemic energy metabolism. Transplantation of BAT, but not WAT from wild-type mice in ADicerKO mice improved glucose tolerance and insulin resistance in the recipient mice which was associated with reduced production and secretion of FGF21 from the liver (12). Further investigation revealed that mRNA expression of Fgf21 in hepatocytes was suppressed by ADEV-derived miR-99b from BAT, suggesting that BAT-derived exosomal miRNAs mediate the adipose-liver crosstalk to modulate glucose homeostasis. However, both animal and clinical studies have shown beneficial effects of FGF21 in improving insulin sensitivity and alleviating hyperglycemia (79). Therefore, it is unlikely that the metabolic benefits of BAT-derived ADEVs are attributed to reduction in hepatic FGF21 expression. Further studies are needed to investigate the detailed molecular mechanism underlying the effects of BAT-derived exosomal miRNAs in the regulation of systemic glucose homeostasis.

ADEVs derived from different adipose depots appear to contain distinct miRNA composition. For example, miR-34a is selectively enriched in the exosomes from VAT, but not in SAT in both rodents and humans (11). Furthermore, high fat diet feeding leads to a progressive increase of miR-34a in the exosomes isolated from adipocytes in VAT in mice. The adipocyte-derived miR-34a is transported to the adjacent macrophages by exosomal delivery and drives the polarization of macrophages toward the pro-inflammatory M1 phenotypes by suppression of transcription factor Krüppel-like factor 4 (KLF4), which is important in maintenance of M2 macrophage phenotypes. Conversely, adipocyte-selective ablation of miR-34a protects mice against obesity-induced adipose inflammation, systemic insulin resistance and NAFLD (11). Selective enrichment of miR-34a in VAT may explain why this adipose depot is more susceptible to inflammation and is more harmful to cardiometabolic health than SAT. In addition, miRNA-containing exosomes released from ATMs can modulate systemic insulin resistance. Administration of obese ATM-derived exosomes in lean mice impaired insulin sensitivity and glucose tolerance (13, 80). Uptake of ATM-exosomes can be detected in the liver, muscle and adipose tissues of mice. *In-vitro* experiments showed that exosomes derived from obese ATMs directly impaired insulin signaling in adipocytes, myocytes and hepatocytes (80). These effects were possibly attributed to obesity-induced changes in miRNA contents in the ATM-exosomes, such as miR-155, which target the nuclear receptor PPARγ (13). Likewise, the distal effects of the exosomal miR-27a released from adipocytes of obese mice on induction of insulin resistance in skeletal muscle were also attributed to its repression of PPARγ (73). Adipocytes also regulate lipid catabolism in skeletal muscle via exosomal miR-130b. miR-130b has been shown to inhibit the expression of PPARγ coactivator 1α (PGC1α), which is important in lipid oxidative capacity and mitochondrial function (72). In addition to the aforementioned roles of miR-155 on adipocytes, hepatocytes and myocytes, exosomal miR-155 derived from ATMs of obese mice also exerts profound regulation on pancreatic β cells, leading to impaired insulin secretion and increased β cell proliferation by repressing the expression of v-maf musculoaponeurotic fibrosarcoma oncogene family protein B (MAFB) (74).

There is emerging evidence showing that environmental changes can alter the composition of adipose-derived exosomal miRNA, which in turn participates in adaptive responses to metabolic stresses. In high-attitude population, hypoxia and cold temperature causes downregulation of exosomal miR-210/92a from WAT, thereby increasing the thermogenic activity

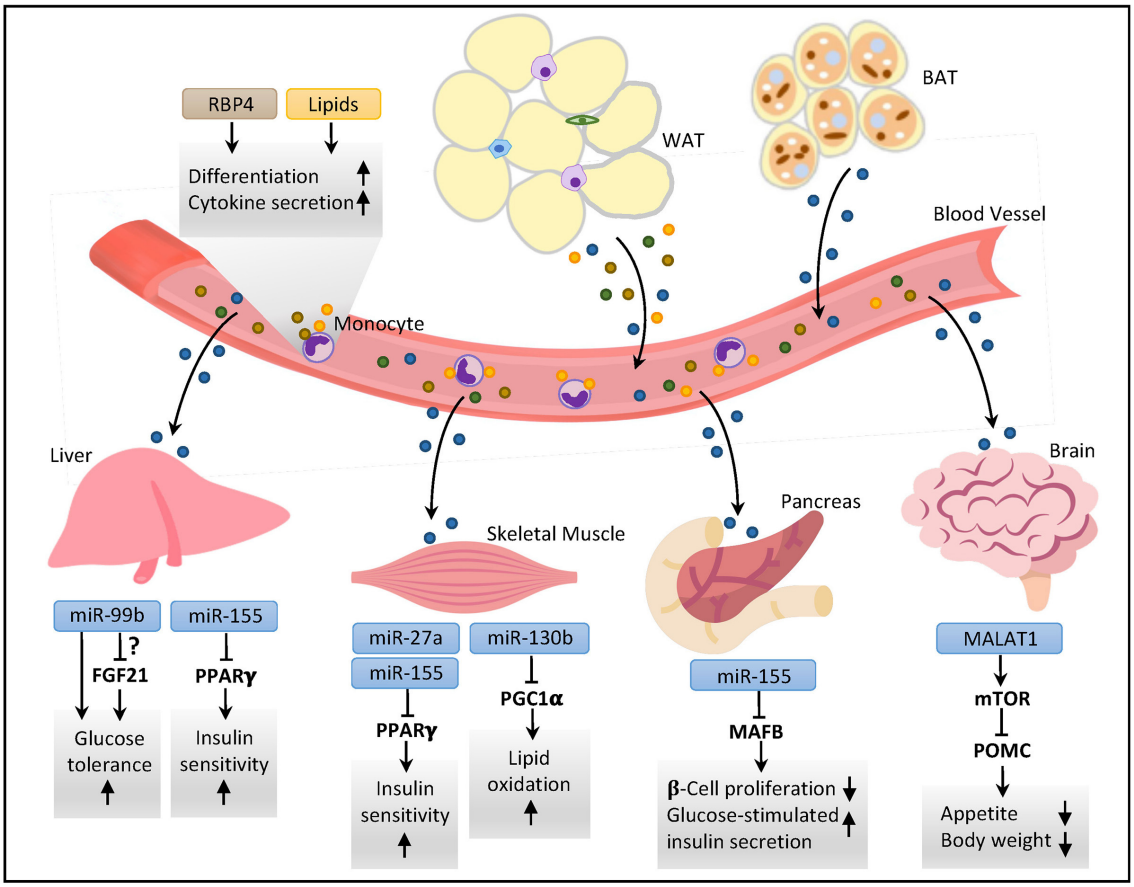

FIGURE 2 | ADEVs-mediated interorgan crosstalk in metabolic regulation. Both white and brown adipose tissues secrete EVs containing various types of vesicular components into the circulation. The ADEVs can act as endocrine factors affecting metabolic profiles in distal organs by sending bioactive vesicular molecules. In the liver, exosomal miRNAs modulate glucose tolerance and insulin sensitivity through modulation of peroxisome proliferator activated receptor gamma (PPARγ) and perhaps fibroblast growth factor 21 (FGF21). In skeletal muscle, miRNAs regulate insulin sensitivity and lipid oxidative capacity through PPARγ and PPARγ coactivator 1α (PGC1α), respectively. In pancreas, ADEV-derived miRNAs modulate β-cell mass and insulin secretion. In brain, ADEVs-derived long non-coding RNA metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) regulates mTOR signaling in hypothalamic pro-opiomelanocortin (POMC) neurons to control appetite and body weight. In the bloodstream, exosomal proteins and lipids affect the differentiation and immunomodulatory responses of monocytes. RBP4, retinol binding protein 4; WAT, white adipose tissue; BAT, brown adipose tissue; MAFB, v-maf musculoaponeurotic fibrosarcoma oncogene family protein B.

of BAT possibly by upregulation of FGFR1 (81). ADEVs may participate in the regulation of the inflammasome activation. EVs derived from both ADSCs and epidural fat-mesenchymal stem cells inhibit Nod-like receptor pyrin domain-containing three (NLRP3) inflammasome activation (82, 83). MiR-223, possibly of ADEV origin, is reduced in blood from patients with T2D and obesity (84). This reduction in miR-223 is believed to contribute to the increased adipose tissue inflammation in obesity as miR-223 can inhibit inflammation by targeting NLRP3, which is a key component of the inflammasome (85). However, it is currently unclear how adipose tissues sense the environmental and nutritional changes to alter the vesicular composition of miRNAs under different pathophysiological conditions.

In addition to miRNAs, mRNAs have been found to be present in EVs. Valadi and colleagues provided the first evidence demonstrating that EVs secreted from mast cells contained substantial amount of mRNAs, which were functional as they can be transferred to other cells and translated into new proteins in the recipient cells (70). Subsequent analysis of EVs derived from adipocytes differentiated from mouse 3T3-L1 cell line revealed that ADEVs also contained mRNAs encoding genes involved in metabolic and inflammatory processes (86). In contrast, exosomal mRNAs were not detected in VAT or SAT from either lean or obese human subjects (87). The discrepancy may be resulted from the difference in cellular sources of exosomes and possible difference in miRNA species that might mediate intrinsic degradation of mRNAs in ADEVs from human subjects.

Long non-coding RNAs (lncRNAs) have been emerged as critical regulators to control the development and functions of various metabolic tissues. For example, brown adipose tissue-specific lncRNA 1 (lnc-BATE1) was induced during brown adipocyte differentiation and enriched in BAT compared to WAT in mice. siRNA-mediated knockdown of lnc-BATE1 impaired differentiation of brown adipocytes *in vitro* (88). Liver-specific

TABLE 1 | Summary of major ADEV cargos and their functions in immune responses and metabolic regulation.

Cargo	Donor	Recipient	Molecular Target	Functions	References
miRNAs					
miR-99b	Adipocytes	Hepatocytes	FGF21	Glucose intolerance ↓	(12)
miR-155	Adipocytes	Macrophages	SOCS1	M1 polarization of macrophages ↑, adipocyte insulin signaling ↓	(54)
miR-34a	Adipocytes	ATMs	KLF4	M2 polarization of macrophages ↓, adipose inflammation ↑	(11)
miR-130b	Adipocytes	Myocytes	PGC1α	Lipid oxidation ↓	(72)
miR-27a	Adipocytes	Myocytes	PPARγ	Insulin resistance ↑	(73)
miR-155	ATMs	Adipocytes, hepatocytes, myocytes, β cells	PPARγ MAFB	Insulin resistance ↑ β cells proliferation ↑, glucose-stimulated insulin secretion ↓	(13, 74)
Proteins					
ACC, FASN, G6PD	Adipocytes	Adipocytes, preadipocytes	*De novo* lipogenesis	Adipogenesis and lipogenesis ↑	(75)
RBP4	Adipocytes	Macrophages	TLR4 signaling	Macrophage activation ↑, adipose inflammation ↑	(46)
STAT3	ADSCs	ATMs	Arginase-1	M2 polarization of macrophages ↑, adipose inflammation ↓, beiging of WAT ↑	(15)
Mitochondrial components	Adipose endothelial cells	Adipocytes	Mitochondrial respiratory chain, lipid metabolism	Respond to changes in systemic nutrient status	(68)
Lipids and others					
Neutral lipids	Adipocytes	Monocytes, ATMs	Lysosomal catabolism	Lipid release, monocyte differentiation to ATM	(52)
Ceramides	Adipose endothelial cells	Adipocytes	Stress-related signaling pathways	Involved in pathological signaling in T2D	(68)
α-KG	Adipocytes	Macrophages	STAT3/NF-κB signaling	M2 polarization of macrophages ↑	(76)

FGF21, fibroblast growth factor 21; SOCS1, suppressor of cytokine signaling 1; ATMs, adipose tissue-resident macrophages; KLF4, krüppel-like factor 4; PPARγ, peroxisome proliferator activated receptor gamma; PGC1α, PPARγ coactivator 1α; MAFB, v-maf musculoaponeurotic fibrosarcoma oncogene family protein B; ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; G6PD, glucose-6-phosphate dehydrogenase; RBP4, retinol binding protein 4; TLR4, toll-like receptor 4; STAT3, signal transducer and activator of transcription 3; ADSCs, adipose-derived stem cells; WAT, white adipose tissue; T2D, type 2 diabetes; α-KG, α-ketoglutarate; NF-κB, nuclear factor kappa B.

triglyceride regulator lncRNA (lnc-LSTR) was identified as an important regulator of hepatic lipid metabolism. Knockdown of lnc-LSTR in mice lowered serum triglyceride levels by induction of apolipoprotein C2 (ApoC2), which promotes lipoprotein lipase-mediated hydrolysis of triglyceride-rich lipoproteins (89). Recently, it has been reported that lncRNAs are transferred by ADEVs to mediate the interconnection between adipose tissue and the central nervous system. In particular, adipocyte-derived exosomal metastasis-associated lung adenocarcinoma transcript-1 lncRNA (lnc-MALAT1), which is elevated in obese mice, has been shown to target hypothalamic pro-opiomelanocortin (POMC) neurons to upregulate mTOR and thereby downregulate POMC expression, resulting in increased appetite and weight gain in lean mice (90).

Proteins

The nature of proteins released from adipocyte-derived exosomes has been characterized by proteomic profiling of exosomes produced by human primary adipocytes in comparison with the overall secretome of the same cells (69). This analysis identified 884 proteins, called as exoadipokines. Among them, 212 proteins commonly found in both secretome of human adipose tissues and exosomes are mainly involved in inflammation and fibrosis, whereas 672 proteins specific for exosomes which are assigned to signaling pathways and membrane-mediated process (69). Notably, exosomes were found to be enriched in proteins without classical signaling peptides that direct proteins to the traditional secretory pathway, suggesting a significant contribution of exosomes to the overall human adipokinome (69). Notably, different proteomic profiles in adipocyte-derived EVs have been observed between obese diabetic rats and obese non-diabetic counterparts (91). Exosomes derived from obese diabetic mice are enriched in proteins and enzymes involved in lipolysis and glycerol export, which may explain ectopic lipid accumulation in major metabolic organs and hence systemic insulin resistance (91). Intriguingly, hypoxia, which is present in obese adipose tissue, has also been shown to alter proteomic composition of ADEVs *in vitro* (75). In particular, enzymes involved in *de novo* lipogenesis such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN) and glucose-6-phosphate dehydrogenase (G6PD) were selectively enriched in exosomes derived from adipocytes under the hypoxic condition, which may increase lipid accumulation in recipient adipocytes and preadipocytes.

Exosomal proteins in ADEVs are functionally involved in the paracrine crosstalk between adipocytes and macrophages in adipose tissue. Adiponectin-positive EVs derived from adipocytes can promote differentiation of monocytes into ATMs, which are associated with the production of immunomodulatory proteins such as TNFα, macrophage-colony-stimulating factor (MCSF) and retinol binding protein 4 (RBP4) (47). The

functional relevance of exosomal protein RBP4 in monocyte differentiation has been substantiated in another independent study showing that ADEVs from *ob/ob* mice contained a higher level of RBP4, and exosomal RBP4 induced macrophage activation and production of pro-inflammatory cytokines *in vitro* (46). Additionally, exosomes from ADSCs contain active STAT3, which can be transported to macrophages to induce polarization of macrophages toward the anti-inflammatory M2 phenotypes through transcriptional activation of arginase-1 (15). Treatment of obese mice with the ADSC-derived exosomes alleviated diet-induced insulin resistance and glucose intolerance by reducing adipose inflammation and enhancing beiging of WAT (15). These studies collectively support the immunomodulatory effects of exosomal proteins, in reminiscence of classical adipokines and chemokines, in the interconnection between obesity and inflammation. However, loss-of-function studies are warranted to confirm the requirement of individual exosomal proteins in the regulation of adipose immune responses and insulin sensitivity.

Lipids and Other Cargos

In addition to aforementioned miRNAs, mRNAs and proteins, lipids and other cargos also act as the signaling molecules conferring the effects of ADEVs. A recent study identified lipid-filled exosomes released from adipocytes, and these lipid-enriched exosomes play an important role in transporting lipids from adipocytes to macrophages (52). Furthermore, these lipid-filled exosomes are also sufficient to induce differentiation of bone marrow-derived monocytes into ATM-like macrophages *in vitro* (52). The number of the lipid-filled exosomes secreted from adipocytes was more than doubled in obese mice relative to the lean mice which might be an additional mechanism for obesity-associated adipose inflammation. This study also proposed these lipid-filled exosomes as a new pathway of lipid release from adipocytes independent of the canonical lipolysis. However, little is known on how different types of bioactive lipids are selectively enriched in ADEVs and exert their local and/or distal effects on immunological and metabolic regulation. Adipocytes are also found to produce exosomes containing α-ketoglutarate. Melatonin, a hormone released from the pineal gland with anti-inflammatory activities, promoted the secretion of exosomes containing α-ketoglutarate from adipocytes, whereas uptake of exosomal α-ketoglutarate by macrophages facilitated the polarization toward the anti-inflammatory M2 phenotype and thus alleviated adipose inflammation in obesity (76).

CLINICAL IMPLICATIONS OF ADEVs IN METABOLIC DISEASES

Measurement of ADEVs in adipose tissue is minimally invasive and can be potentially used as an alternative approach to evaluate metabolic health. Changes in circulating EVs have been associated with various metabolic diseases, including obesity, T2D and NAFLD, making them attractive biomarkers for diagnosis and risk prediction of these diseases (92). However, the extent to which circulating EVs are contributed by ADEVs is currently unclear. Several adipose markers, including adiponectin, FABP4 and perilipin A have been used to identify EVs released from adipose tissues (50, 51). The level of circulating EVs positive for perilipin A and thus of adipocyte origin was dramatically increased in both mice with diet-induced obesity and obese human patients (50). Furthermore, in obese humans, the circulating level of the EVs enriched with perilipin A was positively correlated with plasma insulin level and homeostatic model assessment of insulin resistance (HOMA-IR), supporting potential use of perilipin A-positive EVs as the biomarker of insulin resistance (50).

Metabolic status can be also reflected by changes in vesicular miRNAs in ADEVs. By using FABP4 as a marker to identify ADEVs from the circulation, Hubal et al. found that the miRNA content of circulating ADEVs targeting various genes in the canonical insulin receptor-mediated signaling pathway was significantly altered 1 year-after gastric bypass bariatric surgery, and the changes were closely associated with improvements in insulin sensitivity, suggesting that FABP4-positive ADEVs might be useful to monitor the response of obese patients to the intervention with bariatric surgery (51). However, as FABP4 is also expressed in several types of immune cells and endothelial cells, ADEVs may only account for a proportion of the total FABP4 positive EVs. In addition, the circulating level of exosomal miR-92a possibly of BAT origin was found to be negatively associated with BAT activity as measured by ^{18}F-fluorodeoxyglucose PET/CT in two human cohorts, and thus may represent a potential biomarker for monitoring BAT activity in humans, which is much more cost-effective than PET/CT-based approaches (53).

ADEVs from different adipose depots are differentially associated with metabolic health and disease. The number of EVs derived from omental adipose tissue, but not SAT, correlated positively with HOMA-IR in overweight patients (47). It has also been reported that the abundance of EVs in VAT was positively correlated with serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are the well-established markers for liver injury, whereas the number of EVs in SAT was inversely associated with waist circumference and metabolic syndrome (93). These clinical observations suggest that differential effects of SAT and VAT on metabolic health may be related to the distinct amount and/or composition of EVs from these two adipose depots.

THERAPEUTIC POTENTIAL OF ADEVs FOR METABOLIC DISEASES

Owing to the easy accessibility from the bloodstream, the ability to transport the bioactive cargos and surmount biological barriers, the possibilities to modify the content with bioengineering, and target specificity, EVs have been emerged as a cell-free therapy for treatment of various diseases (94). In particular, EVs from mesenchymal stem cells (MSCs) could fully mimic the immunomodulatory and regenerative functions of parental MSCs, and have therefore been exploited as potential therapeutic agents for various inflammatory diseases

and regenerative medicine targeting lung, liver, bone, kidney, brain and heart (95), and a large number of clinical trials on MSC-derived EVs have been publicly registered in recent years.

Although ADSCs have been shown to possess promising therapeutic efficacy for Crohn's disease, idiopathic pulmonary fibrosis and chronic kidney diseases (NCT03939741) in various clinical studies, the therapeutic application of ADSC-derived EVs are still at the early stage (96, 97). Nevertheless, there is a growing number of preclinical studies suggesting that ADEVs have the great therapeutic potential for obesity-related metabolic diseases. Treatment of obese mice with ADSC-derived exosomes obtained from lean mice caused a significant reduction of adipose inflammation and beiging of WAT, thereby leading to obvious metabolic improvements, including weight loss, alleviation of insulin resistance and hepatic steatosis (15). Administration of exosomes isolated from BAT or serum of wild-type mice significantly improved insulin sensitivity and normalized serum lipids in ADicerKO mice (12). Likewise, treatment of mice with EVs isolated from human ADSCs during adipogenic differentiation to beige adipocytes attenuated diet-induced obesity and hepatic steatosis (67). By contrast, infusion of mice with EVs isolated from human ADSCs during adipogenic induction to white adipocytes promoted adipogenesis and expansion of WAT, suggesting the therapeutic potential for lipodystrophy, a disorder associated with reduced number of circulating exosomes (12).

In addition to use endogenous ADEVs as a therapeutic agent, bioengineering ADEVs by modifying the bioactive cargos may represent another viable approach to develop effective treatment for obesity-related metabolic complications. An example is to deplete those miRNAs causally involved in metabolic inflammation and insulin resistance. In this connection, genetic ablation of exosomal miR-34a, which is highly enriched in exosomes secreted from VAT and contributes to adipose inflammation by inducing M1 macrophage polarization, has been shown to reverse obesity-induced insulin resistance, glucose intolerance and fatty liver in mice (11). Similarly, treatment of dietary obese mice with antisense RNA for miR-34a restores hepatic β-klotho expression and FGF19 signaling, leading to attenuation of fatty liver disease (98). It is also possible to exogenously load ADEVs with transcriptional factors participating in M2 macrophage polarization (15), thereby reducing obesity-related metabolic diseases.

CONCLUSIONS AND FUTURE PERSPECTIVES

Emerging evidence from both *in-vitro* and *in-vivo* studies support the role of ADEVs as important players mediating cell-cell communication within adipose tissues as well as interorgan crosstalk between adipose tissue and other distal organs, thus participating in the regulation of local immune responses, tissue remodeling, systemic insulin sensitivity, and energy homeostasis (**Figures 1**, **2**). Aberrant production and/or function of ADEVs are implicated in the pathogenesis of obesity and its related metabolic complications. ADEVs are heterogeneous in terms of size, composition and origin, with ADEVs derived from different adipose depots exhibiting distinct or even opposite functions. However, we are still in the early stage in understanding biogenesis, regulation and pathophysiological functions of ADEVs, and there are many important questions which remain to be addressed: How is the cargo composition of ADEVs regulated? How does obesity cause dysregulation in the number and cargo composition of ADEVs? What determines the target specificity of ADEVs? How do different cargos affect the functions of target cells? Furthermore, the clinical investigation of ADEVs as diagnostic biomarkers and therapeutics for metabolic diseases are constrained by several technical difficulties: First, there is no well-established, definitive marker(s) for ADEVs, and it is therefore difficult to dissect the contribution of ADEVs to circulating EVs, and to precisely measure the changes of circulating ADEVs in different metabolic diseases. Second, it is difficult to isolate ADEVs with high purity using current experimental approaches, and contamination with other particles such as lipoproteins remains a major concern. Moreover, due to the heterogeneity of ADEVs, a mixture of EV populations with different cargos exists in the same cell, causing the difficulties in obtaining a subtype with a specific set of cargos for functional characterization. Further technological advances in molecular and functional characterization of ADEVs will help to enhance our knowledge in metabolic regulation and to facilitate the development of novel therapeutics for treatment of obesity and its related metabolic complications.

AUTHOR CONTRIBUTIONS

ZH designed the review, collected references, prepared the table and figures, and wrote the manuscript. AX conceptualized the idea, provided critical suggestions and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Clusterin and Its Role in Insulin Resistance and the Cardiometabolic Syndrome

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The cardiometabolic syndrome involves a clustering of metabolic and cardiovascular factors which increase the risk of patients developing both Type 2 Diabetes Mellitus and cardio/cerebrovascular disease. Although the mechanistic underpinnings of this link remain uncertain, key factors include insulin resistance, excess visceral adiposity, atherogenic dyslipidemia, and endothelial dysfunction. Of these, a state of resistance to insulin action in overweight/obese patients appears to be central to the pathophysiologic process. Given the increasing prevalence of obesity-related Type 2 Diabetes, coupled with the fact that cardiovascular disease is the number one cause of mortality in this patient population, a more thorough understanding of the cardiometabolic syndrome and potential options to mitigate its risk is imperative. Inherent in the pathogenesis of insulin resistance is an underlying state of chronic inflammation, at least partly in response to excess adiposity. Within obese adipose tissue, an immunomodulatory shift occurs, involving a preponderance of pro-inflammatory immune cells and cytokines/adipokines, along with antigen presentation by adipocytes. Therefore, various adipokines differentially expressed by obese adipocytes may have a significant effect on cardiometabolism. Clusterin is a molecular chaperone that is widely produced by many tissues throughout the body, but is also preferentially overexpressed by obese compared lean adipocytes and relates strongly to multiple components of the cardiometabolic syndrome. Herein, we summarize the known and potential roles of circulating and adipocyte-specific clusterin in cardiometabolism and discuss potential further investigations to determine if clusterin is a viable target to attenuate both metabolic and cardiovascular disease.

Keywords: adipocyte, clusterin, cardiometabolic disease, type 2 ddiabetes mellitus, inflammation

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INTRODUCTION

Although the exact diagnostic criteria varies (1–3), the metabolic syndrome involves a clustering of abnormalities including obesity, insulin resistance, hypertension, and dyslipidemia. These in turn heighten the risk of cardio- and cerebrovascular disease (CVD) [elevated risk of primary and recurrent stroke (4) and myocardial infarction (5)], Type 2 Diabetes Mellitus (T2D) (6, 7), and non-alcoholic fatty liver disease/steatohepatitis (NAFLD/NASH) (8). Initially termed the metabolic syndrome, Reaven's syndrome, or Syndrome X, among others (9, 10), the ramifications of metabolic disease on CVD risk have subsequently led to a broadening of terminology (i.e., the cardiometabolic syndrome). Although the criteria are the same (**Table 1**), the term cardiometabolic syndrome has

TABLE 1 | Clinical definitions of the cardiometabolic syndrome based on the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults Adult Treatment Panel III, the International Diabetes Federation (IDF), and the World Health Organization (WHO).

	WHO (11)	NCEP ATP III (2)	IDF (12)
	T2D or IFG or IGT or insulin resistance plus ≥ 2 of the following:	3 of the following:	Central obesity defined as WC above the ethnicity-specific cut-off plus ≥ 2 of the following:
Body Weight	• BMI > 30 kg/m2 or WHR > 0.85 (females) or > 0.90 (males)	• WC > 88 cm (females) or > 102 cm (males)	Population specific
Lipid Profile	• HDL < 1.0 mmol/L (< 40 mg/dL) and/or • TG ≥ 1.7 mmol/L (150 mg/dL)	• HDL < 1.3 mmol/L (< 50 mg/dL) and/or • TG ≥ 1.7 mmol/L (150 mg/dL)	• HDL < 1.3 mmol/L (< 50 mg/dL) or specific treatment and/or • TG ≥ 1.7 mmol/L (150 mg/dL) or specific treatment
Blood pressure	• BP ≥ 140/90 mmHg or use of blood pressure medication	• BP ≥ 135/85 mmHg or use of blood pressure medication	• BP ≥ 135/85 mmHg or use of blood pressure medication
Other	• Microalbuminuria > 20 pg/min or Alb/Crea ratio ≥ 30 mg/g		• Fasting plasma glucose ≥ 5.6 mmol/L (100 mg/dL) or previously diagnosed T2D

BP, blood pressure; HDL, high density lipoprotein cholesterol; IGT, impaired glucose tolerance; T2D, type 2 diabetes; TG, triglycerides; WC, waist circumference; WHR, waist to hip ratio.

gained more widespread acceptance due to the intersection of risk factors that contribute to both CVD and metabolic disease and involve similar pathophysiologic processes.

The cardiometabolic syndrome is highly prevalent, affecting over 30% of the adult population in the United States (U.S.) and rising, with especially high prevalence rates (>40%) in patients older than 60 years old (13, 14). Compared to the general population, the relative risk for developing CVD with coexistent cardiometabolic syndrome is doubled (15), with 3-fold the risk of T2D (13). In addition, all-cause mortality is higher in those with the cardiometabolic syndrome. Importantly, factors related to ethnicity/race, gender, and socio-economics affect risk, with the highest rates occurring in non-Hispanic white men and black women (14). In addition, socio-economic factors such as low education level and advanced age are independently associated with a higher risk of the cardiometabolic syndrome. The reasons for these differences are incompletely understood and likely multifactorial, but remain a critical focus of future research with significant public health ramifications (16–19).

THE CENTRAL ROLE OF OBESITY-RELATED INFLAMMATION AND INSULIN RESISTANCE IN CARDIOMETABOLISM

Over 35% of the adult US population is obese (20), and excess adiposity contributes to multiple complications including T2D and accelerated rates of CVD (21). In fact, CVD is the number one cause of mortality in diabetic patients, with a 2–3-fold higher risk of clinical atherosclerosis (22), illustrating a close association between metabolic disease and CV risk. As such, underlying the dysfunction in cardiometabolic disease are four interrelated central features: insulin resistance, excess visceral adiposity, atherogenic dyslipidemia, and endothelial dysfunction (23). Of these, obesity-related insulin resistance appears to the most important trigger. Among all the cardiometabolic risk factors, the relationship between insulin resistance and hypertension is the best established, and end-organ insulin resistance is a central tenet in its pathophysiology (24). Various mechanisms have been put forth to explain this connection including a decrease in insulin-mediated renal artery vasodilatation and uncompensated sodium reabsorption, with a resultant increase in blood pressure. Systemic and vascular insulin resistance occurs in conjunction with inappropriate activation of the renin–angiotensin–aldosterone system (RAAS) (25). Hyperinsulinemia also increases sympathetic nervous system activity (26), contributing further to the development of hypertension, a prominent component of the cardiometabolic syndrome.

Obesity and its associated comorbidities (including T2D and CVD) are associated with a state of chronic low-grade inflammation (27) that is well-recognized as a major cause of decreased insulin sensitivity (28–30). Inflammatory pathway activation has been observed in all classical insulin target tissues, indicating the key role of inflammation in driving the pathogenesis of systemic insulin resistance. Particularly, in adipose tissue (AT), macrophages play a central role (28, 31, 32); however, recent studies have highlighted the importance of several other key immune cells in maintaining lean AT, including immunosuppressive regulatory T (T_{reg}) cells, which contribute to a "Type 2" anti-inflammatory immunoenvironment (33, 34). In obesity, this immunologic milieu is shifted to a more pro-inflammatory state, in which the normal architecture, energy storage, and endocrine activities of adipocytes are profoundly altered. Activation of a proinflammatory pathway in AT leads to the secretion of numerous cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (Il-6) and interleukin-1β (IL-1β) (35) that activate toll-like receptors (TLR2 and TLR4) and impair glucose uptake (36). Cytokines also impair suppression of AT lipolysis, with resultant free fatty acid (FFA) release into the circulation (37–39), which hinders the ability of insulin to stimulate muscle glucose uptake (40) and suppress hepatic glucose production (41), the two major factors in the pathogenesis of insulin resistance. Therefore, disruption

in AT fatty acid metabolism is likely an underlying factor in cardiometabolic disease, by promoting both hyperglycemia and dyslipidemia.

Obesity, the cardiometabolic syndrome, and T2D have also long been associated with higher risk of cerebrovascular disease and cognitive decline (42–52). One potential reason for this connection is that insulin has direct effects on neurotransmission and neuropathology in the brain (53–56), including alterations in the production, degradation and clearance of β-amyloid (Aβ) that lead to plaque deposition in Alzheimer's disease (57). Various murine models of obesity and diabetes (including after high-fat diet feeding) (58–61) have indicated a relationship between peripheral and "central" insulin resistance, and in humans altered metabolic brain activity occurs in peripherally insulin-resistant subjects (62–64), with dysregulation in CNS insulin signaling (65–67). In fact, intravenous insulin infusion (57, 68, 69), inhaled insulin (69, 70), the insulin-sensitizing agent pioglitazone (70, 71), metformin (72, 73), and weight-loss interventions, including bariatric surgery, have demonstrated beneficial effects on memory (74–77). Cerebrovascular disease (78–80) and vascular dementia (81, 82) are also strongly related to insulin resistance, even independent of frank diabetes, and the Insulin Resistance Intervention after Stroke (IRIS) trial established that improving insulin sensitivity can prevent cerebrovascular events (83).

CHARACTERISTICS OF CLUSTERIN AND PHYSIOLOGIC ROLES

The human clusterin (*CLU*) gene (encodes the protein clusterin/apolipoprotein J) was first identified by Blaschuk et al. (84). This highly conserved gene consists of nine exons located on chromosome 8 that encode different isoforms resulting from alternative splicing and post-translational modifications (glycosylation, disulfide bond cleavage, etc.) (85, 86). The *CLU* gene promoter is highly conserved among species, with numerous identified regulatory elements including TGF-β inhibitory element, activator protein-1 and−2, and nuclear factor, but is also responsive to many environmental and cytokines that vary depending on the involved tissue (87–89). Although expressed by nearly every tissue in the human body, clusterin is predominantly made by epithelial tissues during embryonic development and in the testis, ovary, adrenal gland, liver, heart and brain of adults (85, 86). Its identified receptors are varied and often tissue-specific and include the HDL cholesterol receptor, low density lipoprotein-related protein 2 (LRP/megalin) (90), ApoER2 (91), and very low density lipoprotein receptor (VLDLR), many of which are critical to cardiovascular health.

There are two major forms of clusterin: a stress-induced, non-glycosylated, nucleocytostolic 55kDa variant (nCLU) consisting of parallel α and β chains, and a secreted or cytosolic variant (sCLU) that is proteolytically cleaved, connected by five disulfide bonds, and released from cells in an antiparallel fashion (92). Heterodimeric sCLU circulates mainly as a component of high-density lipoprotein (HDL) cholesterol, but has also been found to be bound to apolipoprotein (Apo) A1, various lipids, paroxanase, beta (β)-amyloid protein, and complement proteins, among others [summarized in Trougakos and Gonos (93)]. In healthy subjects, a higher prevalence of sCLU is bound to cardioprotective HDL cholesterol, suggesting that secreted clusterin may play a role in preventing progression of vascular disease (94). In contrast, nCLU predominantly promotes ionizing radiation-induced death of cells and triggers apoptosis in a BAX-dependent mechanism, and has yet to be linked with cardiometabolic pathology (95). Therefore, the remainder of this review will focus on the relationship of CVD and metabolic disease with sCLU.

One of the major roles of clusterin is to act as a molecular chaperone that assists folding of secreted proteins (87). Clusterin may also serve as a sensor of oxidative stress and is reduced upon exposure to acute stress (96). As a result of its ubiquitous nature, it has been implicated in a wide range of pathologic processes including cancer development and progression, complement regulation, and sperm maturation (93, 97, 98). *CLU* gene transcription and protein expression is upregulated in breast cancer (99), ovarian cancer (100), and prostate cancer (101), and inhibition of *CLU* expression protects the cell from apoptosis induced by chemotherapy, radiotherapy, and androgen/estrogen depletion (102–104). Clusterin is also involved in CNS lipid trafficking (105, 106) and is widely expressed in the brain (107). Accordingly, clusterin has clinical associations with Alzheimer's disease (AD) (108, 109) and has been proposed as a biomarker of AD (110). In fact, risk variants in *CLU* are strongly associated with AD (108). In patients with both mild cognitive impairment and AD, clusterin levels are elevated in the brain, cerebrospinal fluid, and blood (111–114), and accordingly *CLU* gene expression is elevated in these pathologic conditions (107).

ROLE OF CIRCULATING CLUSTERIN IN INSULIN RESISTANCE AND METABOLIC DISEASE

There are numerous identified mechanisms by which circulating clusterin could impact the risk of metabolic disease. Leptin resistance has been demonstrated in both murine models and human obesity, with reduced transport across the blood-brain-barrier (BB) (115). In turn, sCLU affects the transport of leptin across the BBB via LDL cholesterol (116), and through its binding to the receptor LRP2 can sensitize leptin receptors in the hypothalamus (117). This suggests that clusterin may play a role in modulating appetite and contributing to obesity (117). Clusterin can also directly affect insulin signaling and inflammation, two factors that can lead to insulin resistance, via its actions on macrophage phosphoinositide 3-kinase (PI3K; a mediator of insulin signaling) and NFκB (a major pro-inflammatory pathway in insulin resistance) (118). Clusterin induces directional migration of macrophages acting as a chemoattractant (119). This stimulates the expression and secretion of TNF-α and various chemotactic cytokines allowing clusterin to serve as a link between inflammation and remodeling of tissues by directing immune cells (120). Therefore, clusterin

plays a significant role in inflammation and immune responses through its molecular interactions with complement factors, immunoglobulins, and inflammatory pathways (121).

In support of these identified mechanistic processes, both murine and human studies have demonstrated a significant link between circulating clusterin and features of the metabolic syndrome. Skeletal muscle and hepatic gene expression of *CLU* increase following high-fat diet feeding in mice, and whole body clusterin knockout mice are insulin sensitive compared to wild-type mice (122). Obese patients without diabetes following a 2 week very low calorie diet have reduced plasma clusterin levels (123), and in obese compared to lean subjects, plasma clusterin levels are elevated and positively relate to body mass index, waist circumference, markers of inflammation (hsCRP and retinol-binding protein-4) (124), and insulin resistance (125). In addition, polymorphisms in *CLU* have been linked to insulin resistance [by the homeostasis model of insulin resistance [HOMA-IR] and impaired insulin secretion [HOMA-β]] (126). In contrast to these deleterious metabolic effects, clusterin has been shown to reduce hepatic fibrosis via stellate cell downregulation of the Smad3 signaling pathway (127).

CARDIOVASCULAR AND CEREBROVASCULAR EFFECTS OF CIRCULATING CLUSTERIN

The mechanistic effects of circulating clusterin on CVD are controversial, due to seemingly paradoxical effects in the existing literature, and the mechanisms behind such a link remain unclear. Clusterin is found in a subset of dense HDL cholesterol particles and has wide-ranging effects on lipid transport (121, 128). In plasma, clusterin forms HDL particles with ApoA-I and ApoE and aids in the transfer of HDL cholesterol from peripheral tissues to the liver, diverting lipoproteins away from atherosclerotic lesions (129, 130). In contrast, clusterin may have a deleterious effect on the antioxidant activity of paroxanase-1 (PON1), whose deficiency enhances atherosclerosis by increasing the accumulation of oxidized phospholipids in atherosclerotic plaques (131).

There are multiple lines of evidence suggesting that human clusterin may have a significant clinical association with multiple facets of cardiovascular risk. Circulating plasma clusterin (sCLU) levels are strongly associated with the pro-inflammatory factor C-reactive protein (CRP) (124), various lipid markers of heightened cardiovascular risk, and increasing systolic and diastolic blood pressure (90, 132). Circulating clusterin is also negatively associated with leptin in obesity-related CVD (133). In addition, clusterin bound to HDL cholesterol is reduced in obese males and is associated with lower levels of HDL cholesterol, higher TGs (134) and low-density lipoprotein (LDL) cholesterol levels, and accelerated atherogenesis (135), and may confer higher cardiovascular risk during the aging process (135). Interestingly, proteomic analysis has shown that higher levels of clusterin are found in carotid atherosclerotic compared to non-atherosclerotic plaques (136). Not all studies, however, have confirmed a beneficial role for clusterin in CVD. A recent study showed that lower serum clusterin was associated with higher rates of mortality in heart failure patients (137), indicating some uncertainty on the importance of circulating clusterin in the CVD process.

ADIPOCYTE-DERIVED CLUSTERIN AND ITS POTENTIAL ROLE IN CARDIOMETABOLIC DISEASE

The adipocyte is no longer viewed as simply a storage depot for lipids, but is now recognized as an important determinant of an obesity-related proinflammatory environment, instigating inflammation in expanding AT (138). Despite significant progress in our understanding of the role of the adipocyte as an immumodulator, and evidence that circulating plasma and HDL cholesterol bound clusterin may be involved in the metabolic syndrome, insulin resistance, atherogenesis, and CV risk, the importance of adipocyte-derived clusterin in human cardiometabolic disease remains largely unknown. In whole human AT, *CLU* gene expression is higher in obese compared to lean subjects, and is decreased following weight loss induced by VLCD or bariatric surgery (123). We have recently shown that clusterin derived specifically from the adipocyte may play an important role in cardiometabolic disease (90). In obese compared to lean human subjects, adipocyte gene expression and protein levels of clusterin were higher and responsive to (FFA) palmitate stimulation (a major component of a high fat diet enriched in fatty acids) (139). In addition, we found strong associations of adipocyte clusterin with systemic insulin resistance, multiple components of the metabolic syndrome (HDL cholesterol, the ratio of HDL cholesterol to total cholesterol, and TGs, and both systolic and diastolic blood pressure), and overall CVD risk and mortality. In this same study, clusterin treatment of human liver cells reduced insulin signaling by lowering Akt phosphorylation and promoting key genes involved in gluconeogenesis; yet hepatic expression of the major regulator of hepatic *de novo* lipogenesis [sterol regulatory element-binding protein-1 [*SREBP-1*]] and *APOA1* were decreased in response to clusterin binding to LRP2. These results suggest that the liver receptor LRP2 may be a key target for the potential cardiometabolic role of clusterin. Knockdown of *SREBP-1* can perpetuate hyperglycemia via enhanced gluconeogenesis and reduced glycolysis and glycogen synthesis (140). APOA1 is a major protein associated with HDL cholesterol particles in plasma which facilitates efflux of cholesterol from cells, notably from macrophages within atherosclerotic plaques, to the liver for excretion. Low plasma APOA1 levels are also a strong predictor of CVD (141). In a mouse model prone to non-alcoholic steatohepatitis (NASH) adipocyte *CLU* expression also paralleled an increase in liver fat, hepatic fibrosis, and steatohepatitis (90).

Although these results suggest several mechanisms by which clusterin could link insulin resistance, metabolic disease, and CVD (**Figure 1**), further investigation is needed to fully elucidate the cardiometabolic role of AT clusterin, and specifically clusterin derived from the adipocyte. Although treatment with the FFA

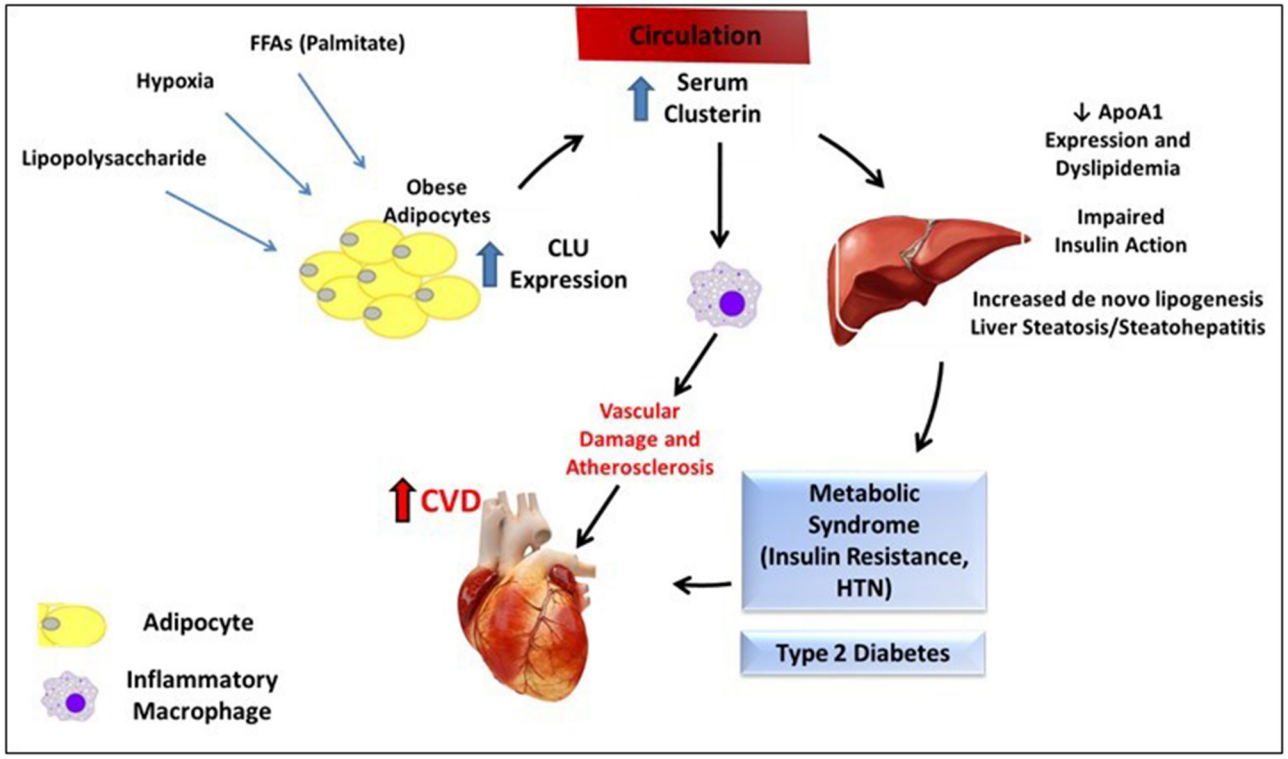

FIGURE 1 | Summary of proposed mechanism for clusterin-mediated cardiometabolic disease. Various stimuli may increase adipocyte expression of CLU from adipocytes in the setting of obesity. Circulating clusterin subsequently has multiple effects on the liver (reduction in ApoA1 expression, dyslipidemia, impaired insulin signaling, and potentially increased steatosis and inflammation) and on macrophages, which may contribute to the cardiometabolic syndrome, and increase CVD risk.

palmitate stimulates clusterin release *in vitro*, other potential triggers for clusterin expression are possible. These include AT hypoxia, which has previously been shown to increase clusterin expression in other cell types outside of AT (142). In addition, the effects of adipocyte-derived clusterin on the AT immunoenvironment and the skewed balance of pro- and anti-inflammatory cytokines observed in human obesity is also unknown.

CONCLUSION

The cardiometabolic syndrome is a clustering of metabolic and cardiovascular abnormalities that increase the risk of CVD, T2D, and all-cause mortality. The rising prevalence of the cardiometabolic syndrome, both in the U.S. and worldwide, make a more thorough understanding of its pathophysiologic underpinnings imperative. Although likely multifactorial, the presence of obesity-related insulin resistance appears to be a central, if not instigating factor. Systemic and tissue-specific insulin resistance not only affect endothelial function and leads to atherogenic dyslipidemia, but propagate a pro-inflammatory environment that includes excess release of detrimental FFAs into the circulation. Clusterin is a ubiquitous protein secreted by many organs/tissues throughout the body. Although studies have implicated circulating clusterin in multiple metabolic and cardio/cerebrovascular abnormalities, a unifying mechanism remains elusive, and the current literature is inconsistent and inconclusive. In particular, the importance of AT derived clusterin, strongly associated with many metabolic and CVD risk factors, requires further investigation. This includes understanding the exact mechanistic processes by which it acts locally within AT and systemically in the liver, endothelial cells, and the vasculature. Isolating its effects, potentially through the development of adipocyte-specific clusterin knockout and overexpression models, will be instrumental in determining if it is a viable target to attenuate features of the cardiometabolic syndrome.

AUTHOR CONTRIBUTIONS

JW and DB co-wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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REVIEW
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Adipocyte Fatty Acid-Binding Protein, Cardiovascular Diseases and Mortality

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It has been increasingly recognized that inflammation plays an important role in the pathogenesis of cardiovascular disease (CVD). In obesity, adipose tissue inflammation, especially in the visceral fat depots, contributes to systemic inflammation and promotes the development of atherosclerosis. Adipocyte fatty acid-binding protein (AFABP), a lipid chaperone abundantly secreted from the adipocytes and macrophages, is one of the key players mediating this adipose-vascular cross-talk, in part *via* its interaction with c-Jun NH2-terminal kinase (JNK) and activator protein-1 (AP-1) to form a positive feedback loop, and perpetuate inflammatory responses. In mice, selective JNK inactivation in the adipose tissue significantly reduced the expression of AFABP in their adipose tissue, as well as circulating AFABP levels. Importantly, fat transplant experiments showed that adipose-specific JNK inactivation in the visceral fat was sufficient to protect mice with apoE deficiency from atherosclerosis, with the beneficial effects attenuated by the continuous infusion of recombinant AFABP, supporting the role of AFABP as the link between visceral fat inflammation and atherosclerosis. In humans, raised circulating AFABP levels are associated with incident metabolic syndrome, type 2 diabetes and CVD, as well as non-alcoholic steatohepatitis, diabetic nephropathy and adverse renal outcomes, all being conditions closely related to inflammation and enhanced CV mortality. Collectively, these clinical data have provided support to AFABP as an important adipokine linking obesity, inflammation and CVD. This review will discuss recent findings on the role of AFABP in CVD and mortality, the possible underlying mechanisms, and pharmacological inhibition of AFABP as a potential strategy to combat CVD.

Keywords: cardiovascular disease, adipocyte fatty acid-binding protein, mortality, inflammation, adipokine

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INTRODUCTION

Obesity is a global health problem. Based on the data from the World Health Organization (WHO), in 2016, more than 1.9 billion adults aged 18 years or above were overweight, and among them, 650 million were obese (1). In a pooled analysis of 19.2 million participants, the age-standardized prevalence of obesity has tripled in men and doubled in women over the last four decades. If these trends continue, around 1 in 5 of the global population will become obese by year 2025 (2).

Obesity leads to increased risks of type 2 diabetes (3, 4), non-alcoholic fatty liver disease (NAFLD) (5), cardiovascular disease (CVD) (6), cancer (7), and mortality. Indeed, high body mass index (BMI) has become one of the top five leading causes of all-cause mortality and disability-adjusted life-years (8). In 2015, high BMI contributed to 7.1% of global deaths. Strikingly, CVD accounted for two-thirds of these deaths and more than half of disability-adjusted life-years related to high BMI (9). Recently, in a Mendelian randomization (MR) study involving more than 360,000 participants from the UK Biobank, each genetically instrumented increase in BMI of 1 kg/m^2 was associated with a significantly higher risk of most cardiovascular outcomes including hypertension, atrial fibrillation, coronary heart disease (CHD), heart failure and peripheral vascular disease (PVD) (10). Genetically predicted fat mass index was associated with an even broader list of cardiovascular outcomes including ischemic stroke. These findings corroborated with another large MR study which demonstrated the causal effects of adiposity on CVD (11). Taken together, both observational and MR studies provided strong epidemiological evidence that obesity, in particular central adiposity, is closely linked with CVD and cardiovascular mortality.

Inflammation, on the other hand, is an established important risk factor of CVD and cardiovascular mortality (12). Previous observational studies had demonstrated that markers of inflammation such as C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α) receptor 1 were independent prognostic markers of adverse cardiovascular outcomes among individuals with and without prevalent CVD (13, 14). Recently, the use of Canakinumab, an anti-inflammatory monoclonal antibody targeting interleukin-1, was also shown in a randomized controlled trial to significantly reduce the incidence of non-fatal myocardial infarction, non-fatal stroke and cardiovascular death, confirming that inflammation plays a crucial role in the pathogenesis of CVD (15). Obesity is a state of chronic low-grade systemic inflammation, which is induced by a cascade of cellular events that occur in the dysfunctional adipose tissue, and perpetuated by dysregulated secretion of adipokines through their local and systemic actions (16). This review will focus on adipocyte fatty acid-binding protein (AFABP) and present the recent data on its role as an important adipokine linking obesity, inflammation and CVD.

AFABP EXPRESSION AND SECRETION

AFABP is a major cytosolic protein of the mature adipocytes (17). As a fatty acid binding protein, it acts as a lipid chaperone that facilitates the trafficking of non-esterified fatty acids throughout cellular compartments such as peroxisome, endoplasmic reticulum (ER), mitochondria and nucleus (18). AFABP also regulates lipid storage and oxidation, and is involved in lipolysis though its interaction with the hormone-sensitive lipase (HSL) and a co-activator of adipose triglyceride lipase (ATGL) (19, 20). The expression of AFABP in adipocytes is induced during adipocyte differentiation, and is transcriptionally activated by fatty acids, glucocorticoids, cyclic adenosine monophosphate (cAMP), and peroxisome proliferator-activated receptor gamma (PPARγ) agonists (21–23).

Studies in recent years have shown that AFABP is secreted from the adipocytes, and circulates in the blood stream in both mice and humans (24) (25). However, since it lacks a signal peptide sequence for classical secretory pathway (25), it has recently been reported that AFABP is secreted unconventionally *via* endosomes and secretory lysosomes in response to lipolytic and fasting related signals, such as adrenergic signaling, beta agonists, branched-chain amino acids and glycerol (25, 26), and the involvement of sirtuin-1 activation has been implicated (27). While it is also expressed in the macrophages (28) and endothelial cells (29), *in vivo* data suggest that the adipocyte is the predominant contributor to circulating AFABP levels (25).

AFABP IN RELATION TO ADIPOSE TISSUE INFLAMMATION AND INSULIN RESISTANCE IN OBESITY

AFABP secretion is dysregulated in obesity, with raised circulating AFABP concentrations being found in obese individuals (24). With chronic nutrient excess, pathological expansion of the adipose tissue causes several maladaptive changes especially in the visceral fat depots. Hypertrophic adipocytes undergo high rates of spontaneous lipolysis (30), which increases free fatty acid (FFA) efflux and stimulates AFABP release. Lipo-toxicity ensues as lipid intermediates such as ceramides and diacylglycerols accumulate. Moreover, adipocyte hypoxia and cell death develop as a consequence of its continuous expansion despite relative under-perfusion and increased mechanical stress (31), and hypoxia is another known stimulus for AFABP release from adipocytes (32). On the other hand, AFABP (33), as a lipid chaperone, has been implicated in ER stress in response to lipotoxic signals, leading to activation of stress kinases such as nuclear factor kappa B (NFκB) and c-Jun NH2-terminal kinase (JNK) (34), enhancing adipocyte insulin resistance that potentiates lipolysis and lipotoxicity. Adipocyte insulin resistance also augments the secretion of pro-inflammatory cytokines including the chemokine monocyte chemoattractant protein 1 (MCP1) (35), which stimulates the recruitment of macrophages into the adipose tissue (36). Furthermore, it induces a phenotypic switch in the macrophages from the anti-inflammatory M2 polarized state to the pro-inflammatory phenotype typical of M1 classical inflammation in metabolically-activated macrophages (MMe) (37, 38).

Both innate and adaptive immunity are activated in obesity. In addition to macrophage infiltration, adaptive immune cells including CD4+ T helper (Th1) cells, CD8+ T cells and B cells also accumulate in the visceral adipose tissue (39). Transient enhancement of AFABP expression has been reported in murine splenic lymphocytes after dexamethasone administration (40). However, among the major human leucocyte subsets, the

expression of AFABP is largely restricted to the macrophages and myeloid dendritic cells (DC) (41). Specifically, owing to its high expression in the macrophages (28), AFABP is more closely linked with the innate immune cells. It has been shown that AFABP perpetuates lipopolysaccharide (LPS)-induced inflammatory responses in macrophages through its interaction with JNK and activator protein-1 (AP-1) forming a positive feedback loop. Upon stimulation by LPS *via* toll like receptor 4 (TLR4), JNK is activated, leading to the induction of c-Jun phosphorylation and its recruitment to a highly conserved AP-1 consensus binding motif located within the AFABP gene promoter. As a result, AFABP gene transcription is upregulated, which further potentiates LPS-induced JNK phosphorylation, activation of AP-1 complex and amplification of pro-inflammatory responses in the macrophages (42). Nonetheless, AFABP can also affect adaptive immunity through the modulation of DC responses. NFκB activation is impaired in AFABP deficient DCs, which exhibit reduced DC function in T cell priming and cytokine production (41). Recently, AFABP was also found to be upregulated in a subpopulation of tissue-resident memory CD8+ T cells which have high requirement for fatty acid metabolism. Importantly, the lack of AFABP in these cells could negatively impact their survival and hence attenuate their function in protective immunity (43). In a viral infection model, mice with genetic deficiency of AFABP had decreased interferon gamma production and increased viral load (41). However, in a rodent model of sepsis, pharmacological inhibition of AFABP in fact was demonstrated to be beneficial, with attenuation of sepsis-triggered inflammatory responses, reduced hepatic and pulmonary tissue injury, as well as improved survival (44). Taken together, these studies highlight the close and complex relationship between AFABP and cellular immunity.

In the adipose tissue, infiltration of these immune cells drives further release of pro-inflammatory adipokines including TNF-α, interleukin-6 (IL-6) and AFABP, and reduces the secretion of the anti-inflammatory adipokine adiponectin. Increased AFABP secretion induces further lipolysis and inflammation in the adipocytes *via* the p38/mitogen-activated protein kinase (MAPK) pathway (45), and contributes to this vicious cycle of adipose tissue insulin resistance and inflammation (46) (**Figure 1**). Whole-body insulin sensitivity was ultimately impaired, accompanied by a chronic state of subclinical systemic inflammation, and the development of an array of obesity-related complications including CVD and cardiovascular mortality (**Table 1**).

AFABP AND CARDIOVASCULAR RISK FACTORS

The detrimental role of AFABP on the development of CVD begins with its effects on traditional cardiovascular risk factors in addition to excess adiposity. AFABP-deficient mice displayed improved glycemia, insulin sensitivity and lipid metabolism in both dietary and genetically induced obesity (47, 48), secondary to a reduced FFA efflux and increased glucose utilization in muscles (49). Moreover, AFABP increases the hepatic expression of gluconeogenic enzymes phosphoenolpyruvate carboxylase 1 (*Pck1*) and glucose-6-phosphatase (*G6pc*), leading to enhanced hepatic glucose production and impaired glucose metabolism (25).

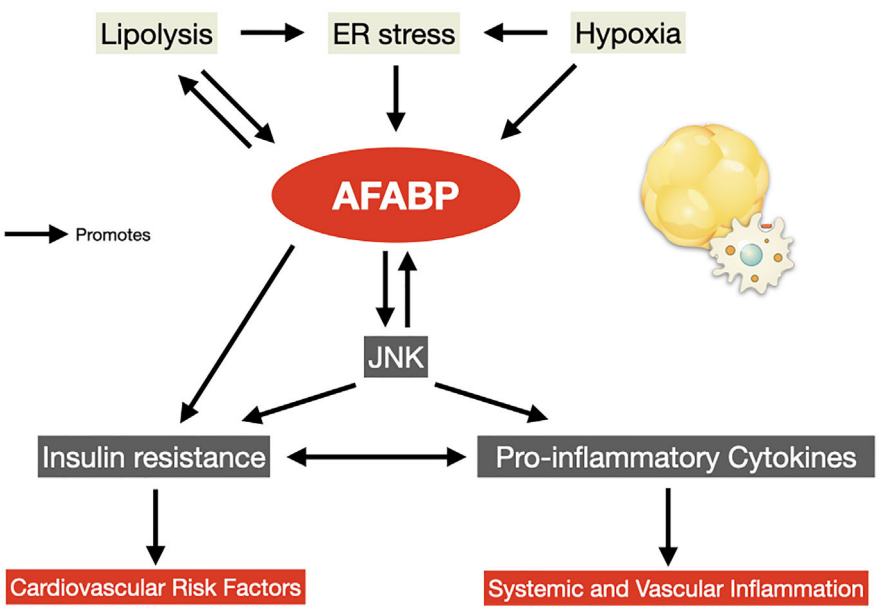

FIGURE 1 | AFABP in the vicious cycle of adipose tissue insulin resistance and inflammation. AFABP, adipocyte fatty acid-binding protein; ER, endoplasmic reticulum; JNK, c-Jun NH2-terminal kinase.

TABLE 1 | Associations of AFABP with cardiometabolic conditions.

	Circulating AFABP level	Potential mechanistic actions	References
Type 2 diabetes	• Predicts the development of type 2 diabetes	• Increases free fatty acid efflux • Reduces glucose utilization in muscles • Increases hepatic expression of gluconeogenic enzymes	(25, 47–51)
Hypertension	• Correlates positively with blood pressure	• Increases endothelial dysfunction • Worsens insulin sensitivity	(47, 48, 50, 52)
Dyslipidemia	• Correlates positively with low-density lipoprotein cholesterol • Correlates negatively with high-density lipoprotein cholesterol	• Increases free fatty acid efflux • Negative effects on lipid metabolism • Worsens insulin sensitivity	(47, 48, 50)
Coronary heart disease	• Predicts the development of cardiovascular diseases • Associates with coronary calcium score in patients with type 2 diabetes • Associates with the coronary plaque burden in patients with coronary heart disease	• Promotes atherosclerosis development: • Alters lipid metabolism in macrophages and facilitates foam cell formation • Promotes saturated fatty acid-induced ceramide production in macrophages • Mediates toxic lipids-induced endoplasmic reticulum stress in macrophages • Increases adipose tissue and systemic inflammation	(33, 53–60)
Stroke	• Associates with the presence of carotid atherosclerosis • Correlates positively with the vulnerable carotid plaque phenotype • Doubles the risk of incident adverse cardiovascular events including cardiovascular mortality, non-fatal myocardial infarction and non-fatal stroke. • Predicts poor functional outcome and mortality from ischemic stroke	• Promotes atherosclerosis development (as above) • Enhances the production of matrix metalloproteinases-9 which degrade the tight junction proteins in the blood brain barrier, leading to cerebral edema, increased neuro-inflammation and poor neurological outcomes	(61–68)
Heart failure	• Correlates positively with circulating levels of N-terminal fragment of pro-B-type natriuretic peptide • Associates with the presence of left ventricular systolic and/or diastolic dysfunction • Associates with increasing severity of clinical heart failure • Predicts incident heart failure among older individuals	• Negative inotropic effect on cardiomyocytes • Reduces phosphorylation of endothelial nitric oxide synthase in acute myocardial ischemia/reperfusion injury • Increases oxidative stress and cardiac inflammation • Increases cardiac hypertrophy and fibrosis	(52, 69–75)
Cardiovascular mortality	• Associates with both short- and long-term cardiovascular morbidity and mortality in patients with established coronary heart disease • Predicts cardiovascular deaths in patients with type 2 diabetes	• See above	(76–80)

In humans, circulating AFABP concentrations also correlate positively with adverse cardiometabolic risk factors including age, obesity indices, hypertension, homeostatic model of insulin resistance (HOMA-IR), low-density lipoprotein cholesterol (LDL-C), and negatively with high-density lipoprotein cholesterol (HDL-C) (50). Moreover, high circulating AFABP concentrations predicted incident metabolic syndrome and type 2 diabetes, both of which are associated with increased risks of CVD and mortality (50, 51).

AFABP AND ATHEROSCLEROSIS

AFABP promotes atherosclerosis, the central event in the pathogenesis of CVD (81). Bone marrow transplant experiments revealed that macrophage-specific AFABP deficiency reduced atherosclerotic lesions in mice with apolipoprotein E (ApoE) deficiency, to a similar extent as those with whole body AFABP deficiency, suggesting that much of the pro-atherogenic effects of AFABP are specific to its actions in macrophages (28). The expression of AFABP in macrophages can be upregulated in response to oxidized LDL (oxLDL) and LPS (82, 83), which are both increased in obesity (84, 85). On the other hand, metformin has been shown to inhibit AFABP expression in macrophages (86). AFABP alters lipid metabolism in macrophages and facilitates the formation of foam cell enriched with cholesterol and triglyceride (53, 54). AFABP also promotes macrophage cell death through saturated fatty acid-induced ceramide production (55). Moreover, AFABP has been shown as an obligatory mediator of toxic lipids-induced ER stress in macrophages, through inhibiting liver X receptor alpha (LXRα) to reduce macrophage *de novo* fatty acid synthesis which confers resistance to ER stress (33), as well as impairing macrophage autophagy by attenuation of Janus Kinase 2 (JAK2) activity (87). The elevated ER stress potentiates JNK activation and further exacerbates inflammation.

However, there was recent evidence suggesting that the negative impact of AFABP on atherosclerosis was not exclusively due to its action in the macrophages. In mice, selective JNK inactivation in the adipose tissue significantly reduced both the expression of AFABP in their adipose tissue, as well as circulating AFABP levels. Importantly, fat transplant experiments showed that adipose-specific JNK inactivation in the visceral fat was sufficient to protect mice with apolipoprotein E (ApoE) deficiency from atherosclerosis, with the beneficial effects attenuated by the continuous infusion of recombinant AFABP, supporting the participation of adipocyte-derived AFABP as a link between visceral fat inflammation and atherosclerosis (56).

In humans, elevated baseline AFABP concentration predicted incident CVD over a median follow-up of around 10 years in a community-based cohort (57). Moreover, high circulating

AFABP concentration was associated with coronary calcium score in patients with type 2 diabetes (58), as well as the coronary plaque burden in patients with coronary heart disease (59). In keeping with observations from preclinical studies, AFABP was not only expressed in macrophages within atherosclerotic plaques of the coronary arteries in patients with CHD, but also in both macrophages and adipocytes in their epicardial and perivascular fat. *In vitro* studies showed that treatment of human coronary artery smooth muscle and vascular endothelial cells with AFABP augmented palmitic acid-induced inflammation, suggesting that AFABP from epicardial and perivascular fat could also participate in the development of coronary atherosclerosis in a paracrine manner (60). Furthermore, individuals who harbored the single nucleotide polymorphism (SNP) T-87C, which reduced AFABP gene expression in their adipose tissue, was found to have a lower risk of CHD (88).

AFABP AND STROKE

The role of AFABP in the development of stroke is multifaceted. First, high circulating AFABP concentration was associated with the presence of carotid atherosclerosis (61, 62), a predisposing condition for cerebral infarction. In patients with carotid atherosclerosis, AFABP concentrations in their carotid plaques correlated positively with the vulnerable plaque phenotype (63, 64), predicted their disease progression (89), and doubled their risk of incident adverse cardiovascular events including cardiovascular mortality, non-fatal myocardial infarction and non-fatal stroke (64). Moreover, circulating AFABP concentration was associated with ischemic stroke in cross-sectional studies, and high AFABP concentration was consistently shown to be predictive of poor functional outcome, as well as short- and long-term mortality in patients who suffered from ischemic stroke (62, 65–67).

Mechanistically, genetic ablation of AFABP in mice was recently found to protect them from severe cerebral ischemic injury induced by surgical occlusion of their middle cerebral artery, which translated to less neurological deficits and improved survival after ischemic stroke. Both circulating and cerebral AFABP concentrations were elevated in response to cerebral ischemia. The increase in AFABP, derived from microglia and infiltrating macrophages, enhanced the production of matrix metalloproteinases-9 (MMP-9) through JNK activity, which degraded the tight junction proteins in the blood brain barrier, leading to cerebral edema, increased neuro-inflammation and poor neurological outcomes (68).

AFABP, HEART FAILURE, AND CARDIOVASCULAR MORTALITY

AFABP plays a critical role in the development of heart failure and predisposes to increased cardiovascular mortality. *In vitro* studies demonstrated that adipocyte-derived AFABP possessed a negative inotropic effect on rat cardiomyocytes and could inhibit their contraction (69). In humans, circulating AFABP concentration positively correlated with circulating levels of N-terminal fragment of pro-B-type natriuretic peptide (NT-proBNP), an established marker of heart failure (70). Moreover, high circulating AFABP concentration was associated with the presence of left ventricular systolic and/or diastolic dysfunction (71–73), as well as increasing severity of clinical heart failure (74). In the Cardiovascular Health Study, circulating AFABP concentration was also shown to be a modest but independent predictor of incident heart failure among older individuals (75).

The negative impact of AFABP on cardiovascular outcomes could also be attributed to their effects on endothelial dysfunction and oxidative stress. Genetic ablation of AFABP protected mice from cardiac dysfunction secondary to diabetes and myocardial ischemia/reperfusion (MI/R) injury. AFABP, whose expression was upregulated in cardiac endothelial cells in response to acute MI/R injury and hyperglycemia, reduced phosphorylation of endothelial nitric oxide synthase (eNOS) in acute MI/R injury, and increased superoxide anions in diabetes. In both situations, endothelial dysfunction ensued, which induced oxidative stress and cardiac inflammation, leading to cardiac hypertrophy, fibrosis and impaired myocardial contractility (52). Indeed, in keeping with findings from studies in mice, high circulating AFABP concentration was associated with both short- and long-term cardiovascular morbidity and mortality in patients with established CHD (76–78),and was an independent predictor of cardiovascular deaths in patients with type 2 diabetes (79, 80).

AFABP AND OTHER OBESITY-RELATED CONDITIONS WITH INCREASED CARDIOVASCULAR RISK

AFABP is also implicated in the pathogenesis of several obesity-related complications with increased cardiovascular risk, such as NAFLD, obstructive sleep apnea (OSA) and chronic kidney disease (CKD) (90–92). In NAFLD, for instance, over-expression of AFABP in Kupffer cells of the liver induced non-alcoholic steatohepatitis in mice, while obesity-induced liver injury was alleviated by pharmacological inhibition of AFABP (93). Similar findings had been observed in humans, where circulating AFABP concentration was associated with increasing lobular inflammation, hepatocyte ballooning and higher stages of hepatic fibrosis on liver histology (94). On the other hand, elevated serum AFABP concentration was also found in patients with severe OSA compared with those with milder disease (95, 96), and the use of continuous positive airway pressure was shown to reduce circulating AFABP concentrations in a recent randomized controlled study (97). Moreover, circulating AFABP was associated with adverse renal outcomes including renal deaths in patients with type 2 diabetes (98), which could possibly be a result of macrophage infiltration in the glomerulus and interstitium, ectopic expression of AFABP in the glomerulus, as

well as AFABP induced increased ER stress in the mesangial cells (99–101). Importantly, high circulating AFABP concentration was also an independent predictor of cardiovascular death in patients with end-stage renal disease (102).

AFABP AS A THERAPEUTIC TARGET FOR CVD

Preclinical studies have demonstrated that there is great potential in targeting AFABP as a therapeutic strategy to combat CVD and its risk factors. Several AFABP inhibitors have been developed, including a few biphenyl azole, indole- and carbazole-based compounds. In particular, BMS309403 (BMS) is a selective, high-affinity small molecule oral inhibitor of AFABP which impedes the ligation of fatty acid to its binding cavity on AFABP (103). Pharmacological inhibition of AFABP using BMS alleviated endothelial dysfunction and atherosclerosis in mice with ApoE deficiency. This was accompanied by reduced cholesterol ester accumulation in macrophages, as well as attenuated expression of pro-inflammatory cytokines including MCP1, IL-6 and TNFα (104, 105). Recently, BMS was also shown to improve stroke outcomes by ameliorating neurological deficits and improving the survival in mice with cerebral ischemic injury after surgical occlusion of their middle cerebral artery (68). Moreover, BMS attenuated non-alcoholic steatohepatitis (93), improved glucose tolerance (105) and decreased toxic lipid-induced ER stress associated inflammation in the skeletal muscle of mice with dietary obesity (106). Another small molecule inhibitor HTS01037, which acts as a competitive antagonist of AFABP mediated protein-protein interactions (107), was shown to alleviate macrophage inflammation and ER stress through upregulating uncoupling protein 2 (UCP2) expression (108). In addition to these oral compounds, alternative approaches of AFABP inhibition have also been investigated. The use of neutralizing antibodies against AFABP was demonstrated to significantly reduce adipose tissue inflammation (34), hepatic glucose production (25), and whole-body insulin resistance in obese mice (109). Likewise, adipocyte targeted silencing of AFABP using short-hairpin RNA treatment resulted in significant weight reduction, improved insulin sensitivity and glycemia in obese mice (110).

Although clinical studies of both BMS and neutralizing antibodies are still not available, several compounds have been found to modulate circulating AFABP concentrations. Treatment with chloroquine in mice diminished AFABP secretion from adipocytes, resulting in a lower circulating concentration (26). In humans, atorvastatin (111), sitagliptin (112), omega-3 fatty acids (113), and angiotensin II receptor blockers (ARBs) including candesartan, olmesartan, telmisartan and valsartan (114) decreased, whereas pioglitazone (115) and canagliflozin increased circulating AFABP concentrations (116). While omega-3 fatty acids and pioglitazone directly affect AFABP expression in adipocytes, it was postulated that ARBs suppressed and canagliflozin promoted catecholamines-induced lipolysis, respectively, causing the changes in the circulating AFABP concentrations despite neutral, if not favorable effects

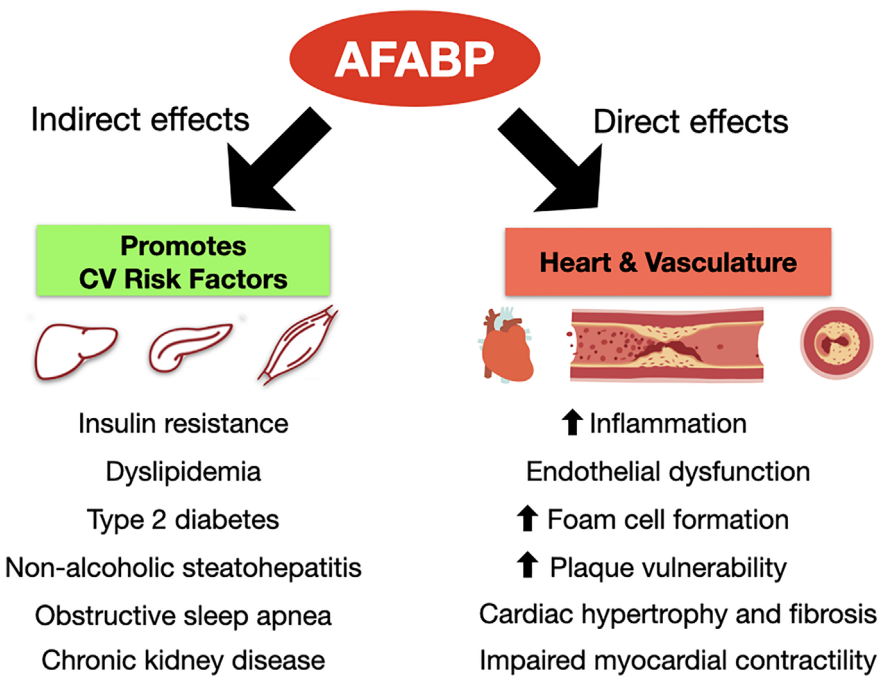

FIGURE 2 | Direct and indirect effects of AFABP to the development of cardiovascular diseases. AFABP, adipocyte fatty acid-binding protein; CV, cardiovascular.

of ARB and sodium glucose co-transporter 2 inhibitors on adiposity (114, 116).

CONCLUSION

Obesity has reached pandemic levels, and so has CVD. Adipose tissue inflammation with dysregulated adipokine secretion is crucial to the pathogenesis of adverse cardiovascular outcomes in obesity. Recent mechanistic and epidemiological studies have provided further insights to support AFABP as a key player mediating this adipose-vascular cross-talk *via* direct and indirect effects (**Figure 2**). However, from a clinical perspective, further validation studies are certainly required to investigate the potential of employing AFABP as a promising marker of CVD and cardiovascular mortality for clinical application. Moreover, standardization of commercial AFABP ELISA assays is also equally important. On the other hand, while preclinical studies have clearly demonstrated AFABP as an attractive therapeutic target in battling against CVD, intervention studies to evaluate the efficacy and safety of pharmacological inhibitors of AFABP and/or neutralizing antibodies in humans are eagerly awaited. In summary, although it may still be a long way before its clinical application as a biomarker or therapeutic target, research in recent years have clearly shown that AFABP is another major adipokine linking obesity with inflammation and adverse cardiovascular outcomes.

AUTHOR CONTRIBUTIONS

C-HL researched the data and wrote the manuscript. DL and KL critically reviewed and edited the manuscript. KL initiated and conceptualized this review and is the guarantor of this work. All authors contributed to the article and approved the submitted version.

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REVIEW
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Adipocyte Oncostatin Receptor Regulates Adipose Tissue Homeostasis and Inflammation

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Adipocytes are the largest cell type in terms of volume, but not number, in adipose tissue. Adipocytes are prominent contributors to systemic metabolic health. Obesity, defined by excess adipose tissue (AT), is recognized as a low-grade chronic inflammatory state. Cytokines are inflammatory mediators that are produced in adipose tissue (AT) and function in both AT homeostatic as well as pathological conditions. AT inflammation is associated with systemic metabolic dysfunction and obesity-associated infiltration and proliferation of immune cells occurs in a variety of fat depots in mice and humans. AT immune cells secrete a variety of chemokines and cytokines that act in a paracrine manner on adjacent adipocytes. TNFα, IL-6, and MCP-1, are well studied mediators of AT inflammation. Oncostatin M (OSM) is another proinflammatory cytokine that is elevated in AT in human obesity, and its specific receptor (OSMRβ) is also induced in conditions of obesity and insulin resistance. OSM production and paracrine signaling in AT regulates adipogenesis and the functions of AT. This review summarizes the roles of the oncostatin M receptor (OSMRβ) as a modulator of adipocyte development and function its contributions to immunological adaptations in AT in metabolic disease states.

Keywords: adipocyte, OSM, Inflammation, OSM receptor, fat, adipose tissue, insulin resistance

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INTRODUCTION

The global obesity rate has nearly doubled since 1980 (1). This high incidence poses a massive economic burden on healthcare systems. More importantly, obesity is frequently accompanied by adverse metabolic effects including hypertension, dyslipidemia, fatty liver, insulin resistance and type 2 diabetes (T2D) (2). In addition, obesity (3) and T2DM (4) are prominent risk factors for the severity of COVID-19 infections. Although obesity is a threat to global health, treatment options remain limited, and they are often ineffective or invasive (*e.g.* bariatric surgery) (5).

Obesity occurs when energy intake exceeds energy expenditure, but this relationship is complex, as many factors influence these two parameters. Positive energy balance causes WAT to expand by adipocyte hyperplasia, hypertrophy, or a combination of these processes. In addition to lipid storage, adipocytes have important endocrine functions whereby they secrete hormones (leptin, adiponectin, etc.), microRNAs, exosomes, and lipids that contribute to systemic metabolic health (6). There is evidence that the release of proinflammatory cytokines, such as Tumor Necrosis Factor α (TNFα) and Monocyte chemoattractant protein 1 (MCP-1) that can occur in obesity is driven by

stress responses related to WAT expansion, although specific mechanisms involved remain to be elucidated (7).

In addition to adipocytes, there are several other cell types within WAT, including different types of macrophages and T cells. The non-adipocyte cells in AT, such as immune, endothelial, perivascular, and stromal cells, as well as preadipocytes, collectively comprise the stromal vascular fraction (SVF). The cell numbers of the SVF are greater than number of adipocytes in white adipose tissue depots. Obesity is associated with changes in the relative abundance and activation states of various immune cell subpopulations in AT, as well as with altered endocrine properties of adipocytes themselves. Many of the proinflammatory cytokines produced in AT act in a paracrine manner and typically do not contribute to circulating levels of these signaling mediators. Proinflammatory cytokines made in AT can inhibit adipocyte differentiation and induce insulin resistance in adipocytes, and modulation of both these processes in AT has systemic effects (8–10). Although less studied than other AT cytokines, OSM clearly contributes to AT homeostasis (11–13), and increased OSM levels in AT promote systemic metabolic dysfunction through effects on both adipocyte development and adipose tissue function.

OSM AND ITS SPECIFIC RECEPTOR OSMRß: SOURCE AND BIOLOGY

The gp130, or interleukin (IL)-6, family is a group of structurally similar cytokines that includes IL-6, IL-11, IL-27, neuropoietin, leukemia inhibitory factor (LIF), OSM, cardiotrophin-1, ciliary neurotrophic factor, and novel neurotrophin-1/B cell stimulating factor-3 or cardiotrophin-like cytokine (14). These cytokines regulate a variety of complex biological processes, including hematopoiesis, immune responses, inflammation, stem cell potency, mammalian reproduction, cardiovascular action, and neuronal survival (15). Also, gp130 cytokines have been proposed as potential therapeutic targets for obesity treatment (16). Hence, there is a strong rationale for studying gp130 cytokines in modulating metabolic processes in WAT and other tissues involved in obesity and related diseases.

All members of the IL-6 cytokine family require glycoprotein 130 (gp130) as a common signal transducer in their receptor complexes. Unlike other gp130 cytokines, OSM has its own specific receptor (OSMRβ) that heterodimerizes with gp130 but is not used by other gp130 cytokines (17) and mediates the majority of OSM effects. OSM and LIF evolved by gene duplication relatively recently (18), and they share substantial sequence identity (19). Though originally identified for its ability to inhibit cancer growth in humans (20), OSM can modulate a variety of other biological processes, including liver development and regeneration (21, 22), hepatic insulin resistance and steatosis (23), inflammation (24), and cardiomyocyte dedifferentiation and remodeling (25). There is some evidence that OSM is the only gp130 cytokine with the unique ability to signal through two distinct receptor units-the gp130/LIFR (26) and the gp130/OSMRβ complex (17). However, other studies have shown that murine OSM signals only through the gp130/OSMRβ receptor complex (27–29).

OSM is produced by activated T cells and macrophages (20, 30, 31), and elevated OSM levels are found in a variety of inflammatory diseases in humans, including inflammatory bowel disease, rheumatoid arthritis, cancer, and obesity (12, 32–35). Our own research has shown that OSM is present in the SVF of AT, but not in adipocytes (11). Purification of immune cells in AT revealed that T cells and macrophages were the main sources of OSM in adipose tissue in mice (36). Although OSM is produced in immune cells, the OSM receptor (OSMRß) is present in both adipocytes and immune cells (36). However, upregulation of OSMRß expression by high-fat diet is observed only in adipocytes (36).

EFFECTS OF OSM-OSMRß INTERACTION IN PATHOLOGICAL CONDITIONS

The molecular signaling caused by OSM-OSMRß interaction has been suggested to modulate several inflammatory processes, including obesity-related insulin resistance (11, 13). One of several mechanisms involved in the ability of excess OSM to promote metabolic dysfunction is the control of adipogenesis. Inhibition of fat cell differentiation and adipose tissue expansion has been recognized as a causative factor for insulin resistance for over twenty years (37). Indeed, factors that inhibit adipogenesis, including OSM, tumor necrosis factor alpha and interferon gamma have been shown to have metabolically unfavorable effects such as insulin resistance (38). It is well established that OSM inhibits adipocyte development of both brown and white adipocytes *in vitro* (39–41). Mice with a global deletion of OSMRβ have increased adipose tissue mass (42), supporting the concept that OSM acts to inhibit adipocyte development and that lack of OSM signaling leads to increased AT expansion. There is also evidence to suggest that OSM treatment of mice reduces body weight and adiposity (42, 43). However, it should be noted that the OSM doses used in these mouse experiments were very high (12.5 ng/g body weight, administered twice daily) and may have caused indirect effects on fat mass. The anti-adipogenic effects of OSM have also been shown in human preadipocytes (13). In regard to the molecular mechanisms involved in the impairment of adipogenesis, OSM has been shown to inhibits C/EBPα and PPARγ (peroxisome proliferator-activated receptor γ) expression, two key transcription factors involved in adipogenesis (40, 44). In terms of modulation of lipid and glucose homeostasis, the anti-adipogenic effects of OSM could have systemic consequences. In addition to AT, the liver is an essential metabolic organ for lipogenesis, lipid uptake, and fatty acid b-oxidation and liver is responsive to OSM signaling (45). Some studies show that the OSMRβ expression levels negatively correlate with mRNA levels of gluconeogenic genes. Moreover, OSMRβ ablation lead to decreased levels of genes related to cholesterol efflux and fatty acid β-oxidation, and increased expression of genes that regulate cholesterol synthesis, fatty acid synthesis, and uptake (45). Hence, it is likely that OSM promotes inflammation and metabolic dysfunction at least in part by inhibiting the development of new adipocytes., but there is also evidence to show OSM also regulates lipid metabolism pathways in the liver.

In addition to regulating adipocyte differentiation, OSM has been proposed to contribute to AT immune response. In contrast to IL-6 which is directly induced through the TLR-nuclear factor k-B pathway (46), OSM is secreted by activated macrophages through a PGE2-cyclic adenosine monophosphate- protein kinase A pathway (47, 48). In adipose tissue from obese mice, OSMRß has been reported to be increased in the SVF, especially in the F4/80-positive ATMs (adipose tissue macrophages), suggesting that OSM signaling is strongly associated with the pathogenesis of obesity and related metabolic disorders (43). OSM binding to OSMRß modulates inflammatory states, both *in vitro* and *in vivo*. Expression of stromal cell-derived factor 1 alpha (SDF-1α) has been reported to be suppressed by OSM treatment of adipocytes (49). SDF-1α, also known as CXCL12, regulates the trafficking of bone marrow progenitor cells, as well as the transendothelial migration of leukocytes (50, 51). Further studies are required to determine whether altered SDF-1 levels play a role in mediating OSM's effects on homeostasis or metabolic dysfunction. In addition to SDF-1, there is evidence that plasminogen-activator inhibitor 1 (PAI1) is also directly regulated by OSM (11). The ability of OSM to induce PAI1 is dependent on OSMRß expression in cultured murine adipocytes (11). Although SDF-1 and PAI-1 may play a role in OSM function in AT, no rigorous studies have identified or directly evaluated OSM-regulated genes in adipocytes. Interestingly, *in vitro* experiments in brown adipocytes have demonstrated that OSM signaling *via* the OSMRß results in an increase in TNFα and MCP-1 (or C-C Motif Chemokine Ligand 2, Ccl2) mRNA levels, and interleukin 6 protein and each of these cytokines are involved in the recruitment and activation of macrophages in AT (13, 41). Therefore, it is reasonable to predict that in obesity, the overexpression of OSM by immune cells, including macrophages, is acting on adipocytes to induce the secretion of inflammatory cytokines that promote infiltration and activation of more macrophages. This vicious cycle leads to a low-grade chronic inflammatory state that contributes to the development of insulin resistance (**Figure 1**). Moreover, in humans with obesity, OSM levels correlate positively with inflammatory markers and negatively with glucose transporter 4 (Glut4), suggesting that signaling through OSMRß could promote an immunological response in AT that impairs glucose homeostasis (13, 41).

In vivo experiments have demonstrated that mice lacking OSMRβ, specifically in adipocytes, have significant increases in AT mass and OSM expression in fat, as well as enhanced adipose tissue inflammation, as compared to floxed littermate controls (36). The latter observation is unexpected, given that OSM signaling is known to promote inflammation. Although data from this study suggests that enhanced OSM-OSMRβ action in other AT cells, including immune populations, is consistent with the increased inflammatory immune response and insulin resistance phenotype in mice that lack OSM receptor specifically in adipocytes (36). Hence, by blocking OSM signaling in adipocytes *via* loss of the OSM receptor, the AT levels of OSM increase and promote metabolically unfavorable effects by acting on non-adipocyte cells present in AT.

One method to assess the importance of an endocrine mediator is to inhibit its activity with an immunoneutralization approach. Immunoneutralizing OSM is a complementary approach to knocking down the OSM receptor in adipocytes. In a recent study, we used high-fat fed C57BL/6J mice to induce OSM expression in AT and performed OSM immunoneutralization. Mice that received a specific anti-OSM antibody had improved inflammatory responses as compared to mice treated with a control IgG antibody (13). Moreover, OSM immunoneutralization normalized glucose levels and decreased expression of inflammatory genes in adipose tissue. However, OSM immunoneutralization did not significantly alter whole-body glucose tolerance or systemic insulin sensitivity (13). Although there are limitations with this approach, these studies underscore the need to understand the cell and tissue specific effects of both physiological and pathological functions of OSM.

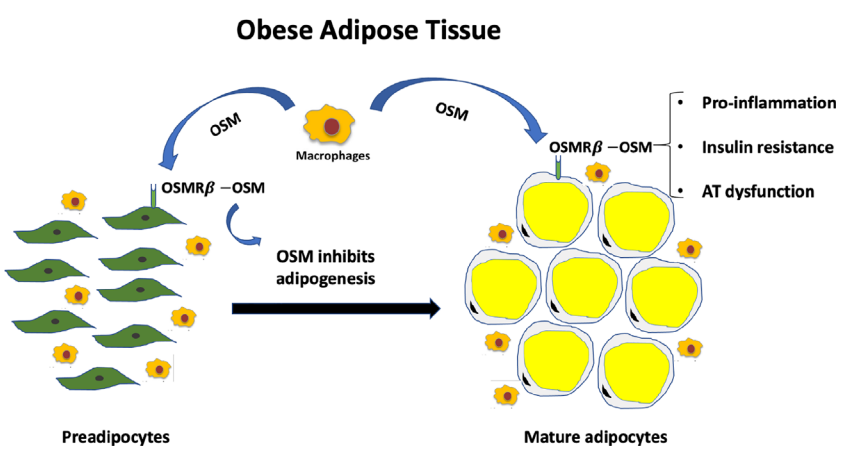

FIGURE 1 | Excess OSM and lack of adipocyte OSM signaling contributes to metabolic dysfunction. Less than half of the cells that comprise white adipose tissue depots are adipocytes. OSM is not produced in adipocytes, but in adipose tissue macrophages in conditions of obesity. OSM acts on preadipocytes to inhibit adipogenesis and acts on mature adipocytes to promote inflammatory signaling and insulin resistance in adipocytes. Both a loss of OSM signaling in adipocytes or excess OSM in adipose tissue promote systemic metabolic dysfunction.

In addition to its functions in AT and association with obesity and Type 2 diabetes, OSM has been shown to play a role in a variety of disease conditions. Several studies have identified the OSM-OSMRß interaction as a potential therapeutic strategy for several pathological conditions. The selective inhibition of OSM by a neutralizing antibody suggested that paracrine actions of OSM in mammary fat played a role in breast cancer progression (34). In addition, OSM has been identified as a potential biomarker and therapeutic target in inflammatory bowel disease (35). The ability to target OSM in inflammatory bowel disease is important as up to 40% of patients do not respond to anti-TNF agents. Of note, an anti-OSM monoclonal antibody has recently been shown to be well tolerated in healthy subjects, and has demonstrated sufficient affinity to achieve target engagement in systemic circulation and target skin tissue, supporting further clinical investigation of anti-OSM antibodies for inflammatory diseases (52).

CONCLUSIONS

In summary, OSM is a member of a large cytokine family, but its unique functions in adipocytes drive its effects on metabolic health. Levels of OSM and its receptor are elevated in AT in conditions of obesity and insulin resistance in mice and man (12). The roles of OSM have been elucidated using a wide range of approaches including global and adipocyte-specific knockout of the OSM receptor, as well as immunoneutralization of OSM in metabolically compromised mice. In AT, elevated levels of immune cell-derived OSM act on adjacent AT cells to inhibit preadipocyte differentiation and to enhance proinflammatory responses in adipocytes. Although adipose tissue OSM levels correlate with systemic metabolic dysfunction, a loss of OSM receptor in adipocytes is also associated with impaired metabolic responses. This finding is consistent with a role for OSM signaling in healthy adipocytes and in AT homeostasis. Of note, there is a precedent for the contribution of inflammatory mediators in normal adipocyte function, as suppressing adipocyte inflammation impairs AT function and promotes insulin resistance (53, 54). Notably, the suppression of macrophage inflammation has little effect on obesity-induced insulin resistance, but inhibition of inflammatory signaling in adipocytes substantially effects systemic metabolic function (54). Inflammatory signaling in adipocytes plays a role in maintaining normal adipose tissue function and OSM signaling in adipocytes and adipose tissue is important for normal adipose tissue function and systemic metabolic health.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the preparation and editing of this review. All authors contributed to the article and approved the submitted versión.

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Adipocytes, Innate Immunity and Obesity: A Mini-Review

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The role of adipose tissue (AT) inflammation in obesity and its multiple related-complications is a rapidly expanding area of scientific interest. Within the last 30 years, the role of the adipocyte as an endocrine and immunologic cell has been progressively established. Like the macrophage, the adipocyte is capable of linking the innate and adaptive immune system through the secretion of adipokines and cytokines; exosome release of lipids, hormones, and microRNAs; and contact interaction with other immune cells. Key innate immune cells in AT include adipocytes, macrophages, neutrophils, and innate lymphoid cells type 2 (ILC2s). The role of the innate immune system in promoting adipose tissue inflammation in obesity will be highlighted in this review. T cells and B cells also play important roles in contributing to AT inflammation and are discussed in this series in the chapter on adaptive immunity.

Keywords: adipocytes, obesity, adipose tissue, inflammation, innate immunity

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INTRODUCTION

Obesity is a growing healthcare problem in the United States and globally. It is a leading cause of preventable death and currently impacts more than 35% of the US population (1). This number is estimated to rise with a projected 42% of the US adult population being obese by 2030 (2). Obesity adversely impacts the entire body leading to increased Type 2 diabetes and associated complications, Alzheimer's disease, vascular dementia, obstructive sleep-apnea, accelerated atherosclerosis, heart failure, fatty liver disease, nonalcoholic steatohepatitis, osteoarthritis, altered immune system, impaired response to vaccines, and increased susceptibility to cancer compared to aged-matched lean individuals (3). Obesity is characterized by an expansion of both visceral and subcutaneous adipose tissue (AT) in the setting of chronic over-nutrition. The ensuing chronic low-grade inflammation sets the stage for many of the extensive complications. Thus, understanding mechanisms that mediate the immunological changes in obesity may unlock new therapeutic strategies. This review places a special emphasis on the innate immune system and the adipocyte.

INNATE AND ADAPTIVE IMMUNITY

The role of the immune system is to identify self- versus non-self to eliminate potential toxins, allergens, and pathogens without destroying the host tissue. The immune system is composed of two key functional responses, innate and adaptive immunity. The innate immune response is the initial

line of defense after the host's external barrier. This immune response is not antigen-specific, but rather recognizes molecular patterns that are inherent to the toxin, allergen, or pathogen. This allows for the rapid activation of an immune response which is followed by the development of an antigen-specific immune response. The adaptive immune system upon the first encounter of a toxin, allergen, or pathogen undergoes expansion to aid the innate immune response. Upon resolution, a subset of these adaptive immune cells persists and creates a distinct population of memory immune cells. Memory cells are faster to respond to future encounters with the same pathogen, allergen, or toxin. The cellular component of both immune responses arises from the hematopoietic stem cell. This pluripotent cell further differentiates within the bone marrow to generate either the common lymphoid progenitor or the common myeloid progenitor. The common myeloid progenitor gives rise to lineage-specific colony-forming cells which then further develop into a majority of the innate immune cells as well as megakaryocytes (platelets) and erythrocytes (red blood cells). The cellular components of the innate immune response include the granulocytes including monocytes, macrophages, neutrophils, basophils, eosinophils, mast cells, and dendritic cells, as well as adipocytes. While the adipocyte is not traditionally viewed as an immune cell, recent research has demonstrated that the adipocyte releases adipokines, microRNAs and lipids to influence the innate immune response (4–7). The adipocyte also expresses MHCII molecules during high-fat diet feeding allowing the adipocyte to interact with naïve T cells resulting in T cell differentiation and activation (8, 9). The common lymphoid progenitor gives rise to the key immune cells within the adaptive immune response including B cells and T cells as well, as more recently discovered innate immune cells including the NK cell and the innate lymphoid cell types 1, 2, and 3 (10, 11). The key link between the innate and adaptive immune system is antigen presentation.

INFLAMMATION IN ADIPOSE TISSUE; CONTRIBUTION OF INNATE IMMUNITY

The AT immune cell microenvironment in the lean state is a well-balanced crosstalk between the adipocyte and the stromal vascular fraction (SVF) or the cellular compartment of AT. In lean mice, the SVF is comprised of mesenchymal stem cells (12), endothelial progenitor cells (13–16) as well as numerous immune cells including anti-inflammatory immunoregulatory T cells, Tregs (17), innate lymphoid type 2 cells (ILC2) (18), alternatively activated macrophages (19, 20), and eosinophils (21). These cells work in concert to ensure the maintenance of homeostasis within AT including maintaining systemic insulin sensitivity. However, upon high-fat diet (HFD) feeding, there is a disruption of the anti-inflammatory milieu with increased differentiation and recruitment of pro-inflammatory immune cells creating a chronic, low-grade inflammatory state. In murine obesity, AT is characterized by the early, transient infiltration of neutrophils (22) followed by the accumulation of pro-inflammatory CD8+ T cells (23), CD4 Th1 cells (8) and M1 macrophages (19, 20) all of which surround the dying adipocyte forming a crown-like structure. Innate immunity is an early and key component in sustaining AT inflammation.

Adipocyte

The adipocyte, unlike most traditional immune cells, links the innate and adaptive immune systems through adipokine, lipid and exosome release and through antigen presentation. While the adipocyte is the primary site of energy storage for the body and performs multiple metabolic activities, it can assume the role of a highly functional immune cell, releasing anti- and pro-inflammatory cytokines and hormones (adipokines), as well as lipids, which also act as signaling molecules (24). Since its discovery as an endocrine cell, the adipocyte has been identified to secrete more than 50 adipokines/cytokines including adiponectin (4, 25), leptin (5), TNFα (26–29), visfatin (30), and resistin (31) among many others (32) which impact local and systemic metabolism and inflammation.

The first adipokine described was leptin which revolutionized our understanding of the critical role that adipocytes play in whole-body energy homeostasis (33). Mutations in the leptin gene in the ob/ob mouse model led not only to hyperphagia and weight gain but also disruptions in fertility and body temperature regulation (34). Treatment of ob/ob mice with recombinant leptin, but not db/db (leptin receptor-deficient) mice, led to improved body weight and decreased food intake (35). However, contrary to what was initially hypothesized, leptin is found in higher levels in obese patients as compared to lean controls (36) suggesting the presence of leptin resistance (37). Increasing evidence supports an immunologic role of leptin. Leptin deficiency is associated with greater susceptibility to death after administration of LPS or TNFα which is partially corrected with leptin administration (38, 39). Macrophages from leptin-deficient mice have impaired phagocytosis and altered cytokine production (40, 41). In neutrophils, leptin appears to increase ROS production (42), inhibit apoptosis (43), and affect neutrophil migration (44) suggesting that leptin impacts cells that mediate the innate immune response. More recently, Scherer and colleagues (45) demonstrated that hyperleptinemia is a driving force for metabolic disorders. Interestingly, a partial decrease of circulating leptin in obesity reestablishes hypothalamic leptin sensitivity and effectively reduces weight gain and enhances insulin sensitivity.

Unlike leptin, adiponectin, a key adipokine involved in energy homeostasis, is reduced in obese subjects and has anti-inflammatory effects. Adiponectin is found in higher levels in AT and blood of lean subjects (46). Ob/ob mice with adiponectin overexpression have an increased ability to expand their subcutaneous AT associated with in a reduction of systemic and local AT inflammation. These mice also develop less ectopic lipid deposition in the liver and skeletal muscle leading to improvements in insulin sensitivity despite greater amounts of AT (47). Within the innate immune system, adiponectin acts primarily on macrophages resulting in a greater polarization of M2-like macrophages, decreased M1-like macrophages, and a reduction in ROS production (48). In neutrophils, adiponectin

functions to decrease the production of the neutrophil chemokine CXCL8 (49) and ROS *via* modulation of NADPH oxidase (50). These observations highlight the yin-yang relationship of leptin and adiponectin, which functions as an anti-inflammatory regulator of the innate immune response. These hormones are the most well-known adipokines, but as discussed above, numerous other have been identified. Consistently obese versus lean humans and mice reveal increased proinflammatory and extracellular matrix gene expression, but the function of many adipokines remains unknown (51, 52).

Lipid release is another important mechanism by which adipocytes can impact immune cells in the AT microenvironment. Lipids such palmitate and other unsaturated fatty acids can bind to toll-like receptors (TLRs) on the surface of immune cells, such as macrophages, and are converted to ceramides and diacylglycerols during states of lipid overabundance as occurs in obesity. These toxic lipids enhance proinflammatory signaling (7). More recently, branched-chain fatty acid esters of hydroxyl fatty acids (FAHFAs) produced by adipocytes were shown to bind to G-protein coupled receptors (GPRs) 40 and 120 to inhibit inflammation and improve insulin secretion and sensitivity (53). Within the blood and subcutaneous adipose tissue of insulin-resistant humans and mice, there is a reduction of several FAHFAs most notably palmitic acid esters of hydroxyl stearic acids. Supplementation of these *via* oral ingestion or subcutaneous administration improves glucose and insulin handling (53). These observations indicate adipocytes release both pro- and anti-inflammatory lipids as a mechanism to modulate the immune system.

Adipocytes are composed of large unilocular lipid droplets containing triacylglycerols and neutral free fatty acids (FFAs), which are a major mechanism for energy storage and release. Adipocytes not only release lipids and secrete adipokines, but they can also employ exosomes- extracellular vesicles (40-150 nm in size) of endosomal origin to participate in this process. Exosomes are increasingly recognized as a novel mechanism by which adipocytes communicate with other cells and target tissues. Their cargo contains adipokines, lipids, and microRNAs. Release is dependent on nutritional status and degrees of adiposity: increased release in obesity and decreased release with caloric restriction or lipodystrophy. Exosomes can be taken up by endocytosis, pinocytosis, or phagocytosis, and can be directed to target cells by adhesion molecules on the exosomal surface (6). Recently, Flaherty, et al. reported that adipocytes of mice release 1% of their lipid content daily ex vivo *via* exosomes, which was increased in obese mice. This release of exosomes contributed to macrophage foam cell formation, suggesting that exosomes contribute to the orchestration of AT immune cells (54). MicroRNAs, which regulate protein translation, are another important component of adipocyte-derived exosomes. Adipocytes are a major source of microRNAs in the circulation with greater than 55 differentially expressed microRNAs between lean and obese individuals. Adipocyte microRNAs contribute to the regulation of metabolism, inflammation, and multiple biologic processes locally and systemically (55). However, controversy exists as to whether adipocyte exosomes represent a minority or majority of circulating exosomes (54, 56).

Finally, one of the most unique features of the adipocyte is its ability to function as an antigen-presenting cell, which is described in detail in Chapter XX of this series by Deng et al. The adipocyte can present antigen to promote differentiation and activation of interferon gamma-producing CD4 Th1 cells. This activity is increased early in obesity, after only 2 weeks HFD in mice, before the AT macrophage increase, suggesting adipocytes both instigate and maintain AT inflammation (8). Adipocytes provide a critical link between the innate and adaptive immune systems.

Neutrophils

Neutrophils are one of the initial inflammatory cells recruited to sites of host injury. As a component of the innate immune system, neutrophils have four primary activities including, phagocytosis, degranulation, reactive oxygen species (ROS) production, and neutrophil extracellular trap (NET) formation (57). Within mouse peripheral blood, neutrophils comprise 10-25% of the circulating immune cells (58), whereas in humans they compromise 50-70% of circulating immune cells. Furthermore, unlike humans, mouse neutrophils do not have defensins (anti-microbial peptides). While there are several differences between mouse and human neutrophils, mouse models are still routinely used for genetic manipulation (59).

Early studies in mice observed the transient infiltration of neutrophils into the AT after the start of HFD resulting in maximal levels by day 3 and undetectable levels by day 28 (22). However, others have suggested a more prolonged presence, contributing to about 2% of the SVF (60). Despite the early and relatively small contribution of the neutrophil in obese mice, loss of neutrophil elastase (60) or myeloperoxidase (61) leads to a decrease in AT inflammation and macrophage recruitment and promotes resolution of insulin resistance. However, loss of neutrophil NET formation does not impact obesity-related inflammation or insulin resistance in HFD-fed mice (62).

Within human AT, there have been fewer studies. One identified the presence of neutrophils within subcutaneous AT and reported that neutrophils were contained within the vasculature with no or limited infiltration into the tissue similar to vascular pools of neutrophils found in the liver (63). Another study showed limited infiltration in obesity in both visceral and subcutaneous AT (64); however, the quantity, cause of recruitment, and function of these cells within human AT remains unknown. Despite this, inflammatory lipids such as leukotrienes are known to attract and activate neutrophils. Under inflammatory conditions adipocyte and macrophages produce increased IL-8, a powerful neutrophil chemoattractant (65, 66). Additionally neutrophils can self-recruit *via* increased production of CXCL2, another known neutrophil chemoattractant (67). Using a mouse peritonitis model, Tynan et al. demonstrated that lipids extracted from human adipocytes promoted migration and accumulation of neutrophils and macrophages, and activated these cells to produce cytokines (68). These effects were similar whether the adipocytes were obtained from lean or obese subjects, as fatty acid profiles, analyzed by gas chromatography, were not different. Oleic acid was also shown to recruit neutrophils in a

similar mouse model (69). Additionally, adipocyte lipolysis has been shown to attract neutrophils and enhance their production of IL-1β leading to the activation of adipocytes and other immune cells (70). Further studies will be useful to determine the types of adipocyte lipids and other factors that attract and activate neutrophils into AT.

Macrophages

One of the most well-studied immune cells in AT and a key component of the innate immune response is the macrophage. Within mouse models of obesity, macrophages comprise up to 40% of the SVF (71) and are shown to be involved in the development of insulin resistance (72), atherosclerosis progression (73), and other obesity-related complications. In mice, macrophages have been classified into the relatively simplistic M1 and M2 phenotypes with obesity increasing the prevalence of pro-inflammatory M1-like macrophages (19). Ablation of these CD11c+ proinflammatory macrophages decreases AT inflammation and interferes with the development of insulin resistance, suggesting that macrophages are key mediators of insulin sensitivity (72). Furthermore inhibiting macrophage recruitment through the genetic depletion of CCR2 (74) or MCP-1 (75) also leads to the repression of AT inflammation and insulin resistance during HFD feeding. In contrast, accumulation of anti-inflammatory PPARγ positive macrophages (M2 macrophages) leads to improvements in AT inflammation and insulin sensitivity (76), while loss of macrophage PPARγ increases AT inflammation and insulin resistance (77). The balance between the pro and anti-inflammatory macrophage subtypes is much less defined within human AT with most AT macrophages expressing both common M1 and M2 markers (78, 79). Although there is still some debate on this topic with others suggesting a greater abundance of CD206 macrophages after weight loss (80). Further research on human adipose tissue macrophage subsets still needs to be done. The interaction between adipocytes and ATMs begins with the formation of crown-like structures characterized by macrophage accumulation surrounding dying adipocytes. This process is mediated by the adipocyte secretion of MCP-1 resulting in macrophage accumulation and activation (81). Once accumulated, these macrophages release TNFα which increases adipocyte release of FFAs (82). FFAs are capable of binding TLR4 on both the adipocyte and the macrophage resulting in NFkB activation and release of IL1β by macrophages (83). Adipocyte turnover occurs with approximately 10% of adipocytes undergoing apoptosis annually (84). These dying adipocytes are removed *via* trogocytosis by ATMs (85). Adipokine secretion by adipocytes also alters macrophage function. Increased leptin in obesity increases the phagocytic function of ATMs and is associated with an increase in circulating C-reactive protein (41, 86). Adiponectin secretion is thought to be inhibited by TNF-alpha secretion which is increased in obesity. In the lean state,

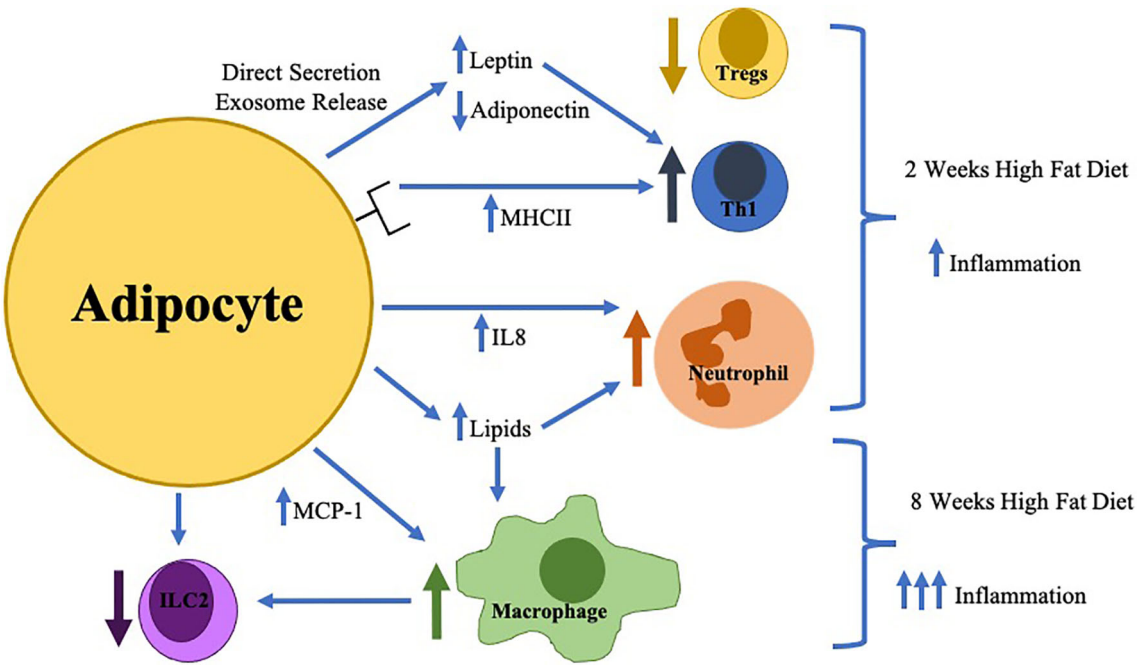

FIGURE 1 | The earliest changes in adipose tissue inflammation include adipocyte hypertrophy with direct secretion/exosome release of key adipokines (increased leptin/decreased adiponectin) which in combination with increased adipocyte MHCII expression result in the differentiation of pro-inflammatory Thelper type 1 cells (Th1) resulting in decreased regulatory T cells (Tregs). Adipocytes during this time frame also increase IL-8 production resulting in neutrophil accumulation. Later in the time course (around 8 weeks), macrophage infiltration is stimulated by toxic lipid production and increased MCP-1 secretion which inhibits innate lymphoid cell type 2 (ILC2) differentiation.

adiponectin is known to inhibit the development of foam cells from macrophages and decreases endothelial cell activation and monocyte adhesion (87). The interaction between adipocytes and ATMs is a key contributor to the chronic low-grade inflammation of obesity (88).

Innate Lymphoid Cells Type 2

Another key innate immune cell that is not well-defined within AT is the innate lymphoid cell type 2 (ILC2). ILC2 cells are important in the maintenance of insulin sensitivity and are decreased in the setting of HFD. Innate lymphoid cells express CD4+ related cytokines, mirror Th1, Th2, and Th3 expression profiles (89), but differ in that they do not express B or T cell receptors despite arising from the common lymphoid progenitor cell (90). The ILC2 cell is similar to Th2 cells in that it contains the transcription factor GATA3 (91) and secretes IL5 and IL13. Within mouse models of obesity, the administration of IL25 leads to improvements in glucose tolerance and weight loss and is associated with the infiltration of ILC2 cells, alternatively activated macrophages, and eosinophils. Depletion of ILC2 cells in obese Rag1-/- mice leads to worsening insulin sensitivity and weight gain, while repletion of ILC2 cells reverses these negative metabolic consequences (18). Furthermore, in murine models, ILC2 cells appear to be the primary source of IL-5 and IL-13 and are necessary for the maintenance of alternatively activated macrophages and eosinophils, two key cells implicated in the anti-inflammatory state of lean AT (92). These cells are thought to contribute to an improved metabolic phenotype through the beiging of WAT characterized by increased expression of Ucp1 leading to an increase in caloric expenditure and attenuation of weight (93, 94). These researchers also confirmed that ILC2 cells are markedly decreased in the SAT of obese compared to lean humans (93); thus highlighting a key role for ILC2 cells in AT (95).

CONCLUSIONS

Multiple changes in the innate immune system are key contributors to inflammation in obese AT resulting in the development of obesity-related diseases as summarized in **Figure 1**. Despite the greater understanding of the immunologic role of AT, further investigation should seek to answer the following questions: (1) what are the causes of AT immune cell infiltration and activation, (2) how does the adipocyte contribute to these changes and interact with AT immune cells, (3) is there a role of the gut microbiota in alteration of AT inflammation and (4) how do AT immune cells change during weight-loss. Through the elucidation of the answers to these key questions, immunologic therapies, potentially targeting the adipocyte, for the treatment of obesity and its inflammatory complications can be developed.

AUTHOR CONTRIBUTIONS

AB, AJ, and WH were involved in all aspects of writing, editing, and decision to publish. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: WH advises for Novo Nordisk and Intercept Pharmaceuticals.

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