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Title

Properties and functions of adipose tissue macrophages in obesity

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Summary

The expansion of adipose tissue in obesity is accompanied by the accumulation of immune cells that contribute to a state of low-grade, chronic inflammation and dysregulated metabolism. Adipose tissue macrophages (ATMs) represent the most abundant class of leukocytes in adipose tissue (AT) and are involved in the regulation of several regulatory physiological processes such as tissue remodeling and insulin sensitivity. With progressive obesity, ATMs are key mediators of meta-inflammation, insulin resistance, and impairment of adipocyte function. While macrophage recruitment from blood monocytes is a critical component of the generation of adipose tissue inflammation, new studies have revealed a role for ATM proliferation in the early stages of obesity and in sustaining adipose tissue inflammation. In addition, studies have revealed a more complex range of macrophage activation states than the previous M1/M2 model and the existence of different macrophage profiles between human and animal models. This review will summarize the current understanding of the regulatory mechanisms of ATM function in relation to obesity, to type 2 diabetes, to depot of origin, and to other leukocytes such as adipose tissue dendritic cells with hopes of emphasizing the regulatory nodes that can potentially be targeted to prevent and treat obesity-related metabolic disorders.

Scope of Review

Since ATMs have been predominantly linked to low-grade, chronic inflammation of obesity, we discuss properties and functions of ATMs in this review with a focus on what we know in mouse models and humans. Our focus on ATM biology will also highlight differences between adipose tissue dendritic cells (ATDC) and other leukocytes.

Introduction

Incidence of obesity and associated co-morbidities (type 2 diabetes (DM), liver and cardiovascular diseases, and certain forms of cancer) remains on the rise[1]. Obesity results from a chronic unbalance between caloric intake and energy expenditure that is characterized as a low grade, chronic inflammatory disease that contributes to metabolic dysfunction and insulin resistance (IR)[2]. Although the molecular

basis underpinning this inflammation is not fully understood, there is consensus that macrophage activation in adipose tissue precedes the development of insulin resistance and contributes to a pro-inflammatory state[3, 4]. Therefore, deciphering macrophage biology and pathophysiology in the obese setting remains a unique challenge to the field of immunology and metabolism research. Transcriptional profiling has advanced the understanding of the plasticity of macrophages suggesting a complex cellular programming in response to stress signals[5] and has emphasized the concept that macrophages can quickly adopt unique properties depending on micro-environmental cues[6]. Nevertheless, the mechanisms underpinning the specialized transcriptional and signaling profiles in macrophages during obesity-induced adipose tissue inflammation are not fully resolved. In this review, we will summarize what is known about adipose tissue macrophage (ATM) biology in the setting of obesity and metabolic disease in a rapidly moving field of investigation.

ATM-driven inflammation links obesity to insulin resistance

Obesity is characterized by a chronic low-grade inflammation which is causally implicated in the development of insulin resistance (IR). Insulin resistance is a central mechanism in obesity associated diseases such as Type 2 diabetes and metabolic syndrome. It is defined as the decline to a normal physiological response to insulin, resulting into a reduction in glucose disposal as well as failure to suppress lipolysis and hepatic glucose production and occurs prominently in adipose tissue. Numerous studies support the role of adipose tissue macrophages and derived inflammatory mediators in the impairment of insulin signaling pathways[7-10]. ATM-derived pro-inflammatory cytokines inhibit insulin action via activating pro-inflammatory kinases, including IkB kinase (IKK) and JNK in adipocytes[11]. Ablation of JNK in macrophages protects mice from obesity-induced IR by reducing macrophages infiltration into pancreatic islets and blocking ATM polarization toward an inflammatory phenotype[12]. Insulin binds to its receptor, the insulin receptor ‘IR’ and its homologous insulin-like growth factor 1 receptor ‘IGFR’, on macrophages to induce a signaling cascade that leads to a metabolic reprograming to promote activation[13]. It was demonstrated that IGFR1 signal transduction promotes alternative macrophage polarization as well as the lack of IGFR1 results into a higher pro- versus anti-inflammatory ATM ratio, less phagocytosis and more infiltration into adipose tissue[14]. Studies in $IR^{\Delta myel}$-mice bearing the conditional inactivation of the insulin receptor in myeloid lineage cells have also demonstrated that these mice remain protected from the development of obesity-associated insulin resistance and exhibit a decreased chronic, low-grade inflammatory state[15]. These findings clearly point out that insulin action plays a key role in regulating macrophage biology in adipose tissue during obesity-driven IR and inflammation[15].

ATM activation states

ATMs in lean and obese/insulin resistant settings were initially described based on the two-dimensional M1/M2 spectrum of macrophage activation[16] (Figure 1). While this paradigm was a useful model initially, advances in the understanding of a spectrum of macrophage activation have challenged the accuracy of this model and its application to *in-vivo* ATM populations[17-19]. Evidence have suggested that obesity-associated ATMs include highly plastic cell populations, whose immuno-phenotype is determined in response to multiple stimuli in their surrounding microenvironment. It is now evident that more than one population of ATMs exist in obese AT[20] and these distinct populations express specific markers, have unique tissue distributions, transcriptional profiles, and functions. The exact number and functions of ATM subsets in obese AT is evolving and the transcriptional mechanisms that define their unique activation states are not fully known. In obesity, ATMs adopt a metabolic activation state with prominent lysosomal activity[17, 18] with the main purpose to clear dead adipocytes[19]. Obese ATMs display surface markers that resemble neither classical (M1) nor alternative (M2) activation, but rather a state of metabolic activation (MMe) induced by diverse metabolic stimuli (i.e. free fatty acids, high insulin, high glucose)[17] (Figure 1). These views will be informed by our understanding of the complexity of macrophage activation[5]. Stimulation of macrophages with many different effectors, including pattern recognition receptor ligands, cytokines, and metabolic cues (i.e. free fatty acids, high-density lipoproteins) generates multiple clusters of activation states[5], and thus may expand and extend the current models.

Adipose tissue macrophage heterogeneity in mice

ATM diversity and heterogeneity can be seen not only between lean and obese states, but also based on the location of the adipose tissue depot (e.g. visceral and subcutaneous adipose tissue) and also if the depot is composed of white, brown, or beige adipocytes. In all depots, ATMs have been classified into two major subtypes: tissue resident and monocyte derived "recruited" macrophages. Number, localization, and properties of ATMs greatly differ depending on the metabolic status[3, 4]. Numerically ATMs population expands from 10% of all cells in lean adipose tissue to more than 50% in severe obesity in mice[3, 21]. The increased number of ATMs in obese AT is due to two distinct processes: recruitment of macrophages from monocyte trafficking and local proliferation of recruited macrophages.

In lean mice, a resident ATM pool originates from yolk-sac progenitors and self-renews by proliferation under homeostatic conditions[22, 23]. Over time, these resident ATMs appear to be replaced with bone marrow-derived macrophages, likely from monocyte intermediates[24]. Mouse monocyte subsets are classified as $Ly6C^{+}$ (further divided as $Ly6C^{high}$ and $Ly6C^{middle}$) and $Ly6C^{-}$ (also called $Ly6C^{low}$)[27]. Based

on the expression of surface markers and chemokine receptors Ly6C^{+} subsets are divided in: CD11b^{+} CD115^{+} and CCR2^{high} CX3CR1^{low}. Ly6C^{-} monocytes are grouped as CD11b^{+} CD115^{+} and CCR2^{low} CX3CR1^{high}[27]. In steady state, Ly6C^{+} monocytes differentiate into Ly6C^{-} monocytes in the circulation. In obese AT in response to inflammatory signals and chemokines such as CCL2, Ly6C^{+} activated monocytes migrate to the site of inflammation where they differentiate into ATMs[28, 29] (Figure 2). CCR2^{high} Ly6C^{+} inflammatory and CCR2^{low} Ly6C^{-} resident monocytes are generally thought to preferentially differentiate into M1 inflammatory and M2 anti-inflammatory macrophages, respectively[27].

Resident ATMs are distributed between adipocytes and along vascular structures in adipose tissue and are F4/80^{+} CD64^{+} CD206^{+} CD301^{+} CD11c^{-} (Table 1). These cells express anti-inflammatory molecules, regulate adipocyte lipid metabolism by secreting factors such as IL-10 and catecholamines and act as efferocytes to clear apoptotic cells and resolve inflammation. With increased lipolysis, resident macrophages contribute to lipid buffering via the control of free-fatty acids release into the circulation. In the obese setting, these cells undergo a polarization shift toward a pro-inflammatory phenotype[25] as well as alterations in death pathways leading to increased cell survival[26].

A major stimulus for this recruitment is adipocyte stress as ATMs accumulate around dead adipocytes forming clusters called crown-like structures (CLSs)[30]. Recruited ATMs have been distinguished from the resident pool based on their localization to CLS as well as their expression of CD11c, CCR2, and TLR4[25, 31, 32] (Table 1). CD11c has been a useful marker in both mouse and human studies to differentiate pro-inflammatory ATMs from the resident pool[21, 33]. Genetic ablation of CD11c expressing ATMs can attenuate obesity-induced inflammation and metabolic dysfunction[34]. In conjunction with infiltrating cells from the periphery, there is evidence indicating that local proliferation contributes to increasing ATM number and this phenomenon occurs predominantly within the CLSs[35-37]. In addition to increased recruitment, proliferation and diminished egress, increased cell longevity is an additional mechanism by which ATM number is modulated in obese adipose tissue[26].

Despite a broad use of CD11c as an inflammatory ATM marker in human and mouse studies, there may be limitations to the classification scheme using CD11c. Due to its expression on dendritic cells, a question in the field has been the ability to differentiate ATMs from adipose tissue dendritic cells (ATDC) given that F4/80 and CD11b are also found on ATDC. Using macrophage specific markers CD64 and MerTK[38], population of CD64^{-} CD11c^{+} ATDCs are identifiable and distinct from CD64^{+} CD11c^{+} and CD64^{+} CD11c^{-} ATM pools[39]. While a CD11c based scheme differentiates ATM subsets in mice and humans, recent transcriptomic studies suggest a revision to this scheme[39]. Single cell RNA sequencing and clustering analysis in murine samples suggested that three main ATM populations can be identified

by the markers CD9 and Ly6C[40]: Ly6c^{+}, Ly6c^{-} CD9^{+}, and Ly6c^{-} CD9^{-}. The Ly6c^{+} identified recruited ATMs with an adipogenic role that resided outside of CLSs, whose adoptive transfer into lean mice was reported to activate gene programs typical of normal adipocyte physiology. The Ly6c^{-} CD9^{-} expressed high levels of *Mrc1/Cd206* and were found in lean and obese settings consistent with the previous definitions of resident ATMs. The Ly6c^{-} CD9^{+} population was described as the subset that accumulated with obesity specifically within the CLSs, had high amounts of intracellular lipid in lysosome-like structures, expressed genes related to lysosomal-dependent lipid metabolism, secreted exosome-size vesicles, and had gene expression and regulatory profiles enriched for pro-inflammatory genes[40]. Thus far, Ly6c^{+} (recruited subset), Ly6c^{-} CD9^{+} (pro-inflammatory subset) and Ly6c^{-} CD9^{-} (anti-inflammatory subset) shared distinct tissue localization and Ly6c^{-} CD9^{+} were also identified in human adipose tissue. The authors note a lack of differential expression of CD11c in these populations, so future studies will need to clarify the functional role of CD9 in ATMs as well as other adipose tissue stromal cells. CD9^{high} adipocyte progenitors[41] have been identified as correlating with adipose tissue fibrosis in humans and CD9 is also found on dendritic cells[42] to control antigen presentation suggesting a potential broader role for CD9 in adipose tissue biology that has yet to be understood.

Human ATM markers

In parallel with an increase in ATMs during obesity in mice, macrophage numbers increase in adipose tissue in obese humans with the percentage of ATMs rising from 5-10% in the lean healthy status to 40-50% of the stromal cells in the obese setting[3, 43]. Similar to mice, there is evidence suggesting the coexistence of diverse ATM population subtypes that shape obesity-induced inflammation in humans. In contrast to the pro-inflammatory phenotype switch of macrophages found in many mouse studies, flow cytometry analysis of human fat has suggested that this phenomenon is fundamentally different in human ATMs in obese individuals and that ATMs are better represented by a mixed inflammatory phenotype. This mixed phenotype is characterized by the simultaneous presence of markers that generally belong to M2- and M1-type macrophages[44], such as CD206 and CD11c. Further ATM heterogeneity is seen when comparing different fat depots (visceral versus subcutaneous) in humans[45].

Human ATMs are generated from peripheral blood monocytes which are divided into three subsets: classical monocytes (CD14^{++} CD16^{-}), intermediate monocytes (CD14^{++} CD16^{+}), and non-classical monocytes (CD14^{+} CD16^{++})[27]. It was suggested that CD14^{++} CD16^{-} and CD14^{++} CD16^{+} monocytes

resemble mouse Ly6C$^+$ inflammatory monocyte subset, whereas CD14$^+$ CD16^{++} monocytes resemble Ly6C$^-$ anti-inflammatory monocytes and have a patrolling role[27].

In lean states, human ATMs are CD16$^-$ and express markers CD14, CD68, CD163, CD204, CD206 analogous to the population of 'resident' or anti-inflammatory ATMs seen in mice (Table 1). CD14$^+$ CD16$^-$ CD163$^+$ ATMs have been described as an anti-inflammatory subset inversely correlated with high BMI[46]. However, studies have suggested that the presence of these cellular markers may not be limited to 'resident' or anti-inflammatory macrophages. In fact, in adipose tissue of obese individuals have been shown to express many markers of M2 macrophages that correlate with ATMs with the capacity of secreting pro-inflammatory cytokines[47], and correlated with increased BMI[44]. In human obese adipose tissue, pro-inflammatory ATMs have been described as CD14 and CD16 positive cells that display high levels of other markers, such as CD11c, CD64, CD40, CD86, HLA-DR, TLR4 and CD36 (Table 1). Importantly, human CLS ATMs have been characterized for having a mixed M1/M2 phenotype[44] expressing the "M1-like" marker CD11c and the "M2-like" marker CD206 or CD163.

Using CD11c to identify pro-inflammatory ATMs[48], the subset of CD11c$^+$ CD206$^+$ ATMs was shown to exhibit a pro-inflammatory phenotype associated with increased number of CLSs and insulin resistance in obese subjects[48]. Interestingly, also CD11c$^+$ CD163$^+$ ATMs were found to accumulate in fat of obese individuals and correlate with high BMI as well as the production of reactive oxygen species[49].

In addition to CD11c$^+$ CD206$^+$ ATMs, it has been indicated that the population of CD11c$^+$ CD64$^+$ ATMs also defines pro-inflammatory macrophages[38]. Additionally, the population of CD14$^+$ CD16$^+$ CD36^{high} ATMs has been shown to include highly phagocytic macrophages[46]. Although, a study identified a strong correlation between CD36 expression on ATMs and metabolic dysfunction[50], CD36 can be found on other cell types, such as adipocytes and platelets and, thus far, might not be an optimal candidate marker. Nevertheless, a study has reported that by using CD163 in addition to CD14, CD16 and CD36, it was possible to distinguish anti-inflammatory macrophages (CD14$^+$ CD16$^-$ CD36^{low} CD163$^+$) from pro-inflammatory macrophages (CD14$^+$ CD16$^+$ CD36^{high} CD163$^-$)[51]. Consistently, the expression of CD163 has been significantly correlated with HOMA-IR[52] but the functional relevance of this marker is unclear.

ATMs properties and functions in lean and obese adipose tissue

Although it is not fully resolved what triggers the activation of ATMs, there is evidence that adipocyte hypertrophy and local hypoxia, following energy unbalance, instigate ATM accumulation[3, 43, 53] and a

switch in the phenotype of ATMs from an anti-inflammatory state to a pro-inflammatory state[21]. Moreover, not only the accumulation of macrophages, but also an impairment in macrophage egress has been implicated in obesity-induced inflammation[54]. It has become critical to understand the mechanisms regulating ATMs polarization and how ATMs functions are altered by obesity, as well as the biology of the macrophage/adipocyte interplay within CLSs, however, the full spectrum of functions of ATMs are still not fully clarified despite many advances in the field.

ATM cytokines production: ATMs actively secrete pro- and anti-inflammatory cytokines[29, 55]. MMe macrophages have been considered a significant source of inflammatory cytokines, whose production can be variably modulated by NADPH oxidase 2 (NOX2) activity during obesity progression[17]. Within lean AT, anti-inflammatory cytokines secreted by resident ATMs help maintain insulin sensitivity by counteracting inflammatory responses. This is demonstrated by the effect of the treatment of adipocytes with IL-10 that alleviates TNFα-induced IR[21]. Similarly, IL-4, known to polarize anti-inflammatory macrophages[29], when administrated to obese mice alleviates inflammatory responses in AT and improves insulin sensitivity[56]. Following adipocyte hypertrophy, the secretion of chemoattractants, such as MCP-1/CCL2, contribute to macrophage recruitment and production of pro-inflammatory cytokines, including TNFα, IL-6 and IL-1β, that act as main effectors of impaired adipocyte function and inflammatory signals[3, 57].

Energy metabolism in ATMs: Profiling of intracellular energy metabolism in ATMs has contributed to further understanding their metabolic functions in obese setting. Pro-inflammatory (M1) macrophages rely on glycolysis for their metabolic demands[58]. Inhibition of glycolysis attenuates the adipocyte release of CCL2 in response to TNFα or LPS[59], demonstrating the connection between metabolism and inflammation. On the contrary, anti-inflammatory (M2) macrophages rely on oxidative phosphorylation (OXPHOS) pathways[60, 61]. Recent studies have characterized the metabolic signatures of ATMs in lean and obese conditions in mice[62]. Transcriptome analysis and extracellular flux measurements of mouse ATMs revealed that fatty acid oxidation, glycolysis, and glutaminolysis participate in cytokine release by ATMs in lean AT[62] (Table 2). Glycolysis and oxidative phosphorylation pathways are both activated in ATMs in obesity. However, glycolysis accounts mostly for the higher cytokine production by ATMs in obese AT[62]. In humans, the relationships of macrophage markers with metabolic parameters and inflammatory biomolecules are not fully clear. However, a recent study has demonstrated that the regulation of M2-ATMs in patients with modest obesity may be closely dependent on mitochondrial biogenesis and OXPHOS through PGC-1α/β signals[63].

Clearance of dead adipocytes: ATMs are known to participate in adipocyte death by promoting the clearance of fragmented cellular contents via phagocytosis and lysosomal activation[30, 64]. Under normal conditions, ATMs phagocytosis of adipocytes debris may be important to maintain AT homeostasis keeping adipocyte turnover and overall tissue health. In obese setting, it is hypothesized that CLSs ATMs clear dead adipocytes that are generated with chronic tissue expansion and perhaps in an attempt to resolve inflammation[65, 66]. Murine studies based on adipocyte-targeted activation of caspase-8 have demonstrated that adipocyte death induces the recruitment of macrophages[67]. Perilipin-negative adipocytes which have undergoing necrosis-like death, plasma membrane rupture, endoplasmic reticulum stress, and cell debris release have showed that most of recruited ATMs specifically accumulate near dying adipocytes[30, 65]. Depletion of mannose-binding lectin has been linked to increased CLSs formation and decreased dead adipocyte clearance[68], implicating a role in phagocytic pathways of ATMs. It has been proposed that these endocytic processes can overwhelm the metabolic capacity of ATMs to take up debris and interrupt the normal process of dead cell clearance, as occurs in foam cell formation and atherosclerosis[69], leading to a maladaptive inflammatory responses[33, 43]. Finally, ATMs populations can exert a complementary role to minimize tissue damage through tight regulation of phagocytosis and tissue repair[70].

Clearance of lipids and lysosomal activity: Since adipocytes are primarily made of large lipid droplets, ATMs play an active role in the clearance of lipid remains from dead fat cells[30, 71], the removal of extracellular lipids from adipose tissue[72] as well as the uptake of triglycerides and non-esterified fatty acids (FFAs) released by enlarged insulin-resistant adipocytes[43]. These phenomena could contribute to the prevention of a potential damage and lipotoxicity caused by ectopic accumulation of saturated FFAs. With increasing adiposity, ATMs store surplus lipid species, giving rise to a population of so-called lipid-laden ATMs[73, 74], which have been considered the primary CLS-forming cells. While these are analogous to atherosclerotic foam cells, the lipid droplets in ATMs are primarily laden with FFA and not cholesterol as in atherosclerotic lesions. Alterations in lipid metabolism in lipid-laden ATMs have been implicated in pro-inflammatory responses linked to insulin resistance[75, 76]. Studies from Coats et al.[19] observed that FFAs induced MMe macrophages are the major subclass responsible of dead adipocyte clearance and inflammatory cytokine production in obesity. Xu et al.[18] demonstrated that the accumulation of ATMs in obesity and insulin-resistant states correlates with the induction of lysosomal biogenesis programs in ATMs. Lysosome biogenesis is found to be tightly coupled to lipid accumulation by ATMs and associated with increased lipid catabolism. Traditionally, ATMs, that accumulate intracellularly a large amount of lipids or cell debris, exert a lysosomal activity through two types of vesicles that mostly differ

based on the pH. Neutral lipid vesicles fuse with primary lysosome forming an acidic-ringed secondary lysosome implicated in lipid catabolism pathways.

Exophagy: A hallmark of CLS ATMs is the ability to internalize and process cell debris and released lipids from dysfunctional adipocytes[18]. However, an exocytosis-based process, namely exophagy, has been added to the spectrum of functions acquired by ATMs in obesity[77]. Exocytosis of lysosomes has been previously reported in the context of macrophage degradation of aggregated LDL in foam cell formation and atherogenesis[78, 79]. Based on this process, CLS ATMs can actively participate in lipid liberation from the adipocytes and form large moieties in an extracellular acidic hydrolytic compartment, called lysosomal synapses. This structure contains lysosomal enzymes, low pH and F-actin-rich seals, that allow the activity of lysosomal acid hydrolases[77-79]. This process results in extracellular catabolism and subsequent uptake of particles from the suffering adipocyte[76]. It has been hypothesized that exophagy in CLS ATMs sustains a feed-forward mechanism of inflammation, linking adipocytes death to pro-inflammatory cytokine release and increased lysosome biogenesis. To this end, studies by Ying et al.[80] have showed that ATMs are also responsible of the secretion of miRNA-containing extracellular vesicles (EVs), namely Exos. These vesicles are taken up by adipocytes, where influence metabolism by directly affecting cellular insulin signaling. Important findings have demonstrated when insulin sensitive mice are treated with obese ATM-Exos develop systemic IR and glucose intolerance. In contrast, treatment of obese insulin resistant mice with lean ATM-Exos normalizes glucose tolerance and enhances systemic insulin sensitivity. *In-vitro* studies showed that treatment with obese ATM-Exos decreases insulin-stimulated glucose transport in adipocytes and myocytes, while lean ATM-Exos treatment improves insulin action. Upon the screening of several miRNA-containing extracellular vesicles, this study proved that miR-155 contributes to the insulin resistant, glucose intolerant state conferred by obese ATM-Exos, indicating an additional mechanism of obesity-induced pro-inflammatory signaling driven by ATM secretory products.

Adipose tissue remodeling and angiogenesis: ATMs participate in the physiologic and pathologic remodeling of adipose tissue[29, 66, 81] via modulating new adipocyte formation (adipogenesis)[82] as well as influencing angiogenesis[83]. Macrophage depletion using clodronate liposomes reduces the formation of blood vessels in adipose tissues through the perturbation of angiogenesis[84]. Other studies based on hypoxia-models have shown that macrophages recruited into the tip of the gonadal adipose tissue promote angiogenesis during tissue outgrowth[85]. These recruited macrophages are lymphatic vessel endothelial receptor 1 (LYVE-1) positive ATMs and participate into tissue remodeling via the secretion of factors such as matrix metalloproteinase (MMP)-7, MMP-9, and MMP-12, and activation of the VEGF-VEGFR2

system to stimulate new vessel formation[85]. Similarly, ATMs isolated from the human adipose tissue secrete MMPs and promote the formation of endothelial cell tubes in matrigel systems[44]. ATMs role in angiogenesis has been linked to the effect of TNFα, which can act as an angiogenic factor[86], as well as to the effect of platelet-derived growth factor (PDGF), that mediates endothelial cell tube formation and capillary maturation by promoting pericyte recruitment[83]. Interestingly, it has been shown in DIO-models that a population of osteopontin over-expressing ATMs could form an adipogenic niche for tissue repair and remodeling in adipose tissue[71]. Consistently, osteopontin-deficient mice fail to form these regenerative adipogenic foci[71].

T cell activation: During the development of obesity, the number of adipose tissue T cells (ATTs) increases as does the $CD8^+$-to-$CD4^+$ T-cell ratio, whereas the percentage of T regulatory cells (Tregs) decreases[87]. Both $CD4^+$ and $CD8^+$ T cells are crucial in the recruitment and polarization of ATMs through cytokines such as IFNγ and the accumulation of $CD8^+$ T cells into AT precedes the increase of ATMs[88, 89]. Several groups have shown that ATMs are functional APCs that promote the clonal expansion of antigen specific T cell activation in AT and the generation of IFNγ–producing Th1 T cells[90-92]. ATMs phagocytose and process antigens for presentation, express costimulatory molecules, and induce antigen-specific $CD4^+$ T-cell proliferation[91]. Both resident $CD11c^-$ ATMs and $CD11c^+$ ATMs contribute to the generation and maintenance of effector/memory ATT cells and the development of IR with obesity[93].

Adipose tissue dendritic cells

Due to shared expression of markers such as CD11b, F4/80, and CD11c[38, 94 and Immgen.org], early studies on adipose tissue leukocytes did not clearly distinguish and differentiate ATMs from adipose tissue dendritic cells (ATDCs). This omission may be significant since ATDCs may be a potent antigen presenting cell in AT[90] crucial in the priming and differentiation of naive $CD4^+$ T cells[95]. The use of the macrophage specific marker CD64 has enabled the clarification of the diversity of ATDC ($CD64^-CD11c^+$) and ATM ($CD64^+CD11c^{+/-}$) in lean and obese states. Transcriptional profiling of ATMs and ATDCs validated their distinct functions and expression profiles[38,39]. Although ATDCs increase quantitatively along with ATMs in obesity[39, 96, 97], the contribution of ATDCs to meta-inflammation and IR remains not completely understood. Diet-induced obesity (DIO) models have demonstrated that a high-fat diet regimen determines an increase in ATDCs number in AT that resemble conventional DCs[39, 92]. Similarly, an increase in ATDCs has been positively correlated with high BMI in humans[90]. Studies in mice have demonstrated that the recruitment of ATDCs during obesity requires the chemokine CCR7 and to a lesser extent CCR2[39]. Experiments with CCR7-deficient mice have attenuated ATDC accumulation and

protection against obesity and IR. In addition, lack of β-catenin in ATDCs is associated with decreased IL-10 levels and suppressed Treg recruitment to AT leading to more inflammation[98]. Based on certain reports, human $CD11c^{+}CD1c^{+}$ and mouse $CD11c^{high}F4/80^{low}$ ATDCs have been considered as an inflammatory subtype of DCs in AT in obesity-associated IR[90]. In DIO models, a population of $CDllc^{high}F4/80^{neg}$ ATDCs have been shown to be responsible of the induction of both Th1 and Th17 cells, whereas $CDllc^{high}F4/80^{low}$ ATDCs of the differentiation of Th17 cells[99]. Induction of a Th17 response via ATDCs results in the production of the cytokines IL-1β, IL-6 and IL-23 leading to more inflammation[90, 100]. Further research is required to address the true function of ATDCs and which subsets are involved in obesity-induced inflammation.

Conclusion

Obesity, which has reached pandemic levels, is viewed as a chronic, low-grade inflammation within the adipose tissue as well as a metabolic disease. Among stromal cells, adipose tissue macrophages play a key role in the pathogenesis of obesity-driven inflammation and metabolic complications. Altered environmental cues and inflammatory phenomena orchestrate macrophage polarization patterns, which generally favor tissue infiltration and acquisition of a metabolic activation state. Recruited ATMs differ from resident macrophages for distribution, transcriptomic programming and functional characterization. In additional to conventional functions, such as clearing cellular debris and participating in tissue remodeling and lipid buffering, the release of secretory products (cytokines and extracellular RNAs) is a critical way that ATMs apply to regulate other cells as well as coordinate inflammatory responses. Although it is now evident that several ATM subsets exist in obese adipose tissue, transcriptional mechanisms, regulatory factors and intracellular pathways that underlie functional differences are not fully defined. Over the years, another limitation in the field has been the shared expression of certain cell markers between ATM and ATDC. Fortunately, recent data have identified specific markers allowing a better characterization of ATDC contribution to AT inflammation. In conclusion, understanding how obesity changes ATM functions and the molecular mechanism underpinning inflammation of obese adipose tissue may lead to novel, therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation. Likely, targeting metabolic capacity and inflammatory phenotype of ATMs may hold a greater potential to restore ATM function in obese adipose tissue.

Conflict of interest statement

Nothing to declare.

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Table 1. Markers for identification of adipose tissue macrophages

	Resident ATMs	Recruited ATMs
Mouse	F4/80	CD11c
	CD206	CD64
	CD301	CCR2
	Arginase 1	TLR4
		CD9
Human	CD14	CD36
	CD68	CD40
	CD163	CD64
	CD204	CD86
	CD206	CD11c
		HLA-DR
		TLR-4
		CD163
		CD206
		CD9

Table 2. Properties of adipose tissue macrophages

	Mouse		Human	
	Resident	**Recruited**	**Resident**	**Recruited**
Phenotype	Anti-inflammatory	Pro-inflammatory	As in mouse	Mixed
Function	Tissue homeostasis, adipose tissue remodeling, angiogenesis modulation, promotion of pre-adipocytes survival, efficient efferocytosis of dead adipocytes, wound healing,	Production of inflammatory cytokines and reactive oxygen species, inefficient dead adipocyte clearance, adipocyte debris scavenging, lipid phagocytosis, surplus lipid storage,	As in mouse	As in mouse

	resolution of inflammation, lipid buffering, regulation of adipocyte lipolysis	exophagy, NEFA spillover, T cell activation		
Secreted factors	IL-10, IL-4, Il-1RA, Tgf-β	Tnfα, IL-6, IL-1, NO	As in mouse	As in mouse
Induced by	IL-4, IL-10, IL-13, Prostaglandin D2	Type I interferons, LPS, TLR4, Saturated FFAs, Ceramides	As in mouse	As in mouse
Metabolic effects	Promote insulin sensitivity	Promote insulin resistance	As in mouse	As in mouse
Metabolism	OXPHOS, Glycolysis, Glutaminolysis	Glycolysis	Not clear	Not clear; evidence of mitochondrial biogenesis and OXPHOS
Dominant polarization	Lean adipose tissue	Obese adipose tissue	As in mouse	As in mouse