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Properties and functions of adipose tissue macrophages in obesity

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Summary

The expansion of adipose tissue (AT) 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 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 AT inflammation, new studies have revealed a role for ATM proliferation in the early stages of obesity and in sustaining AT 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, type 2 diabetes, depot of origin, and to other leukocytes such as AT dendritic cells, with hopes of emphasizing the regulatory nodes that can potentially be targeted to prevent and treat obesity-related metabolic disorders.

Keywords: adipose tissue; inflammation; insulin resistance; macrophages; MMe; obesity.

Introduction

The 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 imbalance 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 (AT) precedes the development of IR 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 AT 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 IR

Obesity is characterized by a chronic low-grade inflammation that is causally implicated in the development of IR. IR is a central mechanism in obesity-associated diseases, such as DM and metabolic syndrome. It is defined as the decline to a normal physiological response to insulin, resulting in a reduction in glucose disposal as well as failure to suppress lipolysis and hepatic glucose production, and occurs prominently in AT. Numerous studies support the role of ATMs 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 in a higher pro- versus anti-inflammatory ATM ratio, less phagocytosis and more infiltration into AT.[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 IR 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 AT during obesity-driven IR and inflammation.[15]

ATM activation states

Adipose tissue macrophages in lean and obese/insulin-resistant settings were initially described based on the two-dimensional M1/M2 spectrum of macrophage activation[16] (Fig. 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 has 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 (e.g. free fatty acids, high insulin, high glucose)[17] (Fig. 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 (e.g. free fatty acids, high-density lipoproteins) generates multiple clusters of activation states,[5] and thus may expand and extend the current models.

ATM heterogeneity in mice

Adipose tissue macrophage diversity and heterogeneity can be seen not only between lean and obese states, but also based on the location of the AT depot (e.g. visceral and subcutaneous AT), 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 the population of ATMs expands from 10% of all cells in lean AT 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 $Ly6C^{low}$).[25] Based on the expression of surface markers and chemokine receptors, $Ly6C^{+}$ subsets are divided into: $CD11b^{+}$ $CD115^{+}$ 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[26,27] (Fig. 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.[25]

Resident ATMs are distributed between adipocytes and along vascular structures in AT, 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,[28] as well as alterations in death pathways leading to increased cell survival.[29]

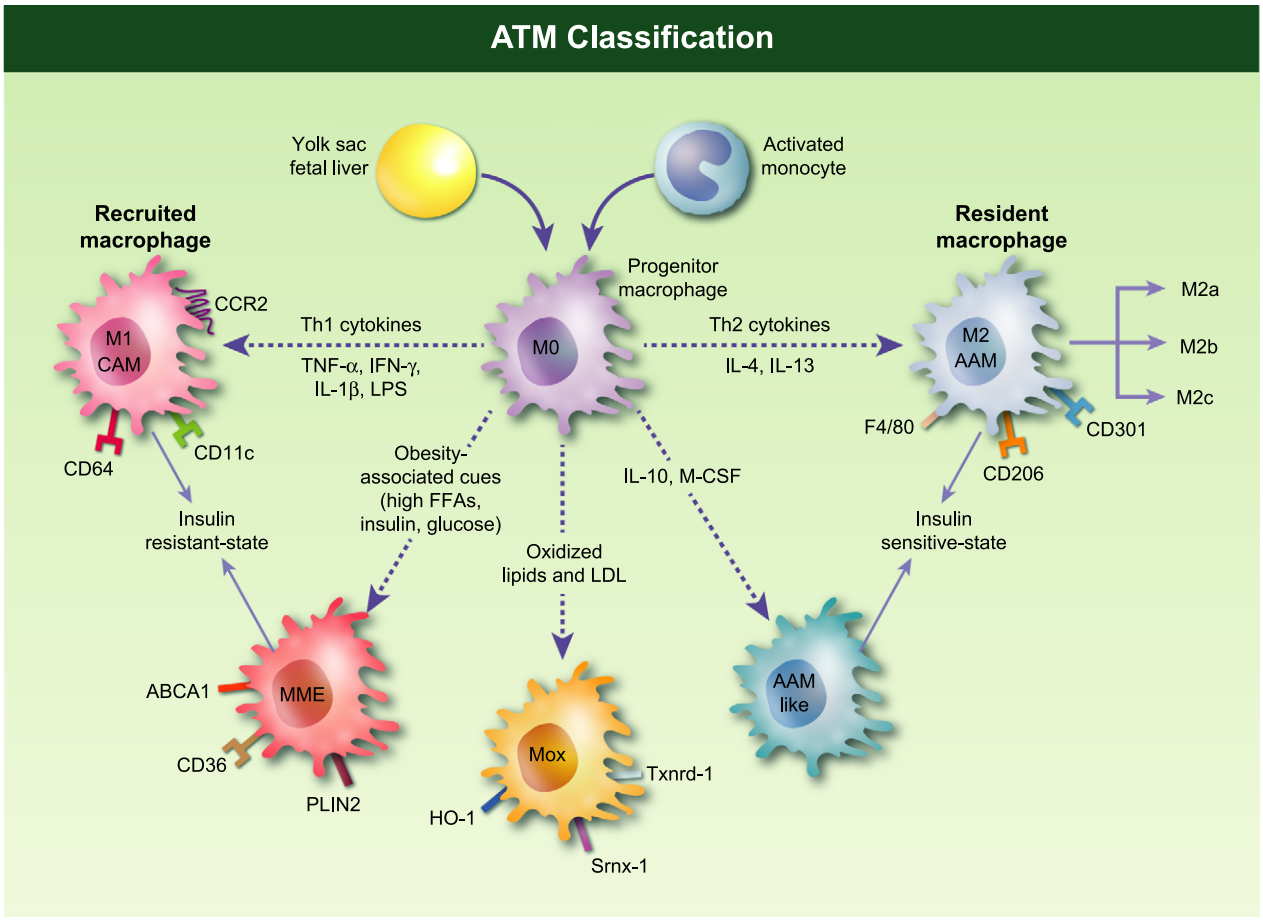

Figure 1. Adipose tissue macrophages (ATMs) classification. 'M0' macrophages originate from bone marrow-derived monocytes or yolk-sac progenitors. Depending on the stimuli, studies in mice have demonstrated that 'M0' macrophages differentiate into several subsets. Based on *in vitro* experiments, 'M1' or 'classical activated' macrophages (CAMs) originate from the stimulation with lipopolysaccharide (LPS), interferon (IFN)-γ or tumor necrosis factor α (TNF-α). These macrophages are characterized by an IL-12^{high}, IL-23^{high} and IL-10^{low} phenotype, display the surface marker CD11c in addition to F4/80 and CD11b, and produce pro-inflammatory mediators like TNF-α, IL-6, IL-1β and nitric oxide (NO). 'M2' or 'alternatively activated' macrophages (AAMs) are macrophages with an anti-inflammatory phenotype derived from IL-4, IL-10, IL-13 and glucocorticoids stimulation. M2 macrophages exhibit an IL-10^{high}, IL-12^{low} and IL-23^{low} phenotype, express the cell-surface markers CD11b, F4/80, CD301 and CD206, and secrete IL-4, IL-10 and IL-1 receptor antagonist (IL-1Ra). 'M2' macrophages are further divided into three major variants: 'M2a', elicited by type II cytokines IL-4 or IL-13; 'M2b', obtained by triggering of Fc gamma receptors in the presence of a Toll receptor stimulus; and 'M2c', elicited by glucocortocoids, IL-10 or TGF-β. In obese setting, metabolic cues (e.g. free fatty acids, high insulin, high glucose, oxidized phospholipids, oxidized LDL) give rise to a population of metabolic activated (MMe) or oxidized (Mox) macrophages associated to an insulin-resistant state. For instance, saturated fatty acids, which are released from hypertrophied adipocytes during the course of obesity, can act as a danger signal to MMe macrophages via the TLR4 complex. MMe macrophages exhibit a phenotype strikingly different from the typical M1/M2. Cell-surface proteins specifically overexpressed by MMe macrophages include ABCA1, CD36 and PLIN2, while conventional M1/M2 markers are suppressed. Mox macrophages are characterized by high expression of heme oxygenase-1 (HO-1), sulforedoxin-1 (Srnx-1), thioredoxin-1 reductase (Txnrd-1), all redox-regulatory genes under the control of the Nrf2 transcription factor, IL-10 and VEGF.

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 CLSs, as well as their expression of CD11c, CCR2 and TLR4[28,31,32] (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 AT.[29]

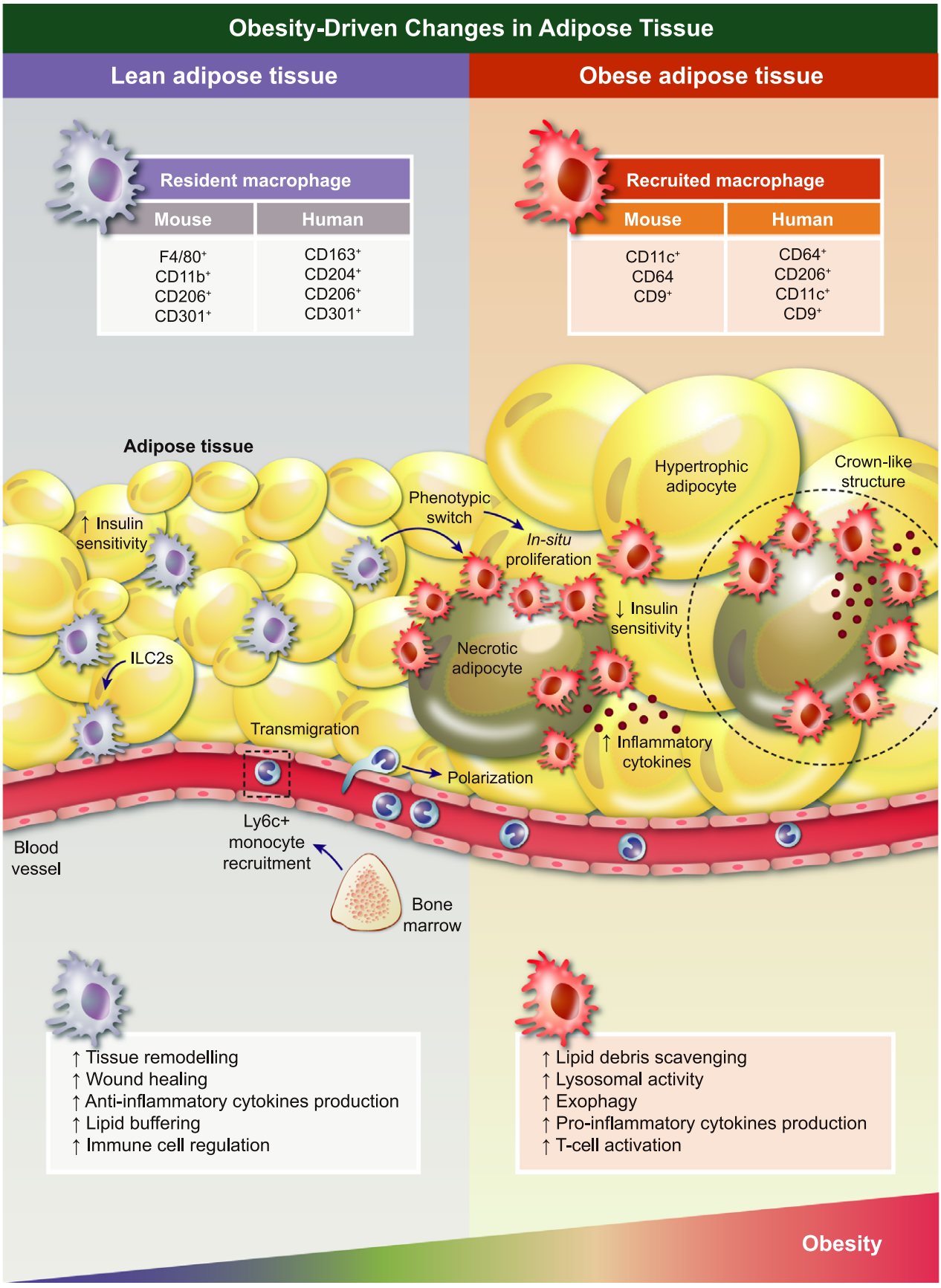

Figure 2. Obesity-driven changes in adipose tissue-associated macrophages (ATMs). In lean adipose tissue (AT), eosinophils and type 2 innate lymphoid cells (ILC2s) constitutively produce Th2 cytokines interleukin-4 (Il-4) and interleukin-13 (Il-13), respectively, which promote anti-inflammatory polarization of macrophages. As adiposity increases, obese AT manifests adipocyte hypertrophy, which leads to hypoxia, endoplasmic reticulum stress and lipotoxicity resulting in excessive leptin, cytokine and chemokine secretion. Among others, monocyte chemotactic protein-1 (Mcp-1) induces Ly6C$^+$ monocytes recruitment giving rise to tissue macrophages. Recruited macrophages, increased in number due to augmented infiltration and local proliferation, undergo a drastic change in distribution, forming crown-like structures (CLSs) around dead adipocytes. Additionally, they adopt a differential spectrum of functional properties and transcriptomic programming more prone to inflammation and metabolic dysregulation. This includes increased production of pro-inflammatory cytokines and other secretory products.

Table 1. Markers for identification of ATMs

	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

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 (DCs), a question in the field has been the ability to differentiate ATMs from adipose tissue dendritic cells (ATDCs) given that F4/80 and CD11b are also found on ATDCs. Using macrophage-specific markers CD64 and MerTK,[38] the population of CD64$^-$ CD11c$^+$ ATDCs is 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 AT. 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 AT stromal cells. CD9^{high} adipocyte progenitors[41] have been identified as correlating with AT fibrosis in humans, and CD9 is also found on DCs[42] to control antigen presentation, suggesting a potential broader role for CD9 in AT 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 AT 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 that are divided into three subsets: classical monocytes (CD14^{++} CD16$^-$); intermediate monocytes (CD14^{++} CD16$^+$); and non-classical monocytes (CD14$^+$ CD16^{++}).[25] 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.[25]

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 body mass index (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, AT of obese individuals has 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 AT, 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 IR 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.

Properties and functions of ATMs in lean and obese AT

Although it is not fully resolved what triggers the activation of ATMs, there is evidence that adipocyte hypertrophy and local hypoxia, following energy imbalance, 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 ATM polarization and how ATM 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

Adipocyte tissue macrophages actively secrete pro- and anti-inflammatory cytokines.[27,55] MMe macrophages have been considered a significant source of inflammatory cytokines, whose production can be variably modulated by NADPH oxidase 2 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 tumor necrosis factor (TNF)α-induced IR.[21] Similarly, IL-4, known to polarize anti-inflammatory macrophages,[27] 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, contributes to macrophage recruitment and production of pro-inflammatory cytokines, including TNFα, IL-6 and IL-1β, which 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 an 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 TNF-α or lipopolysaccharide,[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 OXPHOS 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

Table 2. Properties of ATMs

	Mouse		Human	
	Resident	Recruited	Resident	Recruited
Phenotype	Anti-inflammatory	Pro-inflammatory	As in mouse	Mixed
Function	Tissue homeostasis, AT remodeling, angiogenesis modulation, promotion of pre-adipocytes survival, efficient efferocytosis of dead adipocytes, wound healing, resolution of inflammation, lipid buffering, regulation of adipocyte lipolysis	Production of inflammatory cytokines and reactive oxygen species, inefficient dead adipocyte clearance, adipocyte debris scavenging, lipid phagocytosis, surplus lipid storage, exophagy, NEFA spillover, T-cell activation	As in mouse	As in mouse
Secreted factors	IL-10, IL-4, Il-1RA, TGF-β	TNF-α, 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 AT	Obese AT	As in mouse	As in mouse

AT, adipose tissue.

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

Adipose tissue macrophages 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 an 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 that have undergone necrosis-like death, plasma membrane rupture, endoplasmic reticulum stress and cell debris release have shown that most of the 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 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

Because 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 AT,[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 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 the 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 IR.[75,76] Studies from Coats *et al.*[19] observed that FFAs-induced MMe macrophages

are the major subclass responsible for 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 shown that ATMs are also responsible for the secretion of miRNA-containing extracellular vesicles (EVs), namely Exos. These vesicles are taken up by adipocytes, where they influence metabolism by directly affecting cellular insulin signaling. Important findings have demonstrated that when insulin-sensitive mice are treated with obese ATM-Exos they 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 EVs, 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.

AT remodeling and angiogenesis

Adipose tissue macrophages participate in the physiological and pathological remodeling of AT[27,66,81] 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 ATs through the perturbation of angiogenesis.[84] Other studies based on hypoxia models have shown that macrophages recruited into the tip of the gonadal AT promote angiogenesis during tissue outgrowth.[85] These recruited macrophages are lymphatic vessel endothelial receptor 1 (LYVE-1)-positive ATMs and participate in 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 AT secrete MMPs and promote the formation of endothelial cell tubes in matrigel systems.[44] The role of ATMs in angiogenesis has been linked to the effect of TNF-α, which can act as an angiogenic factor,[86] as well as to the effect of platelet-derived growth factor, that mediates endothelial cell tube formation and capillary maturation by promoting pericyte recruitment.[83] Interestingly, it has been shown in diet-induced obesity (DIO) models that a population of osteopontin over-expressing ATMs could form an adipogenic niche for tissue repair and remodeling in AT.[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 interferon (IFN)-γ, and the accumulation of $CD8^+$ T cells into AT precedes the increase of ATMs.[87,88] Several groups have shown that ATMs are functional antigen-presenting cells that promote the clonal expansion of antigen-specific T-cell activation in AT and the generation of IFNγ-producing Th1 T cells.[89–91] ATMs phagocytose and process antigens for presentation, express co-stimulatory molecules, and induce antigen-specific $CD4^+$ T-cell proliferation.[90] 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.[92]

Adipose tissue dendritic cells

Due to shared expression of markers such as CD11b, F4/80 and CD11c,[38,93] and Immgen.org, early studies on AT leukocytes did not clearly distinguish and differentiate ATMs

from ATDCs. This omission may be significant as ATDCs may be a potent antigen-presenting cell in AT[89] crucial in the priming and differentiation of naive $CD4^+$ T cells.[94] 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,[39,95,96] the contribution of ATDCs to meta-inflammation and IR remains not completely understood. DIO models have demonstrated that a high-fat diet regimen determines an increase in ATDCs number in AT that resemble conventional DCs.[39,91] Similarly, an increase in ATDCs has been positively correlated with high BMI in humans.[89] 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.[97] 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.[89] In DIO models, a population of $CDllc^{high}F4/80^{neg}$ ATDCs has been shown to be responsible for the induction of both Th1 and Th17 cells, whereas $CDllc^{high}F4/80^{low}$ ATDCs for the differentiation of Th17 cells.[98] 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.[89,99] 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 AT as well as a metabolic disease. Among stromal cells, ATMs 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 addition 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 AT, 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 AT may lead to novel, therapeutic strategies to prevent or treat obesity-induced AT inflammation. Likely, targeting metabolic capacity and inflammatory phenotype of ATMs may hold a greater potential to restore ATM function in obese AT.

Disclosures

The authors declare having no competing interests.

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