Abstract

Obesity is associated with systemic chronic low-grade inflammation, a major contributor to the aetiology of insulin resistance (IR). An inflammatory response in the presence of obesity appears to be triggered by, and to reside predominantly in, adipose tissue (AT). The discovery that the AT in obese mice and humans is infiltrated with macrophages has provided a major advance in our understanding of how obesity propagates inflammation.Interestingly, AT-infiltrating macrophages exhibit a proinflammatory phenotype (classical activation) whereas macrophages residing in AT have a reparative phenotype (alternative activation). In this review, the processes involved in monocyte/macrophage recruitment into the AT, and the events underlying the activation of infiltrating and/or resident AT macrophages (ATM) are described. Also, the localized roles of ATM on AT growth, metabolism and remodelling, as well as their systemic effects in promoting IR, are revealed. Finally, the new therapeutic targets that have recently emerged, and which have the potential to modulate the recruitment and/or activation of ATM, are discussed.

Keywords: Adipose tissue; Inflammation; Adipokines; Endotoxaemia; Free fatty acids; Tissue remodelling; Review

1. Introduction

Obesity is associated with chronic low-grade inflammation that is characterized by increased serum concentrations of acute-phase reactant proteins combined with metabolic alterations such as type 2 diabetes mellitus [1]. An inflammatory response in the presence of obesity appears to be triggered by, and to reside predominantly in, adipose tissue (AT) [2].

In 2003, the discovery that the AT in obese mice and humans is infiltrated with macrophages provided a major step forward in our understanding of how obesity propagates inflammation [3,4]. Indeed, AT was shown to contain bone-marrow-derived macrophages, which correlated with the degree of obesity. In addition, macrophage infiltration was associated...
with increased expression of tumour necrosis factor (TNF)-α, monocyte chemotactic protein (MCP)-1 (or CCL-2), inducible nitric oxide synthase (iNOS) and 1κB kinase (IKKβ) in the ATstromal-vascular fraction (SVF) that preceded or coincided with decreased insulin sensitivity. The accumulation of macrophages within AT in obese mice and humans has subsequently been reported in many studies [5–10].

Recently, it was observed that obese subjects with AT inflammation characterized by macrophage accumulation exhibited a marked systemic arterial dysfunction and insulin resistance (IR) compared with obese subjects with quiescent, non-inflammatory adipose architecture [11]. In addition, the central role of myeloid cells in the genesis of IR was demonstrated in mice lacking IKKβ—a central coordinator of inflammatory responses through activation of nuclear factor-κB (NF-κB)—in myeloid cells [12], by NH2-terminal kinase (JNK1) removal from haematopoietic cells [13] and by ablation of CD11c+ cells [10].

In this review, we discuss the potential mechanisms by which macrophage accumulation in AT may link obesity to IR. The processes involved in macrophage recruitment to fat tissue and the events underlying the activation of AT macrophages (ATM) are also described. Finally, hypotheses of the local and systemic roles of ATM in promoting IR are proposed.

2. Characterization of adipose tissue macrophage

2.1. Accumulation of macrophages in adipose tissue in obesity

Both adipocyte hyperplasia and hypertrophy contribute to AT expansion; however, in adults, hypertrophy appears to predominate [14]. Indeed, increased adipocyte size has clearly been associated with IR and progression to type 2 diabetes [15,16]. In both rodents and humans, adipocyte size is a strong, direct predictor of macrophage accumulation in AT [3,17]. Some of the consequences of adipocyte hypertrophy are altered adipokine production, adipocyte cell death and local adipose hypoxia—all potential contributors to ATM accumulation. Moreover, ATM accumulation in the fat mass could be the consequence of increased blood monocyte recruitment and infiltration, decreased tissue macrophage emigration and/or proliferation of resident tissue macrophages. Intravital microscopy performed in a mouse model has shown active monocyte recruitment and infiltration within AT [18], while some human ATM have been found to express a specific proliferative marker, suggesting that local proliferation occurs [19].

Adipocyte size is an important determinant of the production of several adipokines, with leptin being the most well described. Leptin itself may initiate the recruitment of macrophages to AT through direct effects on endothelial cells via promotion of oxidative stress, activation of NF-κB and activator protein (AP)-1 pathways, and increased expression of chemokines and adhesion molecules [5,20]. In addition, leptin has proved to be a direct chemoattractant for monocytes and macrophages in vitro [21]. Further support for the role of leptin in ATM infiltration in vivo comes from a report by Xu et al. [4] showing that, although obese mice deficient in leptin production or action (Lepob/ob and LepRdb/db) had increased macrophage infiltration of AT compared with their lean counterparts, the degree of macrophage accumulation and inflammation was less than expected for their body weight [4]. In contrast, adiponectin—the adipocyte expression and production of which are decreased in obesity—has been reported to reduce the expression of adhesion molecules on endothelial cells and to suppress the expression of proinflammatory mediators in monocytes/macrophages [22]. Also, adiponectin transgenic ob/ob mice, which show massive increases in subcutaneous fat mass, are protected against diet-induced IR [23]. Interestingly, the AT in these mice revealed reductions in both average adipocyte size (hyperplasia, but not hypertrophy) and local inflammation, with minimal macrophage infiltration. However, whether this effect is due to a direct action of adiponectin on endothelial cells and/or monocytes, or is a consequence of metabolic improvement, remains to be determined.

Larger fat cells secrete more proinflammatory adipokines, mostly chemoattractant and immune-related products (such as serum amyloid A [SAA], interleukin [IL]-8 and MCP-1) [24,25]. In contrast, weight loss that results in smaller adipocytes is characterized by beneficial alterations in adipocyte secretory patterns [26], together with a decrease in ATM [27]. Among the inflammatory-related adipokines secreted in excess by hypertrophied adipocytes, MCP-1 appears to play a role in ATM infiltration and obesity-induced IR. Indeed, MCP-1 and C-C chemokine receptor-2 (CCR2, a receptor for MCP-1) knockout (KO) mice are both characterized by decreased ATM infiltration, reduced proinflammatory gene expression in AT, decreased hepatic triacylglycerol (TAG) content and improved insulin sensitivity compared with wild-type animals fed a high-fat diet (HFD) [28,29]. However, conflicting results have also been reported [30–32]. AT-specific overexpression of MCP-1 has been reported to increase ATM content and decrease insulin sensitivity in murine models [28,33]. In humans, MCP-1 in AT is expressed predominantly in non-adipose cells [34] and mainly in ATM [6]. In the same way, osteopontin (OPN) is increased in the AT of mice fed an HFD, and ATM were the main producers of OPN during the development of diet-induced obesity (DIO) [35]. OPN has been shown to amplify MCP-1-mediated migration of macrophages, and mice lacking a functional OPN gene, despite being obese, are insulin-sensitive, with AT showing less macrophage infiltration and inflammation [35]. This suggests that local ATM could contribute, through their production of chemoattractants such as MCP-1 and OPN, to the accumulation of additional cells.

Increased adipocyte size in obese mice and humans is associated with greater adipocyte death [36], and similar observations have been made in hormone-sensitive lipase-deficient (HSL−/−) mice (a model of adipocyte hypertrophy without increased adipose mass). As more than 90% of all macrophages in the white AT of obese mice and humans are found in crown-like arrangements around large dead or dying individual adipocytes [36], increased cell death subsequent to adipocyte hypertrophy could be a major event involved in ATM accumulation in obesity. However, a mechanism by which hypertrophy can promote adipocyte death is not clear, although increasing evidence points to the potential involvement of stress-dependent pathways in response
to excess nutrients, including free fatty acids (FFA) and proteins.

Obesity is characterized by increased plasma concentrations of FFA. FFA are thought to promote the expression of various chemokines by the adipocyte in part through interaction with the adipocyte toll-like receptor (TLR)4 pathway. In addition, FFA are essential building blocks for glycosphingolipid, and a reduced glycosphingolipid content in adipocytes has been associated with reduced adipocyte size and death, and promotion of adipocyte insulin sensitivity. As a consequence, ATF content is reduced, as well as MCP-1 and OPN expression [37]. Excess energy intake is also associated with induction of endoplasmic reticulum (ER) stress [38]. ER stress activates the unfolded-protein-response (UPR) cascade, leading to transcriptional induction of genes involved in the assembly, folding, modification and degradation of proteins to alleviate this stress [39]. However, in chronic conditions in which ER stress cannot be relieved, the UPR induces cell death [40]. Furthermore, ER stress and the UPR are linked to the major inflammatory and stress-signalling networks, including the JNK–AP-1 and IKK–NF-κB pathways, and the production of reactive oxygen species (ROS). Ozcan et al. [41] were the first to demonstrate that, in both dietary and genetic obesity, the UPR is increased, and leads to activation of JNK and impaired insulin action. Links between chronic excess energy intake, ER stress-induced inflammation and IR have been confirmed in other studies in vivo of mice and humans [42–46].

There is accumulating evidence that AT local hypoxia may play a major role in macrophage accumulation. At least three different obese mouse models (DIO, KKAy and Lepob/ob) have demonstrated that hypoxia occurs in obese AT [47,48], and increased hypoxia-inducible factor (HIF)-1α expression has been reported in obese human AT [27,49]. Reduced tissue perfusion capacity rather than cell size appears to be the main culprit for tissue hypoxia, as pimonidazole (hypoxia probe) staining was detectable in small as well as large adipocytes in obese animals [47]. This is in agreement with the reduction of AT blood flow per unit of tissue reported in both human and animal obesity [50–52]. Hypoxic conditions can alter the production of various adipokines (increased production of chemoattractants such as leptin and macrophage inflammatory protein [MIP]-1α, and production of macrophage migration inhibitory factor [MIF], a recognized inhibitor of macrophage emigration from hypoxic regions) [51]. In fact, dysregulation of adipokine production under hypoxic conditions may be related to the induction of ER stress [47]. Hypoxia has also been reported to induce cell necrosis in the 3T3-L1 murine preadipocyte cell line [51]. Also, ATM have been predominantly found in hypoxic areas of AT in obese mice [53], and ATM markers have been found to be inversely correlated to AT pO2 in humans [52]. Therefore, hypoxia could be a primary event involved in AT inflammation.

In addition to hypoxic- and stress-related events occurring within adipocytes, recent studies point towards a potential role for another immune-cell population in the infiltration of macrophages—namely, T lymphocytes. The studies of Kinitscher et al. and Rocha et al. [54,55] have shown that accumulation of T cells precedes macrophages in AT in obese mice and humans.

Nutritional induction of metabolic endotoxaemia may play a key role in obesity and its associated low-grade inflammation. Indeed, one report observed that young adult mice have 40% more total body fat than their germ-free counterparts fed the same diet [56]. Similarly, lean axenic mice colonized with microbiota from genetically obese mice put on weight while axenic mice fed an obesogenic diet did not. This suggests that a bacteria-related factor could be responsible for obesity induced by an HFD [57]. Indeed, the metabolic concentration of plasma lipopolysaccharide (LPS) is a sufficient molecular mechanism for triggering HFD-induced metabolic diseases [58]. In addition, changes in the gut microbiota leading to a reduction of metabolic endotoxaemia have been correlated with reduced glucose tolerance, fat-mass development, macrophage infiltration and inflammation [59]. In healthy men, endotoxaemia was independently associated with energy intake [60]. Furthermore, endotoxaemia (LPS challenge) has been shown to modulate inflammation-related adipokine production, including MCP-1 and leptin, in humans and mice, and to increase AT hypoxia [61,62].

2.2. Adipose tissue macrophage phenotypes

Macrophages are highly heterogeneous cells found in nearly all tissues of the body [63]. They are differentiated from circulating monocytes by their ability to migrate into tissue in steady state or in response to inflammation. Their heterogeneity reflects their specialized functions at different anatomical locations (as microglial cells, osteoclasts, alveolar macrophages and Kupffer cells). In addition, macrophages have a remarkable plasticity that allows them to efficiently respond to environmental signals. Their phenotype and physiology can be markedly altered by both innate and adaptive immune responses. Mirroring the T-helper type 1 (Th1)/type 2 (Th2) concept of T-cell activation, an M1/M2 macrophage activation state has been devised [64,65]. Stimulation of macrophages with Th1 cytokines, such as interferon-γ, or bacterial byproducts, such as LPS, promotes maturation of ‘classically’ activated macrophages (M1), which have high inflammatory and bactericidal potential. In contrast, Th2 cytokines, including IL-4 and IL-13, promote ‘alternative’ activation of macrophages (M2), which have antiparasitic capabilities and functions in tissue repair and remodelling. However, the M2 designation has now been expanded to include virtually all other types of macrophages, encompassing cells with dramatically different biochemistry and physiology [66,67]. For this reason, a new classification of macrophages has been proposed by Mosser and Edwards [68] that is based on function: host defence, wound-healing and immune regulation.

In AT, the evidence that newly recruited activated M1 and resident M2 are involved in the promotion and attenuation, respectively, of IR has led to extensive studies aimed at characterizing more precisely the ATM phenotype and how they change in obesity, as well as the mechanism involved in the modulation of their phenotypes [69]. In several murine models, macrophage pulse-labelling studies in vivo showed that newly recruited ATM fed an HFD were more proinflammatory than were the resident ATM in lean mice. Comparison of the gene
expression of macrophages in obese mice and CCR2 KO obese mice suggests that the inflammatory phenotype requires an intact MCP-1/CCR2 axis [8]. Further investigations in the same animals showed that newly AT-infiltrating macrophages are positive for the surface markers F4/80, CD11b and CD11c, and are preferentially recruited to clusters surrounding necrotic adipocytes, while AT-resident macrophages are more interstitial, and express genes characteristic of M2 (Ym1, arginase 1 and IL-10) [70,71].

The acquisition and maintenance of the M1 inflammatory phenotype of newly recruited ATM in mice clearly involve several ‘partners’ such as adipokines, hypoxic conditions, LPS and FFA [9,72,73]. FFA, released from hypertrophied adipocytes, can trigger proinflammatory responses in macrophages by acting on TLRs [74]. TLR4 KO mice are partially protected from diet-induced IR, and are characterized by reduced adipose and liver inflammation [75]. In addition, the myeloid cell subpopulation characterized as F4/80⁺CD11b⁺CD11c⁺ is more susceptible to induced IR, and are characterized by reduced adipose and liver genes characteristic of M2 (Ym1, arginase 1 and IL-10) [70,71].

3. Roles of adipose tissue macrophage in obesity and insulin resistance

The products derived from murine inflammatory macrophages—and especially TNF-α—decrease adipocyte sensitivity to insulin and promote FFA production through direct lipolytic effects or indirectly via inhibition of the insulin-mediated antilipolytic effect [74,82,83]. Also, they promote the appearance of the adipocyte ‘inflammatory’ phenotype, suggesting that the cross-talk between ATM and adipocytes generates a vicious circle of aggravating AT inflammation and IR [74]. Fatty-acid-binding proteins (FABP) have been considered critical regulators in such cross-talk [84], although there are little relevant data for human cells. Acute-phase serum amyloid A (A-SAA) has been recently described as a player in the dialogue between human hypertrophied adipocytes and macrophages through the regulation of adipocyte cholesterol efflux [85].

High expression of scavenger receptors and CD206, together with high endocytic activity [5,78], suggest a role of ATM in the uptake of lipids and lipoproteins, glycoproteins and apoptotic cells [86]. Consistent with such a function, macrophages have been shown to accumulate at sites of adipocyte death [36,71]. Interestingly, Strissel et al. [87] also reported widespread collagen deposition that coincides with adipocyte death and macrophage infiltration. Large-scale transcriptomic analyses of human obese AT, together with immunohistochemical analyses and studies in vitro [88,89], have suggested that phenotypic alterations of human preadipocytes, induced by macrophages, may lead to excess synthesis of extracellular matrix components that, in turn, lead to an increase in interstitial fibrosis. Matrix remodelling and angiogenesis are features of the homoeostatic programme in which macrophages have been previously
implicated, particularly under inflammatory and/or hypoxic conditions [90]. Indeed, human ATM have been found to specifically express and secrete into plasma the matrix metalloproteinase-9 (MMP-9), a key enzyme involved in angiogenesis and atherosclerosis [19]. In addition, they express the hyaluronan receptor LYVE-1, a marker thought to be expressed in a particular subset of macrophages involved in tumour growth and wound-healing, as well as in mouse AT angiogenesis [91,92]. Furthermore, human ATM-conditioned media can stimulate AT-derived endothelial-cell migration and organization in capillary-like structures. Thus, in humans, ATM exhibit a remodelling phenotype with proangiogenic properties. The role of ATM in promoting angiogenesis for remodelling in obesity is supported by a recent report by Pang et al. [93]. In addition to their effects on matrix remodelling and angiogenesis, ATM, through their production of soluble factors, were shown to modulate the viability [94] and proliferation of adipocyte progenitor cells, and to impair adipocyte differentiation (adipogenesis) [19,95,96].

This suggests that ATM may limit the plasticity of AT through direct effects on adipocyte progenitor cell numbers and by their ability to give rise to new adipocytes, angiogenesis and matrix remodelling. ATM may also favour the release of FFA into the circulation. Both effects could contribute to ectopic lipid storage.
in the liver, muscles and pancreas, leading to lipotoxicity and IR [39,97].

In addition, the accumulation of macrophages in AT may contribute to increased systemic concentrations of the inflammatory cytokines involved in obesity-associated pathologies (reviewed extensively elsewhere [39,73,86,98]). In rodents, evidence is accumulating that resistin impairs insulin sensitivity. Circulating levels of resistin are increased in obesity, and an increase in serum resistin levels can induce IR in several rat and mouse models [99,100]. However, although resistin is produced by adipocytes in rodents, it is expressed mainly in macrophages in humans, and human ATM have been shown to express resistin [6]. Recently, a transgenic mouse model with macrophage-specific expression of human resistin, but no murine resistin (‘humanized resistin mice’), was found to be characterized by accelerated AT inflammation and IR. In mice with DIO, macrophage-derived human resistin was capable of exacerbating the pathophysiological consequences of obesity [101].

4. Differences between subcutaneous and visceral adipose tissue macrophage

Visceral AT depots are more lipolytic, with greater fat turnover and FA release and, hence, are more metabolically adverse than subcutaneous depots, especially given their proximity and direct access to the liver via the portal vein [102]. Visceral AT is also known to express more IL-6, IL-1β, IL-8, MCP-1 and other macrophage- or inflammation-related genes [90,103]. Macrophage infiltration was found to be higher in visceral compared with subcutaneous fat pads in a mouse model of obesity [3,71,87]. Increased macrophage infiltration has also been reported in human visceral AT and is associated with clinical parameters of obesity co-morbidity [7,103]. A positive correlation between adipose cell diameter and the number of infiltrating macrophages has also been found within each fat depot. However, omental adipocytes exhibit smaller adipocyte cell size [7,104]. Therefore, fat-cell hypertrophy is not the sole determinant of macrophage recruitment to AT; it is also dependent on the anatomical location of the fat depot. The mechanisms underlying such discrepancies are yet to be defined. In mice, adipocytes from visceral fat pads exhibit a greater susceptibility to death compared with cells from a subcutaneous depot [87,104]. In humans, an activated stress-sensing pathway is shown in omental versus subcutaneous adipocytes from obese patients [105].

Taken altogether, these data suggest that macrophage deposition in visceral AT is more likely to be responsible for enhanced systemic cytokine/chemokine levels than has been previously postulated [6], and may be mechanistically linked to the increased pathogenicity of intra-abdominal fat.

5. Roles of skeletal muscle and liver macrophages in insulin resistance

Skeletal muscle is the biggest consumer of glucose at the whole-body level. Some reports have observed infiltration of macrophages in skeletal muscle that is suspected of producing local proinflammatory cytokines that affect insulin sensitivity. Indeed, Weisberg et al. [3] were the first to show a threefold increase in macrophage (F4/80+ cells) content in skeletal muscle in obese compared with lean mice through immunohistochemistry approaches. Macrophages were rarely detected in areas surrounding myofibrils, but were found in the intermuscular adipose depot. Although conflicting results have been reported [4,106], the presence of macrophages (CD11c+ cells) in skeletal muscle and their accumulation in HFD-fed mice have been associated with increased expression of inflammatory factors [9,76], whereas ablation of myeloid CD11c+ cells reduced muscle macrophage content and decreased cytokine expression [10]. In-vitro findings have further demonstrated the direct interaction of myotubes and macrophages [9,107], and suggest that the inflammatory phenotype of muscle cells promoted by macrophages as well as by FFA results in impaired insulin signalling [107]. In humans, however, the data are scanty. A recent study by Varma et al. [107] found that muscle macrophage numbers are increased with obesity (significantly correlated with BMI) and IR in humans.

In contrast to AT and skeletal muscle, obesity does not appear to be associated with macrophage infiltration in the liver [3,4,10,108]. However, inflammatory activation of liver-resident macrophage-like cells—Kupffer cells—has been suspected in both obesity-induced IR and fatty liver disease [12,13]. Further data concerning activation-state changes of these liver-residing macrophages in obesity are needed to clearly delineate their causes, and their consequences for inflammation and hepatic IR.

6. Conclusion and perspectives

It is now evident that chronic low-grade inflammation is an important contributor to the aetiology of IR associated with obesity, and that ATM may play a key role in orchestrating the tissue inflammatory response. Macrophage accumulation in AT (recruitment, proliferation and/or retention of resident macrophages) as well as macrophage differentiation are the results of distinct mechanisms involving adipocyte-derived products, adipokines, chemokines and FFA, as well as local AT hypoxia and nutrient-induced metabolic endotoxaemia. Accumulation of ATM, in turn, locally affects AT plasticity and also exerts systemic effects by favouring the release of FFA into the circulation and ectopic lipid storage in non-AT. Also, in addition to cell-specific production (resistin, MMP-9), ATM may promote systemic IR and cardiovascular disease (Fig. 1).

The discovery that distinct subsets of macrophages are involved in the promotion and attenuation of IR suggests that pathways controlling macrophage activation are potential targets in the treatment of the co-morbidities of obesity. The absence of alternatively activated ATM in Mac-PPARγ KO mice, and the observation that Mac-PPARγ KO mice are more susceptible to obesity and IR, suggest that this platform of macrophage activation protects against the metabolic consequences of obesity. Similar results were recently found in myeloid-PPARδ-depleted mice [109,110], providing evidence that macrophage PPAR are
critical regulators of inflammation and IR and, thus, are potential therapeutic targets. Indeed, in the context of excess caloric intake, homeostatic functions performed by alternatively activated ATM could allow organisms to store and oxidize incoming lipids more efficiently, thereby maintaining insulin sensitivity and glucose tolerance.

However, our findings raise additional issues that require further investigation. First, to establish whether or not alternatively activated macrophages can be therapeutically exploited to treat type 2 diabetes, genetic and pharmacological approaches that activate or inhibit IL-4 signalling in macrophages need to be assessed. Second, although the presence of alternatively activated ATM attenuates inflammation, we cannot exclude the possibility that the remodelling activities of these cells may themselves lead to improvement in AT function. Finally, in addition to their paracrine effects, it is plausible that peptides or lipids secreted by alternatively activated ATM act in an endocrine fashion to modulate peripheral insulin sensitivity.

Another promising avenue of investigation is the new field of the therapeutic development and use of synthetic aP2 (FABP4) inhibitor against IR, diabetes, fatty liver disease and atherosclerosis. Indeed, FABP4 is specifically expressed (with FABP5) by both adipocytes and macrophages, and is critical for functional interactions between these cells in the genesis of the metabolic syndrome. In fact, chemical inhibition of FABP4 improved glucose metabolism and enhanced insulin sensitivity in both dietary and genetic mouse models of obesity and diabetes [111]. Fatty liver infiltration and the expression of obesity-associated inflammatory mediators, as well as JNK1 activity, were also suppressed. Moreover, insulin action in both adipose and liver tissues was improved. Thus, targeting FABP4 might reduce the vicious circle of negative cross-talk between the metabolic status of adipocytes and the inflammatory status of macrophages.

In conclusion, despite the advances made in this area in recent years and the subsequent perspectives, more human data are needed to turn the basic biological knowledge obtained from rodents into potentially useful therapeutic options against the inflammation and IR associated with obesity. However, caution is required when comparing the acute fat-mass growth and related inflammation observed in mice fed a HFD, and the development of chronic obesity in humans.

7. Conflict of interests

The authors have no conflicts to declare.

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References


