Macrophage infiltration into adipose tissue:

initiation, propagation and remodeling

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Abstract

It has long been known that adipose tissue in obesity is in a heightened state of inflammation. Recently, our understanding of this has been transformed by the knowledge that immune cells such as macrophages and T cells can infiltrate adipose tissue and are responsible for the majority of inflammatory cytokine production. These seminal findings have opened up a new area in biology that is garnering the interest of scientists involved in research relating to cell motility, inflammation, obesity, physiology, diabetes and cardiovascular disease. Some important general questions relevant to this field are: how are macrophages recruited to adipose tissue in obesity? What are the physiological consequences of macrophage—adipocyte interactions? Do these inflammatory macrophages contribute to pathophysiological conditions associated with obesity, such as insulin resistance, dyslipidemia, diabetes and cardiovascular disease? This review focuses on the first of these important questions.

Keywords

activation; adipose tissue; chemokines; crown-like structures; hypoxia; inflammation; insulin resistance; leptin; lymphocytes; macrophages

During the past two decades, the complex nature of adipose tissue (AT) has become an area of intense investigation. This is in part due to the growing worldwide obesity epidemic, and in part to the identification of leptin as an adipokine secreted from AT. Since the discovery of leptin, many other adipokines, such as adiponectin, resistin, visfatin and omentin, have also been identified. These discoveries have led to the first revolution in the field of AT biology, the identification of AT as an endocrine organ. More recently, it has been discovered that not only adipocytes, but also immune cells, such as macrophages [1,2] and T lymphocytes [3–5], reside in AT, and that these cells may induce insulin resistance by promoting a proinflammatory milieu within the AT. This discovery has led to the second revolution in the field of AT biology,

the identification of AT as an organ at the interface of inflammation and insulin resistance. In this review, we focus on what has been discovered in the past 5 years regarding macrophage infiltration into AT. In particular, we will discuss the continuum of AT physiology, from initiation and propagation of macrophage recruitment to AT, ultimately leading to the remodeling of AT.

Adipose tissue structure & function

Although adipocytes make up the bulk of AT mass, many other cell types are also found in AT. These include preadipocytes, vascular cells, such as endothelial cells (ECs), and immune cells. The major functions of AT include insulating and cushioning internal organs as well as storing excess energy in the form of triglycerides. This process is highly regulated, such that plasma nonesterified fatty acid levels are tightly controlled during fasting and feeding [6]. In addition to these functions, AT has an endocrine role, secreting many different adipokines and cytokines into the circulation that impact whole body physiology in significant ways.

History of macrophage infiltration into adipose tissue

The presence of macrophages in AT was first reported less than 8 years ago [7]. Subsequent studies have demonstrated that macrophage infiltration into white AT is increased in obesity [1,2]. Xu *et al*. demonstrated increased levels of macrophage markers in AT of leptin-deficient (Lep$^{ob/ob}$), leptin receptor-deficient (LepR$^{db/db}$), and diet-induced obese (DIO) mice compared with lean controls [1]. Ferrante and colleagues demonstrated a similar relationship between obesity and macrophage infiltration into AT in lethal yellow Agouti (Ay), Lep$^{ob/ob}$ and DIO mice [2]. Using bone marrow transplantation, they demonstrated that the recruited AT macrophages (ATMs) were bone marrow derived [2]. These original discoveries have subsequently been confirmed and expanded on by other groups [8–18].

Consequences of macrophage recruitment into white adipose tissue

In the seminal papers by Xu [1] and Weisberg [2], important observations were made regarding the consequences of macrophage infiltration into AT. First, both groups showed that expression of inflammatory cytokines such as TNF-α and macrophage inflammatory protein (MIP)-1α in AT was almost entirely derived from macrophages rather than adipocytes [1,2]. Xu *et al*. demonstrated that macrophage infiltration into AT temporally preceded elevations in plasma insulin levels [1]. Based on this, they proposed that ATMs contribute to whole-body insulin resistance by inducing inflammation and insulin resistance starting locally within the AT. This hypothesis has gained momentum and been supported by additional studies in recent years. For example, mice with an absence of TNF-α expression from ATMs following bone marrow transplantation of TNF-α-deficient bone marrow, exhibited reduced epididymal fat pad weight and enhanced insulin sensitivity compared with control mice receiving TNF-α sufficient marrow [19]. Using *in vitro* studies, Suganami *et al*. demonstrated that macrophages and adipocytes interact in a paracrine manner, whereby TNF-α secretion from macrophages interferes with adipocyte insulin signaling and induces fatty acid lipolysis. These fatty acids then further exacerbate the inflammatory pheno-type of the macrophages, creating a vicious cycle of inflammation and insulin resistance [20]. Thus, it is widely believed that macrophage infiltration into AT leads to increased local inflammation, which in turn reduces insulin sensitivity both in the AT and systemically.

Initiation of macrophage recruitment

Both adipocyte hyperplasia and hypertrophy can contribute to AT expansion; however, in adults, hypertrophy appears to predominate. Some of the consequences of hypertrophy include fatty acid flux, vascularization, increased leptin secretion, hypoxia and adipocyte cell death.

These adipocyte-related consequences of AT expansion are all possible contributors to the initiation of macrophage recruitment. All stages of macrophage recruitment to AT in obesity are depicted in (Figure 1).

Fatty acid flux

Implicit when discussing weight gain and AT expansion is the idea of overnutrition. As previously mentioned, excess nutrients are ultimately stored as triglycerides in the AT; thus, fatty acid flux within AT may be an important regulator of inflammation and macrophage recruitment to AT. Furthermore, the fatty acid content of the diet may contribute to this effect, and many of the models used to induce ATM accumulation involve the use of diets rich in saturated fatty acids [1,2,13,21,22]. Toll-like receptor (TLR) 4 has recently been shown to be a receptor for saturated fatty acids and to mediate inflammatory cytokine production in macrophages exposed to fatty acids [23–25]. Furthermore, polyunsaturated fatty acids protect against saturated fatty acid-induced inflammatory cytokine production [23–25]. Suganami *et al*. have shown *in vitro* that macrophage TLR4 expression mediates their inflammatory responses to fatty acids released from adipocytes [26]. In addition, several groups have used TLR4-deficient mice to demonstrate *in vivo* that TLR4 plays a role in AT expansion and inflammation to varying degrees depending on the mouse strain, type of high-fat diet and length of feeding [25,27–30]. Our own data have extended these findings to demonstrate that deficiency of myeloid TLR4 can result in reduced macrophage infiltration and inflammation in AT under certain dietary conditions [Hasty *et al*., Unpublished Data]. In contrast to saturated fatty acids, polyunsaturated fatty acids, such as fish oils, have been shown to reduce macrophage infiltration into AT [31,32]. Thus, dietary fatty acid composition can potentially impact the initiation of macrophage recruitment to AT.

Endothelial cell-mediated adhesion

Infiltration of immune cells into tissues occurs by a process of adherence to blood vessel ECs, rolling along the EC layer and extravasation into the underlying tissue; thus, capillary ECs likely influence the transport of immune cells into AT. Adhesion molecules such as intercellular adhesion molecule (ICAM)-1 have been shown to be expressed in murine AT [33]. Furthermore, it has been shown in mice that within 3 weeks of high-fat feeding, AT expression of ICAM-1 was increased, and after 6 months of feeding, soluble ICAM-1 in plasma correlated with bodyweight and fat mass [34]. Most recently, Nishimura *et al*. used *in vivo* imaging to show that leukocyte—EC interactions are increased in the microcirculation of the subcutaneous AT of obese mice, and that administration of antibody to ICAM-1 normalized these interactions [35]. By contrast, it has also been shown that macrophage infiltration into AT is not altered in ICAM-1-deficient mice [33]. Despite these conflicting studies, the cumulative *in vivo* and *in vitro* evidence supports a role for capillary ECs in the initiation of macrophage recruitment to AT, although further studies are required to define the role of ECs and adhesion molecules more clearly.

Leptin

Although leptin deficiency causes morbid obesity in mice and humans, what is more commonly found in obesity is an increase in plasma leptin levels, indicating the presence of central leptin resistance. The impact of hyperleptinemia on peripheral cells and tissues is not completely understood; however, there is some evidence to suggest that leptin itself may initiate the recruitment of macrophages to AT. For example, Curat *et al*. demonstrated that leptin can increase adhesion molecule expression in ECs [36]. Leptin has been previously shown to be a chemoattractant for neutrophils, smooth muscle cells, ECs and cancer cells [37–41], and we recently demonstrated that leptin can act as a potent chemoattractant for monocytes and macrophages *in vitro* [42]. *In vivo* support of this concept derives from the papers of Xu and

Weisberg showing that while Lep[ob/ob] and LepR[db/db] mice were obese and had increased macrophage infiltration into AT compared with lean mice [1,2], the degree of macrophage infiltration was less than what would be expected for their bodyweight. In fact, Xu and colleagues suggested a possible role for leptin in mediating macrophage recruitment to AT based upon this observation [1]. Thus, although further *in vivo* evidence is warranted, leptin may contribute to the initiation of macrophage recruitment to white AT.

Hypoxia

At least three different obese mouse models (DIO, KKAy and Lep[ob/ob]) have been used to demonstrate that hypoxia occurs in obese AT [43,44]. Decreased vascular density, which has been observed in obese mice [17,45], may contribute to this. Furthermore, protein and mRNA levels of hypoxia-inducible factor (HIF)-1α are elevated in AT from obese mice, as are mRNA levels for other hypoxia inducible genes [43,44]. Ye *et al*. have demonstrated *in vitro* that hypoxia may contribute to AT inflammation by showing that exposure of primary adipocytes and macrophages to hypoxia increases their expression of multiple inflammatory genes [44].

Recently, Wang, Wood and Trayhurn investigated the effect of hypoxia on human adipocytes and preadipocytes [46,47]. Like murine adipocytes, human adipocytes also have increased expression of HIF-1α and other hypoxia-related genes when exposed to low oxygen levels (1%) [47]. Human preadipocytes exposed to hypoxia had elevated HIF-1α mRNA, increased secretion of vascular endothelial growth factor, and although leptin expression and secretion was nearly absent in preadipocytes under normal oxygen conditions, hypoxia stimulated their expression and secretion of leptin [46]. Therefore, hypoxia is potentially involved in several of the initiating factors of macrophage recruitment including leptin secretion and adipocyte death (discussed below), as well as an initial upregulation of inflammatory genes.

Adipocyte death

A prominent hypothesis regarding the reasons for which macrophages enter the AT is that they are recruited to phagocytose dead or dying adipocytes present in the expanding AT depot. Whereas adipocyte death is rare in lean humans and mice, it is a common hallmark of obesity and is positively correlated with adipocyte hypertrophy [48–50]. In fact, hormone-sensitive lipase-deficient mice, which have adipocyte hypertrophy but are not obese, also exhibit increased adipocyte death [51]. Within AT, crown-like structures (CLS), composed mostly of macrophages [48], surround dead adipocytes, identified by the absence of perilipin staining [48–50]. CLS stain positive for F4/80, MAC-2, TNF-α and IL-6, indicative of their macrophage content and inflammatory properties [48–50]. Both adipocyte death and secretion of inflammatory cytokines are increased in visceral and epididymal fat depots relative to inguinal (subcutaneous) fat depots [49,50], and these two fat pads also have more ATM accumulation than subcutaneous AT [9,52,53]. Thus, it is likely that adipocyte cell death, which may be caused by the hypoxic conditions that occur during rapid AT expansion (reviewed in [54]), is a signal that attracts phagocytic macrophages.

Neutrophils

Since the recruitment of neutrophils generally occurs prior to the recruitment of macrophages during inflammatory responses, Elgazar-Carmon and colleagues evaluated AT for the presence of neutrophils at early time points following high-fat diet feeding in mice [55]. They found that neutrophils transiently infiltrated abdominal AT. In human studies, Leik *et al*. demonstrated that hypertensive pregnant women have an increase in neutrophils adhered to endothelium in their AT compared with control patients [56]. Thus, there is some evidence that neutrophils can also infiltrate AT and may be one of the initiating events in the recruitment of macrophages.

T lymphocytes

It was recently shown that the number of CD3$^+$ T cells is increased in AT in obesity [3–5]. Wu *et al*. demonstrated that CD3$^+$ T cells infiltrate white AT of DIO mice [5]. Based on the upregulation of the chemokine RANTES and its receptor, CCR5, in the AT of obese mice as well as the results of *in vitro* migration experiments, they suggested that adipocyte secretion of RANTES may contribute to the recruitment of T cells to the AT [5]. Obese humans also have increased gene expression of RANTES and CCR5 in subcutaneous and visceral AT, and this expression is positively correlated with CD3 and CD11b, a macrophage marker [5].

To understand the function of T cells in AT some investigators have further characterized them as either CD4$^+$ or CD8$^+$. Rausch *et al*. found a three- to four-fold increase in cytotoxic CD3$^+$/CD8$^+$/CD4$^-$ T lymphocytes in both DIO and Lep$^{ob/ob}$ mice [4]. However, in patients with Type 2 diabetes, Kintscher *et al*. detected only moderate expression of CD8 in AT, but demonstrated the presence of CD4$^+$ T cells, which positively correlated with bodyweight [3]. Furthermore, they found that the majority of the macrophages were human leukocyte antigen-DR-positive, suggesting that they may have been activated by IFN-γ, a cytokine released by CD4$^+$ helper T cells [3].

How T lymphocytes contribute to the sequence of changes in AT that occur as it expands is still in question. As mentioned above, macrophage infiltration of the AT has been shown to temporally precede systemic insulin resistance in DIO mice [1]. In a different study, T-cell accumulation in AT, as well as impaired glucose tolerance and insulin sensitivity, preceded macrophage accumulation in AT in DIO mice [3]. Thus, the sequence in which immune cells infiltrate AT, and whether they are a cause or consequence of insulin resistance is not yet clearly defined. Fully understanding the contribution of T cells to the inflammatory nature of obese AT remains an intriguing challenge for continued study.

Propagation of macrophage recruitment

While capillary ECs and adipocytes contribute to the initiation of macrophage recruitment, ATMs themselves likely contribute to propagation of the signals that promote further attraction of new macrophages. In support of this notion, we have recently utilized the Ay mouse model and shown that ATM content was highly correlated with AT mass when total fat mass ranged from 2 to 15 g [21]. When total fat mass was greater than 15 g, the expression level of macrophage marker F4/80 spanned a fourfold range but was no longer positively correlated with total fat mass, indicating that something other than fat mass must contribute to continued recruitment of macrophages to AT. Therefore, it is possible that factors secreted by macrophages, rather than adipocytes, may have a more important role in the propagation of ATM accumulation. Owing to the relative abundance of studies on the roles of chemokines in macrophage infiltration, this review focuses on their contribution; however, the literature indicates that other molecules are also important in this process.

Chemokines

Chemokines are small, 8–10 kDa, chemotactic cytokines that are well established to play a role in macrophage mobilization out of bone marrow and into many different tissues during the inflammatory process. Although they can be secreted by adipocytes, studies in which adipocytes were separated from stromal vascular fractions have demonstrated that the majority of chemokine secretion in AT is from the stromal vascular population [1,9]. Thus, expression of chemokines from ATMs likely contributes to propagation of macrophage accumulation in the AT.

Mouse studies—Numerous chemokines exhibit elevated mRNA and protein levels in the AT and plasma of genetically or diet-induced obese mice. Expression of the macrophage chemokines monocyte chemotactic protein (MCP)-1 and MIP-1α have been shown to be increased in the white AT of LepR[db/db], Lep[ob/ob] and DIO mice [1,10,13,57–60]. Other chemokines upregulated in AT from DIO and/or Lep[ob/ob] mice are MCP-2 [12], MCP-3 [12], RANTES [5] and CXCL14 [60]. Receptors for these chemokines, such as CCR2, CCR3 and CCR5, are also elevated in the white AT of DIO mice [5,12]. Furthermore, Chen *et al.* have performed microarray analyses of epididymal and inguinal white AT from DIO mice, and have shown altered gene expression of 60 inflammatory genes, which included MCP-1 and MCP-3 as the two inflammatory genes that were most highly upregulated [10].

To date, MCP-1 and its receptor CCR2 are the best studied of the chemokine ligands and receptors for their role in macrophage accumulation in white AT and obesity-induced insulin resistance. Yu *et al.* demonstrated that MCP-1 was upregulated at the mRNA and protein levels in the mesenteric, epididymal, subcutaneous and perirenal white AT of DIO mice, with the highest upregulation being in the mesenteric fat [59]. They likewise observed increased gene expression of the macrophage markers F4/80 and CD68 in AT beds [59]. In addition, they performed *in vitro* migration experiments showing increased macrophage migration to mesenteric AT-conditioned media, which was blocked in response to an antibody for MCP-1 [59].

Two different groups have engineered transgenic mice using the aP2 promoter to over-express MCP-1 in AT [13,58]. Both groups found increased numbers of macrophages within the AT of the MCP-1-overexpressing mice, and these mice were insulin resistant and glucose intolerant, suggesting that MCP-1 may promote macrophage infiltration and insulin resistance during obesity. On the other hand, experiments with MCP-1-deficient (-/-) mice have yielded inconsistent results. Kanda *et al.* found that DIO MCP-1^{-/-} mice had fewer ATMs and were more insulin sensitive and glucose tolerant, although they demonstrated no differences in bodyweight relative to wild-type controls [13]. By contrast, Inouye *et al.* observed significant increases in bodyweight in the DIO MCP-1^{-/-} mice and possibly a slight increase in macrophage accumulation in AT combined with decreased insulin sensitivity [57]. Kirk *et al.* found similar results to Inouye *et al.* except that they observed no differences in insulin or glucose tolerance [61]. The role of MCP-1 has also been examined using a dominant-negative approach, where improved insulin sensitivity and glucose tolerance were found in both LepR[db/db] and DIO mice [13].

Similar to MCP-1 studies, experiments with CCR2^{-/-} mice resulted in opposing conclusions regarding the importance of CCR2 in macrophage infiltration of AT. Weisberg *et al.* observed decreased macrophage accumulation in the epididymal, subcutaneous and perirenal AT of CCR2^{-/-} mice on a high-fat diet, decreased expression of TNF-α in epididymal AT, as well as improved insulin sensitivity and glucose tolerance relative to CCR2^{+/+} controls [12]. When they treated wild-type DIO mice with the CCR2 antagonist INCB3344 for 2 weeks, they noted improved insulin sensitivity and a small but significant decrease in immunohistochemical staining for F4/80 in the AT [12]. Chen *et al.* did not observe any differences in the amount of ATMs or in plasma insulin or glucose levels in CCR2^{-/-} mice [10]; however, the mice from this study were on a DBA1/J background, which may make them less susceptible to diet-induced obesity, in contrast to the mice in Weisberg's studies, which were on a C57BL/6J background.

A chemokine outside of the CC subfamily, CXCL14, is also upregulated in white and brown AT in genetic and diet-induced obesity [60]. DIO mice deficient in CXCL14 had fewer ATMs, were more insulin sensitive and had a faster glucose disposal rate [60]. This supports CXCL14 having a role in macrophage accumulation and insulin resistance in diet-induced obesity. In conclusion, it is clear that obese mice have elevated chemokines in their AT and that many of

these chemokines are secreted by macrophages. However, there appears to be a significant amount of redundancy in their functions, such that alteration of only one chemokine or chemokine receptor at a time may have only minor effects on ATM accumulation. More work needs to be done to clarify which chemokines are important in the recruitment of macrophages and the mechanisms by which they induce insulin resistance.

Human studies—Multiple human studies have demonstrated an elevation in gene expression of various chemokines in AT in obesity, including MCP-1, MIP-1α, MIP-1β, MCP-2, MCP-4, MIP-2α and pulmonary and activation-regulated chemokine [15,62,63]. The expression of MCP-1 from the stromal vascular fraction of fat has been shown to be greater than its expression from the adipocyte fraction, and MCP-1 expression is positively correlated with the expression of macro-phage/monocyte markers CD68 and CD14 [9], providing evidence that macrophages are the main source of this chemokine in human AT. Westerbacka *et al*. have demonstrated that insulin-resistant humans have increased expression of macrophage markers in AT in comparison with insulin-sensitive individuals, and that AT expression of MCP-1 and MIP-1α is elevated during hyperinsulinemic euglycemic clamps in people with insulin resistance [63]. Furthermore, a significant positive correlation has been observed between gene expression of the chemokines MCP-1, MIP-1α and MCP-2 and fasting serum insulin as well as whole-body glucose disposal rate [62]. Finally, a recent study by Stulnig and colleagues demonstrated that expression of six different chemokines and their receptors was higher in obese compared with lean subjects, and the expression of CCL3 (MIP1-α) and CCL5 (RANTES) positively correlated with fasting plasma insulin levels independent of waist circumference [64]. Thus, in agreement with murine studies, analysis of human AT has demonstrated a potential role for macrophage-derived chemokines in the propagation of macrophage recruitment and in insulin resistance.

Adipose tissue remodeling

Although much attention has been given to the inflammatory nature of AT in obesity, some evidence indicates that the number of ATMs can decrease in association with AT remodeling and repair [50]. A similar phenomenon has been observed in renal inflammation and in athero-sclerotic lesions, where macrophages have a role in both inflammation and tissue repair [65–67]. The capability of macrophages to secrete both pro- and anti-inflammatory cytokines contributes to their dual role, and, in fact, ingestion of apoptotic cells has been shown to reprogram macrophages to become anti-inflammatory [68].

Alternatively & classically activated macrophages

Like T cells, macrophages are now understood to be a heterogeneous population of cells that can be polarized toward an M1 'classically activated' or an M2 'alternatively activated' phenotype [69,70]. The M1 macrophage phenotype is induced by inflammatory stimuli and results in secretion of pro-inflammatory cytokines, whereas the M2 phenotype is induced by exposure to IL-4 and IL-13 and results in the secretion of anti-inflammatory cytokines. One hallmark feature of M2 macrophages is the production of arginase, which blocks inducible nitric oxide synthase (iNOS) activity and is thought to allow M2 macrophages to contribute to tissue repair [71]. Interestingly, M1 and M2 macrophages are also present in AT. Recent publications by Saltiel and colleagues [22,72] have demonstrated that ATMs in lean mice express arginase-1 and IL-10, indicating that they are M2 or alternatively activated. Upon high-fat feeding, expression of these genes was decreased, while expression of TNF-α and iNOS was increased, indicating that the ATMs were of the M1, or classically activated, phenotype [22]. Furthermore, using a pulse-label technique, these authors elegantly showed that the macrophages recruited to AT during high-fat feeding highly express IL-6, iNOS and CCR2, indicating that they had characteristics of migratory phagocytic cells [72]. In addition to

providing evidence for the recruitment of M1 macrophages to AT, this observation provides support for the concept that macrophages are recruited to AT to phagocytose dead adipocytes.

Subsequent to these studies, Chawla and colleagues demonstrated that macrophage deficiency of PPAR-γ results in an inability to develop an alternatively activated M2 phenotype [73]. Furthermore, mice with macrophage PPARγ deficiency showed a predisposition to diet-induced weight gain, glucose intolerance and insulin resistance. Interestingly, despite the increased AT mass, total ATMs were reduced in the PPARγ-deficient mice, and this appeared to be due to a reduction in M2 macrophages. Further evidence for a role of PPARγ in macrophage activation stems from studies by Stienstra *et al.* demonstrating that treatment of high-fat fed mice with the PPARγ agonist rosiglitazone led to a repolarization of AT macrophages to an M2 phenotype [74]. Kang *et al.* demonstrated that myeloid deficiency of PPAR-δ results in a loss of M2 macrophages leading to proinflammatory cytokine production from adipocytes, increased weight gain and increased systemic insulin resistance [75]. Together, these studies indicate that M1 macrophages promote inflammation and insulin resistance in AT, while M2 macrophages are required to protect against these physiological consequences of the increasing AT mass. Thus, the repertoire of M1 and M2 macrophages appears to control the inflammatory status of AT and may contribute to tissue repair and remodeling of AT during its expansion.

As is commonly observed in biology, human macrophage populations cannot always be classified simply as 'M1' or 'M2'. Zeyda *et al.* and Bourlier *et al.* have recently demonstrated that human ATMs have both M1 and M2 characteristics, as evidenced by their secretion of both pro- and anti-inflammatory cytokines [76,77]. Thus, although human macrophages may undergo phenotypic changes similar to murine macrophages, it will be critical to characterize ATMs carefully to determine their inflammatory properties.

Cell death & hyperplasia

Strissel *et al.* have examined the progression of cell death in the AT of DIO mice and found that after 16 weeks on a high-fat diet approximately 80% of the adipocytes were dead (perilipin-negative and surrounded by CLS) in the epididymal AT [50]. An intriguing aspect to this finding was that after 20 weeks on high-fat diet, the percentage of dead cells was reduced to approximately 16%, similar to the level observed after 12 weeks on high-fat diet. Collagen deposition was also greater at week 16 than at week 20, and although the number of adipocytes increased from week 16 to 20, the cell size distribution changed to include a majority of small adipocytes ($<5000 \ \mu m^2$) by week 20. The authors suggested that AT remodeling had occurred and, based on the differences in distribution of adipocyte size, that after the peak of dead adipocytes at week 16, the AT transitions from expanding through mostly hypertrophy to mostly hyperplasia [50]. Perhaps the dramatic increase in cell death and subsequent ingestion of dead adipocytes by macrophages is partially responsible for the trend towards decreased inflammation that Strissel *et al.* observed at week 20, since ingestion of dead cells can induce an M2 phenotype in macrophages. An important note regarding this study is that Strissel *et al.* found a negative correlation between epididymal fat mass and hepatic mass after the AT was remodeled [50], suggesting that the apparently beneficial changes within the AT may be associated with ectopic lipid storage.

Adipose tissue vascularization

As mentioned earlier, reduced vascular density has been observed in the AT of obese mice [17,45]. Pang *et al.* demonstrated that ATMs significantly contribute to the elevated expression of the angiogenic factor platelet-derived growth factor in obese Lep[ob/ob] mice, however in lean mice most of this expression comes from preadipocytes [45]. Therefore, the increased presence of macrophages in obese AT may assist in AT remodeling by stimulating angiogenesis [45],

a suggestion which Bourlier *et al.* has further supported with *in vitro* evidence [77]. It will be informative to find out whether macrophages have an important *in vivo* role in the vascularization of AT in obese humans.

Matrix metalloproteinases & tissue inhibitors of metalloproteinases

As in other tissues, AT remodeling likely involves the matrix metalloproteinase system. Expression studies in AT from DIO, Lep$^{ob/ob}$ and LepR$^{db/db}$ mice demonstrated an increase in mRNA levels of matrix metalloproteinases (MMPs), such as MMP-2, -3, -11, -12, -14 and -19 in obese compared with lean animals [78,79]. By contrast, MMPs such as -7 and -9 were downregulated [78,79]. Furthermore, tissue inhibitors of MMPs (TIMPS) are also differentially regulated in AT of obese mouse models [78,79]. A more direct role for MMPs and TIMPs has been shown in gene knockout studies. For example, MMP-2$^{-/-}$ mice gained less weight and had smaller fat pads when placed on a high-fat diet compared with MMP-2$^{+/+}$ controls [80]. TIMP-1$^{-/-}$ mice also had a similar phenotype [81]. Finally, studies utilizing inhibitors of MMPs have shown a reduction in AT mass in mice that have reduced MMP activity [82,83]. Interestingly, dietary fish oils have been shown to inhibit the increase in MMPs noted in high-fat fed mice [84]. These data indicate that the MMP/TIMP system may be activated in obesity to allow for adipocyte hypertrophy, AT expansion and remodeling.

Human studies also point to a potential role of MMPs in obesity as evidenced by an increase in plasma levels of MMP-2 and MMP-9 in obese compared with lean individuals [85]. Furthermore, TIMP-1 protein levels were found to be significantly higher in obese individuals, and plasma TIMP-1 levels correlated positively with BMI, plasma free fatty acids, cholesterol, leptin and IL-6 levels [86].

Conclusion

There are many different molecules and processes that have been shown to play a role in macrophage recruitment to AT. In this review, we have broken macrophage infiltration into three primary stages (Figure 1):

- Initiation
- Propagation
- Remodeling

However, this process most certainly occurs on a continuum, such that certain characteristics of the macrophages and adipocytes are similar in all three stages. In addition, there appears to be a great degree of compensation among the factors responsible for attracting macrophages to the AT, such that removal of one player generally has only minimal effects on the cumulative accrual of macrophages. Although knowledge in the field continues to expand, much work is needed to identify the role of each key contributor.

Future perspective

Despite the advances that have been made in the field regarding initiation and propagation of macrophage recruitment to AT, there are possible factors that remain to be analyzed. For example, it has not been proven *in vivo*, whether fatty acid flux, leptin or hypoxia play a role in initiating macrophage recruitment to AT. Furthermore, it is not known whether macrophage-derived chemokines other than MCP-1 might be involved in propagating the signals that stimulate the influx of additional macrophages. Finally, the area of AT remodeling is very new, and future studies will certainly highlight the role of the remodeling process in AT physiology.

In addition to understanding the mechanisms by which macrophages are recruited to AT, it is imperative that we understand the physiological relevance of the paracrine loop between macrophages and adipocytes. Furthermore, our knowledge of whether the macrophages contribute to the pathophysiological consequences of obesity is very rudimentary and must be expanded. Finally, although it has clearly been shown that inflammatory macrophages infiltrate AT in humans, the model we propose is based primarily on studies performed in mice. Thus, the continued translation of this research from rodents into humans is necessary to turn this basic biological knowledge into useful therapeutic options for inflammation and insulin resistance associated with obesity.

Executive summary

Initiation of macrophage recruitment to adipose tissue

- Uncontrolled fatty acid flux may contribute to macrophage recruitment by increasing the proinflammatory status of the adipose tissue (AT).

- Immune cells must enter tissue via blood vessels, and changes in endothelial cells may increase macrophage adhesion and migration into AT.

- Leptin secretion from AT is increased in obesity and could contribute to macrophage recruitment by increasing adhesion molecule expression on endothelial cells or by acting as a chemoattractant.

- Rapid expansion of AT mass could lead to hypoxia, which could ultimately promote adipocyte cell death and the infiltration of macrophages to phagocytose the debris.

- Other immune cells, such as neutrophils and T cells, may enter the AT first and contribute to macrophage recruitment.

Propagation of macrophage recruitment to adipose tissue

- After a certain degree of AT expansion, AT macrophage (ATM) content no longer correlates with bodyweight or AT mass. Thus, chemokine secretion from ATMs themselves likely contributes to propagation of macrophage recruitment to AT.

Remodeling of adipose tissue

- M1 'classically activated' and M2 'alternatively activated' macrophages are both present in AT and help control inflammatory status and remodeling as AT expands.

- Phagocytosis of dead adipocytes may promote ATM polarization toward an M2 phenotype.

- Adipose tissue vascularization and the matrix metalloproteinase/tissue inhibitor of matrix metalloproteinase system probably contribute to AT remodeling.

Acknowledgement

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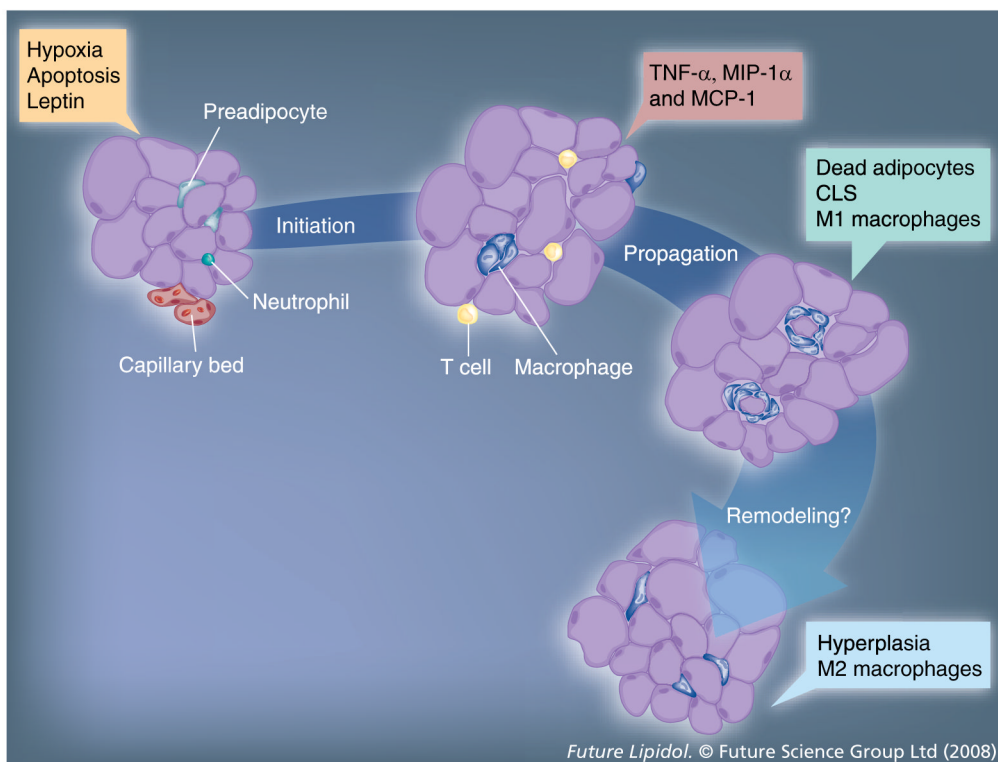
Figure 1. Stages of macrophage infiltration into adipose tissue obesity
During weight gain, macrophages are recruited to the expanding adipose tissue (AT) bed.
Factors that initiate this process are thought to be derived from the adipocytes or capillary
endothelial cells within AT and include adipocyte secretion of leptin, hypoxia, adipocyte cell
death and initial infiltration of other immune cells, such as neutrophils and T cells. Adipose
tissue macrophages (ATMs) are highly inflammatory, secreting cytokines such as TNF-α, and
likely contribute to propagation of the recruitment of additional macrophages by secreting
chemokines such as MCP-1 and MIP-1α. A dramatic increase in ATMs results in the formation
of CLS that surround the dead adipocytes. These macrophages appear to contribute to
remodeling of AT, during which fewer CLS are present and AT expansion may occur via
hyperplasia more than hypertrophy. In addition, a repolarization from an M1 toward an M2
phenotype may occur.
CLS: Crown-like structures; MCP-1: Monocyte chemotactic protein-1; MIP-1α: Macrophage
inflammatory protein 1α.