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Review article

Is obesity an inflammatory illness? Role of low-grade inflammation and macrophage infiltration in human white adipose tissue

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There are at least two scientific evidences of human obesity as a chronic inflammatory illness: first, the well-described moderate increase of inflammatory factors in the circulation in obese subjects, and second, the recent identification of macrophage cells infiltrating the white adipose tissue (WAT). These observations led to a revision of the physiopathology of obesity and its co-morbidities. It has been suggested that the 'low-grade' inflammatory state associates with metabolic and cardiovascular complications of obesity. Weight loss is able to improve this inflammatory state by both significantly decreasing circulating inflammatory molecules and macrophage cell infiltration in WAT depots. However, the mechanisms of WAT macrophage recruitment into the adipose tissue and their role in obesity complications have not been defined. This review aims to point out the knowledge on inflammatory cytokines associated with obesity and focuses on macrophage infiltration in human WAT, discussing their recruitment and role. The interactions of macrophages with adipocytes will certainly be the subject of intense investigations in the future.

Keywords Inflammation, macrophage, obesity, white adipose tissue.

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Introduction

The existence of an inflammatory state involving the adipose tissue and its potential role in obesity and obesity complications has been demonstrated for the first time by Hotamisligil *et al.*[1] in 1993. This study showed the constitutive production by the white adipose tissue (WAT) of tumour necrosis factor alpha (TNFα), a proinflammatory cytokine. Its expression increases in adipocytes of obese animals, and the neutralisation of TNFα by a TNFα soluble antibody leads to an improvement of insulin sensitivity in these animals.[1] These observations exhibit the existence of a strong link between a proinflammatory cytokine, produced and secreted by the WAT, and the development of insulin resistance associated with obesity progression. These findings opened a new field of research in the domain of inflammation and obesity, which became more important after the discovery, in 1994, of the leptin hormone. Leptin is a cytokine specifically produced and secreted by WAT, having a fundamental role in the regulation of food intake and energy balance.[2] The concept of 'adipocytokines' or 'adipokines' has been proposed to qualify small protein molecules, or cytokines, produced and secreted by WAT. These secretory products are susceptible then to circulate in plasma, having a systemic action.[3] Among these proteins, there is a distinction between molecules specifically (or exclusively) produced by WAT (such as leptin and adiponectin) and others produced in abundance by the adipose tissue, also by other tissues and organs (Figure 1).

Origin of chronic inflammation in obesity and contribution of the WAT

Generally, the liver and the lymphoid organs are the major sites of production of inflammatory mediators. Recent data have shown that WAT also expresses many proinflammatory and anti-inflammatory factors, and in this respect, it probably contributes to the increase of circulating levels of inflammatory molecules in obesity. Indeed, it has been shown that the

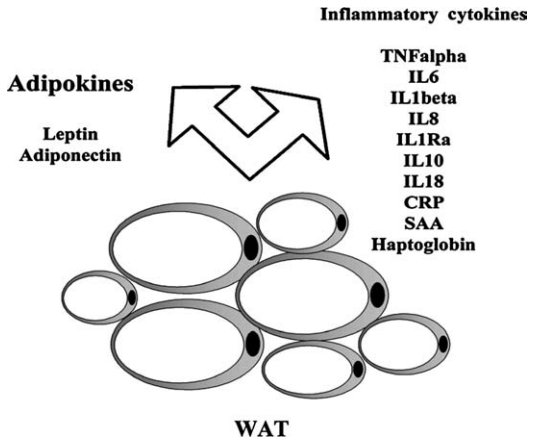

Figure 1. List of adipokines specifically expressed and secreted by WAT and of inflammatory cytokines produced and secreted by WAT and other tissues or organs (TNFα, IL6, IL8, IL1Ra, IL10, IL18, CRP, SAA and haptoglobin).

adipose tissue is able to produce inflammatory cytokines (such as TNFα, transforming growth factor β and interferon-γ), interleukins (IL) (such as IL1, IL6, IL10 and IL8), factors of the complement cascade (plasminogen activator inhibitor-1 [PAI-1], fibrinogen, angiopoietin-related proteins, metallothionein, complement factor 3) and chemoattractant cytokines (monocyte chemotactic protein-1 [MCP-1] and macrophage inflammatory protein-1alpha).[4] Some of these are recognised as acute-phase proteins of inflammation (e.g. IL6,[5] C-reactive protein [CRP][6] and haptoglobin[7,8]). Furthermore, we have observed that different isoforms of the serum amyloid A (SAA) protein, usually known to be produced by the liver, can also be produced by the WAT. The SAA protein levels are increased in human obesity, correlate with adiposity and are modulated by weight loss.[9]

On the other hand, obesity is also associated with a reduced production of adiponectin,[10] usually considered as a factor with anti-inflammatory properties. In contrast to what is seen in recognised inflammatory diseases, obesity is associated with a moderate, but chronic, increase of this 'cocktail' of inflammatory factors. The relative contribution of the different organs and tissues (such as liver, lymphoid system, subcutaneous and visceral adipose tissue) in the circulating levels of inflammatory cytokines is difficult to determine in obesity and during the different phases of its evolution. Indeed, especially in cases of extreme obesity, where all the different adipose depots of the organism are enlarged, the respective contributions of both adipose tissue mass and other tissues in the production of proinflammatory factors are unknown.

Subcutaneous or omental WAT?

The accumulation of the adipose tissue in different anatomic localisations certainly plays a role in the development of obesity co-morbidities. Excess adipose mass in the upper parts of the body (also indicated as 'android obesity' or 'central obesity') usually constitutes a risk factor for type II diabetes, hypertension, dyslipidaemia and cardiovascular disease, especially in women.[11,12] Anthropometric measurement of body mass index (BMI) and waist circumference are potentially useful tools for clinicians in counselling patients regarding type II diabetes risk and risk reduction.[11] Glucose intolerance is significantly higher in women with predominantly upper body obesity than in women with lower body obesity, and fasting plasma triglyceride levels are also significantly higher in women with central obesity.[12] On the contrary, the excess of adipose mass in the lower parts of the body ('gynoid obesity') seems not to have major metabolic consequences.[11,12] Thus, in women, the sites of fat predominance offer an important prognostic marker for many metabolic complications. The adipose tissue has strong anatomical specificities,[12,13] in particular for the expression and the secretion of many adipokines. Leptin[14] is preferentially secreted by the subcutaneous adipose tissue, while the expression of adiponectin,[15] PAI-1, IL8, and IL1β[16,17] is more important in the visceral adipose depot.[18] There have been discrepancies in results for the IL6 secretion and modulation,[19] since both visceral and subcutaneous WAT of obese and nonobese subjects release this cytokine. The differences in the production and secretion capacities between the subcutaneous and visceral WAT and the respective abundance of these depots in some individuals certainly contribute to the relative risks for the occurrence of metabolic and cardiovascular complications and the development of other complications, such as hepatic diseases.[13] However, the roles of key molecules linking obesity to specific complications still remain to be identified. In addition, other adipose depots (e.g. the epicardial WAT) also express inflammatory mediators in the absence of obesity and established diabetes.[20] For instance, this local inflammation of adipose tissue and its proximity to coronary vessels, for example, may contribute to the aetiopathogenesis of coronary artery disease. Thus, plasma inflammatory biomarkers may not always adequately reflect local tissue inflammation. Current therapies do not decrease or inhibit the local inflammation in epicardial adipose tissue.[20]

Adipocytes or stroma vascular fraction cells?

The WAT is a very heterogeneous tissue. It is composed of several cell types: mature adipocytes and various other small cells (i.e. preadipocytes, fibroblasts, endothelial cells, histiocytes and macrophages), usually grouped and indicated as the 'stroma vascular fraction' (SVF). Due to this heterogeneity, the cellular origin of different secreted inflammatory factors by whole adipose tissue is debated. Some *in vitro* studies indicate that isolated adipocytes express inflammatory factors, such as the TNFα.[1] We have showed that the SAA

protein was over-expressed and secreted by isolated adipocytes rather than by SVF cells.[9] Moreover, the importance and the contribution of the SVF cells in the secretion of inflammatory factors are now well recognised, especially in pathologic conditions such as obesity.[19]

The infiltrating macrophages of WAT

Recent investigations published by American and French research teams have given evidences that the WAT of obese rodents and humans is the target of an important macrophage infiltration and that this infiltration increases in proportion to BMI and to adipocyte hypertrophy.[21–23] The origin of the macrophage infiltration of adipose tissue is currently under investigation. Similarities in the gene expression profiles between macrophages and mature adipocytes have been reported, indicating that adipocytes could have some 'macrophage properties', in particular proinflammatory microenvironments.[24,25] However, bone marrow cell transplantation experiments in irradiated mice (chimera generation) demonstrated that the macrophages infiltrating the adipose tissue are essentially derived from bone marrow.[21]

Our team has shown that WAT-infiltrating macrophages in obese women typically aggregate in 'crown-like structures', completely surrounding adipocytes.[23] These aggregates resemble the macrophage arrangement characteristic of local chronic inflammatory states. These WAT-infiltrating macrophages express activation markers like CD68, CD87 and IL12A.[23] The infiltration in obese WAT of a small proportion of other inflammatory cells (such as lymphocytes, granulocytes or giant cells) cannot therefore be excluded.

Mechanisms of macrophage infiltration in WAT

The factors inducing macrophage infiltration and activation in WAT are most probably multifactorial (Figure 2). Paracrine, autocrine, endocrine signals as well as mechanical modifications (i.e. adipocyte hypertrophy and hyperplasia) may play a role in such phenomena. Moreover, numerous chemoattractant cytokines, like MCP-1, colony stimulating factor 3 (CSF-3) and some specialised cytokines could be involved in WAT macrophage recruitment. *In vitro* studies suggest that leptin itself is a powerful adherence-inducing and transmigration-inducing factor for macrophages derived from bone marrow, on cultured endothelial cells.[22] On the contrary, adiponectin inhibits this process in a model of cultured aortic endothelial cells.[26,27] These observations raise the hypothesis

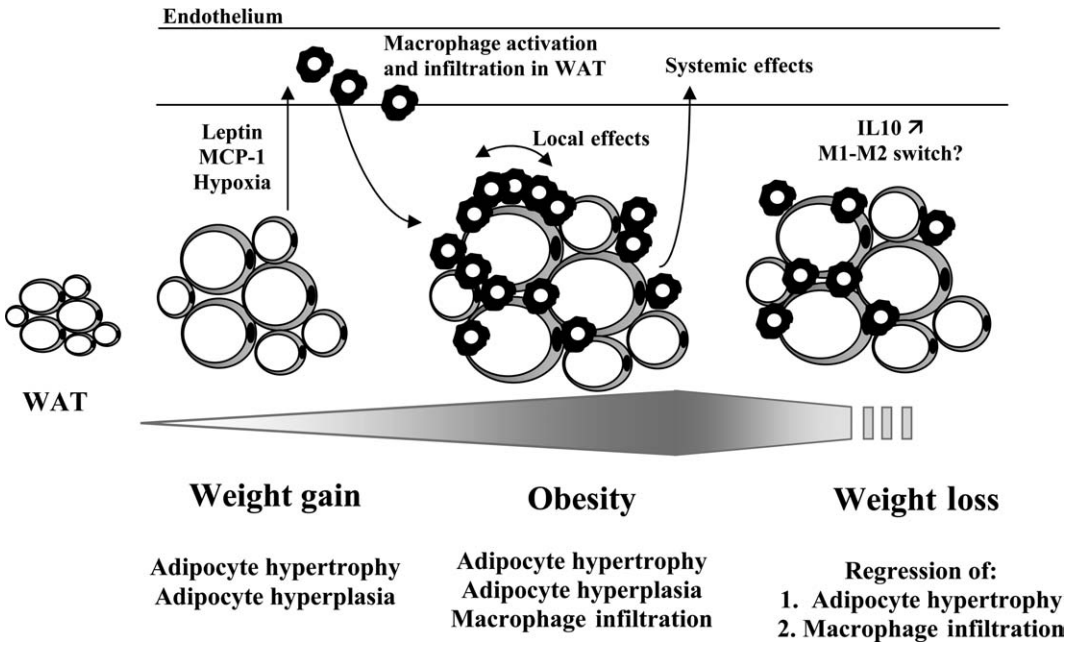

Figure 2. Schematic model of macrophage infiltration into WAT in obesity and during weight loss. Macrophage infiltration occurs after activation of circulating monocytes by several factors: MCP-1 secretion, leptin secretion, adipose tissue hypoxia and stress of endothelial cells. Monocytes differentiate into macrophages after transmigration into WAT and the cross-talk with adipocytes, macrophages and endothelial cells may aggravate the inflammatory local and systemic state, resulting in an increased secretion of proinflammatory cytokines/chemokines, adipokines and angiogenic factors. Weight loss induces a significant regression of both adipocyte hypertrophy and macrophage infiltration in WAT.[25] This regression may be the 'starting point' for the improvement of systemic inflammation observed in human obesity. In slimmed WAT, a molecular switch, 'M1/M2-like', of resting macrophage phenotype (from a proinflammatory versus an anti-inflammatory profile) could be suggested.[25]

of a local and dual action of these adipokines, respectively, exercising some stimulating or inhibitory effects on macrophage recruitment in the adipose tissue. Selectins and integrins may also intervene in the macrophage attraction phenomenon, but they are still poorly explored in the adipose tissue.

Thanks to their exploratory nature, the large-scale gene expression studies also provide some new targets for the identification of the molecular actors involved in these inflammatory processes. Using these techniques, we observed that the expression levels of molecules such as MCP-1, CSF-3 and urokinase plasminogen activator receptor (CD87) clustered together and were strongly increased in the WAT of morbidly obese subjects, with a high degree of macrophage infiltration.[23] This may suggest a co-regulation of the expression of these inflammatory factors. MCP-1 (also known as CCL2) is the most powerful chemoattractant cytokine and its action is mediated via its receptor CCR2. Interestingly KO mice for *CCR2* gene ($CCR2^{-/-}$) present a reduction of the macrophage infiltration into WAT.[28] In these mice, the expression of adiponectin is increased compared with the wild type, and an improvement of the hepatic steatosis of the glucose homeostasis and insulin sensitivity is observed. The receptor CCR2 and the MCP-1 cytokine then seem to contribute in a relevant way to the macrophage accumulation in the WAT.[28] However, other candidates still remain to be defined.

There are important hints that local adipose hypoxia can also be involved in the attraction and retention of macrophages in WAT.[23] We showed that hypoxia inducible factor-1α (HIF-1α): a transcription factor strongly induced by hypoxia is over-expressed in obese WAT and its expression decreases during weight loss.[23] Local tissue hypoxia is a well-known inducer of macrophage attraction and maintenance in some solid tumours and in atheroma plaques.[29,30] These observations suggest that the adipose tissue of obese subjects may present hypoxic areas with an increased local expression of hypoxia-stimulated chemoattractant factors.

Interestingly, leptin itself is a well-known HIF-1α inducible gene[31] with chemoattractant properties.[22]

Role of infiltrating macrophages in WAT

The population of macrophages in the WAT may contribute to enhance the 'low-grade' chronic inflammation associated with obesity since macrophages, and adipocytes, are secretory cells. Various observations support the hypothesis of a potential deleterious role for adipose-infiltrated macrophages in the genesis of obesity-associated pathologies.[32] Nevertheless, their phenotype in adipose tissue neither has been defined yet nor has their exact biological role in different adipose tissue depots. The development of techniques facilitating the isolation of resident adipose tissue macrophages will help us in the near future to determine their phenotypes.

A recent study showed that the majority of adipose tissue macrophage aggregates are located around 'dead' adipocytes, suggesting that one of their normal functions is to clear necrotic adipose fragments from adipose tissue.[33] In an earlier study, we had observed WAT-infiltrated macrophages by transmission electron microscopy: adipocytes surrounded by macrophages contain in their cytoplasm granules of lipofuscin,[23] a typical feature of stressed and aged cells.[34–36] This is in good agreement with the well-known effects of macrophages in other inflammatory states.

Macrophage accumulation seems also to be required for the formation of new blood vessels, notably at the site of inflammation[37] and in ischaemic areas.[38] The role of macrophages in the angiogenesis may occur in rapidly expanding adipose tissue, eventually leading to some degree of hypoxia. As a consequence, macrophages could contribute to the local control of fat mass expansion and of its biology. A dual effect of macrophage in WAT can be envisaged: 1) a local 'beneficial' effect in controlling and eventually in limiting the fat mass development and 2) a simultaneous 'deleterious' systemic effect, through the increased production and secretion of chemokines and inflammatory cytokines that facilitate the genesis and progression of obesity complications.[32]

In vitro co-culture of differentiated 3T3-L1 adipocytes and macrophage cell line (RAW264) results in a significant upregulation of proinflammatory cytokines and a downregulation of anti-inflammatory cytokines.[39] Such inflammatory changes are induced by the co-culture without direct contact, suggesting a powerful role of soluble factors.[39] To our knowledge, no published survey has aimed to systematically define the role of macrophages on the proliferation and differentiation of adipocytes in humans.

Systemic consequences of macrophage accumulation in adipose tissue

The accumulation of macrophages in the adipose tissue may indeed contribute to the increased systemic concentrations of some inflammatory cytokines. The action of these inflammatory molecules may represent the molecular 'link' between adipose tissue and the metabolic, cardiovascular or even hepatic complications of obesity. In particular, increased levels of TNFα, IL6 and resistin, produced by activated macrophages,[40] may directly contribute to the mechanisms of change in the insulin sensitivity in different adipose depots.[41,42] Unfortunately, a systemic circulating factor which strongly correlate with this macrophage infiltration of WAT is still not been found.

Effects of weight loss on systemic 'low-grade' inflammation and macrophages infiltrating adipose tissue in human obesity

Obesity is considered as a 'chronic low-grade' inflammatory state, like numerous other associated pathologies such as atherosclerosis, type II diabetes and some hepatic lesions (e.g. steatosis, nonalcoholic fatty liver disease and nonalcoholic steatohepatitis). In addition to diabetes, hypertension and heart disease, obesity is independently associated with reduced fertility and is a component of polycystic ovarian syndrome, which is a cause of infertility in women.[43] Obese women who become pregnant have higher risks of fetal malformations, gestational diabetes, pre-eclampsia, prolonged labour and surgical birth.[43] The role of chronic inflammation in these obesity complications remains unexplored.

Weight loss, even if modest, improves numerous complications of obesity. Ziccardi *et al.*[44] have shown a clear improvement in the endothelial stress factors during weight loss. There are numerous data indicating that decreasing the energy intake and increasing the physical activity may be effective for reducing overall inflammation.[45] This was clearly shown for CRP, TNFα and IL6.[46–49] Similarly, the serum concentrations of PAI-1, CRP and of the alpha 1 acid glycoprotein are strongly decreased in massively obese subjects after bariatric surgery.[50] We have accordingly observed similar plasma modifications in a cohort of about 60 obese subjects 1 year after weight loss (reduction of 30% of the initial weight). We have also observed a marked reduction of the CRP, SAA, the orosomucoid protein, IL6, TNFα, fibrinogen and an increase in the adiponectin levels.[9,51] The kinetics of these modifications was different from one molecule to another. For example, after the surgical intervention, level of IL6 decreased slowly whereas the level of SAA or the CRP decreased very rapidly in plasma.[52] Furthermore, a moderate weight loss induced by a very low calorie dietary intake is also associated with a reduction of cytokines derived from adipose tissue . The contribution of fat mass variations in the modulation of the inflammatory gene profile in human adipose tissue and the modifications of macrophage infiltration into WAT remained largely unknown, until recent works of our research group.

We have studied the effects of moderate weight loss induced by a very low calorie diet (VLCD) on the expression of inflammatory factors gene in subcutaneous WAT of obese women.[53] About 100 genes related to inflammatory processes were modulated after 4 weeks of VLCD (41% increase and 59% decrease). These genes belong to 12 functional families including cytokines, IL, complement cascade factors, acute-phase proteins and some molecules involved in cell–cell contacts or extracellular matrix remodelling. In our studied cohort, the classes of inflammatory genes were essentially downregulated after weight loss.[53] After 4 weeks of VLCD, leading to an average of 5 to 6 kg of weight loss, the proinflammatory gene expression profile of the subcutaneous adipose tissue of the obese patients became closer to that of the nonobese subjects, despite the persistent differences in the clinical and biologic phenotypes of these two groups. This improvement of the inflammatory profile not only results in a reduction of the expression of the proinflammatory factors but also corresponds to an increase of the expression of anti-inflammatory factors, such as IL10 or IL1-Ra.[53] This modification in the inflammatory profile of the subcutaneous WAT could provide the first biologic support to the observation of an improvement of metabolic parameters during weight loss, even when the loss of weight is modest.

In agreement, the reduced expression of the inflammatory genes by 'slimmed' WAT and the systematic morphological analysis of subcutaneous WAT biopsies (performed during gastric reduction surgery) also revealed a significant decrease of the macrophage infiltration into WAT.[23] This reduction was associated with a macrophage proinflammatory phenotype switch (IL10 expression increase after weight loss) and with the suppression of several proinflammatory and chemoattractant genes[23] (Figure 2).

Conclusions

The discovery of the 'low-grade inflammatory state' in obesity and of the macrophage infiltration in WAT opens new perspectives in the research of the physiopathological mechanisms involved in the development of obesity comorbidities, their evolution and maintenance.

A future goal will be to identify the molecular mechanisms of this inflammatory state and of the very early signals of macrophage infiltration into WAT, in order to control the interaction of macrophages with adipocytes and other target tissues.

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