M1/M2 macrophage polarization in human obese adipose tissue

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Obesity and insulin resistance are closely associated with chronic inflammation in adipose tissue, where macrophages play an important role. Adipose tissue macrophages can be divided into two main phenotypes: the classical M1 macrophages and alternatively activated macrophages M2. M1 macrophages produce pro-inflammatory cytokines (TNF-α, interleukin IL-6 and MCP-1) and thus contribute to the development of insulin resistance. On the other hand, M2 macrophages, anti-inflammatory, are involved in the maintenance of tissue homeostasis and are typical in the adipose tissue of slender individuals. Macrophages can also play a role in the pathogenesis of other serious illnesses such as cardiovascular diseases or cancer. This article reviews the latest data on macrophage polarization in adipose tissue.

Key words: M1/M2 macrophages, adipose tissue, obesity, inflammation, chemokines

Received: December 12, 2017; Accepted with revision: March 27, 2018; Available online: May 16, 2018
https://doi.org/10.5507/bp.2018.015

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INTRODUCTION

Macrophages are present in all tissues and show a huge functional diversity. In recent years a large amount of new information about the origin of macrophages in the steady state and in the context of the inflammation has opened up a numerous new directions in research of new therapeutic interventions. More than hundred years have passed since the discovery of macrophages and their ability to carry out phagocytosis, the discovery of which resulted in a Nobel prize being awarded to Russian zoologist Ilya Ilyich Metchnikoff.

Fourteen years ago Weisberg et al.[1] first described macrophages infiltration in adipose tissue as the main trigger of the inflammation associated with obesity and insulin resistance. After a search of the phrase "adipose tissue macrophages, M1, M2" in the databases of Medline/PubMed, Scopus and Web of Science we found that 110, 118, or 137 scientific articles respectively have been published dealing with the issue over the past five years. Evidence has accumulated which demonstrates the important role of the polarization of macrophages in the development of metabolic diseases. We now know that the resident macrophages in normal adipose tissue belong to alternatively activated, M2 type, which are important in the homeostasis of fatty tissue. On the other hand, the number of classically activated M1 macrophages increases in the adipose tissues of obese individuals, contributing to the inflammation processes and insulin resistance. However, there is evidence revealing a more complex scenario within the scale of the functional statuses of the macrophages, which goes beyond the classic subdivision of M1/M2 macrophages and the significant differences in macrophage profiles in animals and human models[2].

In this article, we review the latest knowledge in the area of the macrophage polarization in adipose tissue. Adipose tissue is composed of mature adipocytes and other smaller cells including, pre-adipocytes, fibroblasts, endothelial cells, macrophages and histiocytes, which form the so-called stromal vascular fraction. Macrophages are mononuclear phagocytotic cells involved in the inflammatory and immune processes. Infiltration of a huge quantity of macrophages into the adipose tissues of obese individuals, was described for first time, by Weisberg et al.[1] and Xu et al.[3] in 2003. In adipose tissue of lean individuals, the percentage of macrophages in the stromal vascular fraction is around 5-10%, while for the obese this may be up to 40% or 50% in the case of the morbidly obese. In mice and people the content of macrophages in adipose tissue is closely related to the size of adipocytes and body weight. Analysis of upregulated genes for chemokines CCL2, CCL3, CCL4, CCL 18 and IL8/CXCL8 in cells isolated from adipose tissue of obese revealed that adipose tissue of obese is a major source of inflammatory cytokines[4]. In addition, the accumulation of macrophages in the adipose tissue is associated with the development of insulin resistance and progression of type II diabetes[5,6]. However, not all individuals with high BMI develop insulin resistance.

As a result of the relatively recent discovery of the important role macrophages have in adipose tissue, many experiments were carried out with the goal of increasing or decreasing the number of macrophages in the adipose tissue. This was done in order to determine whether there is an accompanying change of sensitivity to insulin following the alteration of macrophage numbers. Xu H. et al. demonstrated, using RT PCR and Northern blot analysis of six macrophage pro-inflammation genes isolated from stromal vascular cells, that there is downregulation

of the mRNA levels of inflammation genes, increase sensitivity to insulin, with a decrease in the number of macrophages in adipose tissue in a mouse model following Rosiglitazone (insulin-sensitizing drug) treatment[3]. A decrease of inflammatory gene expression (monocyte chemotactic protein-1 [MCP]-1, plasminogen activator urokinase receptor [PLAUR], colony-stimulating factor-3 [CSF]-3) in white adipose tissue has been observed following the loss of macrophages as a result of bariatic weight reduction surgery carried out in morbidly obese indiviuals. Similarly, a decrease in macrophages in adipose tissue was observed in obese individuals after modifying their lifestyle (diet, exercise) (ref.[6]). Kanda H. et al. found a slight decrease in macrophage numbers and increased sensitivity to insulin, as well as decrease in hepatic steatosis associated with obesity, in mice after inhibiting monocyte migration into adipose tissues by disrupting the MCP-1 gene. On the other hand, increased expression of monocyte chemoattractant protein-1 (MCP-1) led to an increased number of macrophages in adipose tissue and reduced sensitivity to insulin, as demonstrated in transgenic mice where MCP-1 is overexpressed in adipocytes[7]. Based on these discoveries, we can conclude that the number of macrophages in adipose tissue is closely related to insulin resistance.

In cases of obesity, not only do the number of macrophages in adipose tissue increase, but they also take on an inflammatory phenotype (M1). Lumeng C.N. et al. found that adipose tissue macrophages in lean mice express genes characteristic of the anti-inflammatory M2 type or "alternatively activated" macrophages. These genes include: protein Ym-1, Arginase-1 and IL-10. While in obese mice, increased expression of TNFα and iNOS was found, which is characteristic of the M1 (pro-inflammatory) or "classically activated" macrophages. In cases of obesity, adipose tissue macrophages transition from the M2-(anti-inflammatory) phenotype to the M1-(pro-inflammatory) phenotype. These M1 macrophages produce inflammatory cytokines that inhibit the ability of adipocytes to respond to insulin. Conversely, weight loss is associated with the reverse change, with M1 macrophages transitioning to the M2-anti-inflammatory phenotype[8]. These discoveries highlight that obesity plays an important role in the development of insulin resistance, due to the number and phenotype of the macrophages involved.

However, in cases of human obesity there remains an unanswered question as to whether the presence of M1 macrophages in the adipose tissues is caused by development from precursor monocytes, or whether it occurs as result of repolarization of M2 macrophages already present in the tissue. Experimental studies in obese mice have shown that M1 macrophages are continuously replenished by precursor monocytes from the blood stream[6,7,9-12]. These macrophages accumulate around adipocytes in the "crown-like" structures. Macrophages in these crown-like structures contain a lot of vesicles, which implies the presence of intracytoplasmic lipids associated with phagocytic activity[3,9].

Adipose tissue in healthy lean subjects contains anti-inflammatory M2 macrophages, which maintain tissue homeostasis and insulin sensitivity by secreting anti-inflammatory cytokine IL-10. On the basis of kinetic studies carried out on obese mice, it is assumed that before infiltration of adipose tissue by M1 macrophages other cells are present including: neutrophils, CD8 + T lymphocytes and mast cells. In addition, the hypertrophic adipocytes become inflammatory or necrotic, thereby attracting M1 macrophages, which are organized into the “crown-like” structures. Obesity is therefore associated with destabilizing the balance between M1 and M2 macrophages, leading to the number of M1 macrophages greatly exceeding the number of M2 macrophages[10].

Macrophages infiltrate both visceral and subcutaneous adipose tissue. As development of visceral adipocytes differ from differentiation of subcutaneous adipocytes, it can be assumed that these adipocytes differ in their potential to attract macrophages in cytokine secretion stimulation. It is known that visceral adipose tissue expresses more: IL-6, MCP-1, MIP-α and CSF1 and other genes associated with macrophages and inflammation[13]. Harmann-Boehm et al. found that in humans, preferential macrophage infiltration in omental fat is a common phenomenon extending from lean individuals to obese individuals. Omental fat macrophage infiltration is particularly exaggerated in central type obesity where it is associated with the comorbid states that usually accompany severe obesity[14,15].

What is the cause of the infiltration of macrophages in adipose tissues in obesity? Adipocyte hyperplasia and hypertrophy can both contribute to adipose tissue expansion. Sun et al. predict four different mechanisms for initiation of macrophage infiltration: adipocyte death, hypoxia, enhanced chemokine secretion and dysregulation in fatty acid fluxes[16].

Cinti et al. assumed that adipocyte death is the main stimulus that regulates macrophage infiltration of fat tissue. Numerous macrophages are found in obese humans in close proximity to the dead adipocytes. They fuse creating so-called the "crown-like" structure. The "crown-like" structures express F4/80, MAC-2, TNFα and IL6, which proves the presence of M-1 macrophages and inflammation[11,17-19]. The death of adipocytes is more prevalent in visceral adipose tissue than in subcutaneous adipose tissue[17,19]. It is therefore likely that the cell death of adipocytes, which may be caused by hypoxia during the rapid increase in adipose tissue, is a signal that attracts macrophages.

As a result, human obesity leads to dysregulation of the intercellular milieu through the secretion of cytokines, chemokines and adipokines by adipose tissue. Even though they may be released by adipocytes, studies have shown that the majority of secreted chemokines in adipose tissue comes from the stromal vascular population[3,15,16]. Production of chemokines in adipose tissue macrophages thus presumably contributes to the accumulation of macrophages in adipose tissue. Numerous studies in humans have shown increase of gene expression of different cytokines in adipose tissue of obese individuals (TNFα, IL-1, IL-6,6), chemokines, including MCP-1, MIP-1α (macrophage inflammatory protein), MIP-1β, MCP, MCP-2-4. Increased transfer of chemokines from

dysregulated adipose tissue into blood results in chronic low-grade inflammation, insulin resistance and atherosclerosis[16,18,20].

It is well known that obesity leads to dysregulation in adipocytokine production: increased secretion of leptin levels and reduced secretion of adiponectin. Leptin in humans has a slight ability to trigger macrophage phagocytosis and cytokine production. Additionally adiponektin has anti–inflammation effect inhibiting production of TNFα and IL6 (ref.[5]).

Several recent studies have shown significantly increased adipocyte volumes in obese patients. It causes deficiency of O_2 (cytoplasmic hypoxia) in the center of hypertrophic adipocytes[21,22]. Hypoxia leads to substantial increase of HIF1α (hypoxia-inducible factor-alpha) and decrease of adiponectin in both humans and rodents[6,23]. Ye et al. have demonstrated (using mice adipocytes cell culture, and qRT-PCR) that hypoxia increases adipocytes expression of HIF-1a, VEGF, GLUT1, Hemox and PDK inflammatory genes. Hypoxia also induces leptin expression in adipocytes[23]. Hypoxia is involved in the initiation of several processes, including adipocyte death, the infiltration of leukocytes, activation of leptin secretion and suppression of adiponectin expression[23-25].

Fatty acids, stored in the form of triglycerides in adipose tissue, may also be an important regulator of inflammation and the infiltration of macrophages into adipose tissue. Fatty acids can serve as ligands for the complex of TLR4 (toll-like receptor), which trigger an inflammatory reaction, together with an increase in the concentration of extra-cellular lipids, which ultimately leads to the infiltration of macrophages into adipose tissue[12].

Despite the evidence that adipose tissue macrophages lead to chronic inflammation and worsen insulin resistance, they may also have a protective role. Kosteli et al. published that the loss of fat stored in adipocytes, which is caused by a restricted food intake or by medication, is associated during early weight loss with increase in local release of free fatty acids (FFA), inducing macrophages recruitment. Macrophages phagocytose excess lipids and reduce local concertation of FFA, may thus protect local adipocyte function. In another study carried out in obese mice, macrophages in adipose tissue accumulate lipids leading to the formation of the "foam like" cells in adipose tissue[26,27]. Additional evidence confirms the hypothesis, that M1 macrophages, which in the adipose tissue of obese individuals are localized around the dead and the dying adipocytes, form the so-called "crown like" structure. It was found that macrophages in these structures contain lipid phagosomes. These reports support the role of macrophages in protecting the body from the toxic effects of free fatty acids released from adipocytes[11,17].

Macrophages are distributed in tissues throughout the body. They play a major role in diseases and homeostasis. They have an important effect on the growth of tissues as well as tissue remodeling and organization. These functions are, unfortunately, often corrupted in many chronic diseases, some of which are associated with aging, and their rampant activity can worsen many pathologies. A complete understanding of the role of macrophages in the development and maintenance of tissues, especially with the use of methods describing the gene expression in specific macrophages subpopulations, may bring light deeper into the biology of macrophages and also allow more precise targeted anti-macrophage therapy.

Despite all the knowledge, there are still many unanswered questions. There are limitations to animal studies including: differences in study design, molecular method used, alterations to gut microflora (due to sterile environment during studies) and dietary induced obesity in mice as not comparable with human obesity. Due to these limitations, results from animal studies are often not utilizable in therapies for patients. Additionally, human studies can produce conflicting data and design flaws seriously undermine their validity. Several interleukins and adipokines have been described as potential targets for therapies.

CONCLUSION

In this article we summarize recent findings concerning the role of macrophages polarization in obese adipose tissue. Macrophages are classified into two basic population: M1 classically activated and M2 alternatively activated. Recent studies described a broad range of macrophage subpopulation. It is generally accepted that the M1 macrophage accumulation in adipose tissue is associated with metabolic changes linked to obesity. Insulin resistance correlated with levels of pro- inflammatory cytokines, such as TNF-α, IL-1β and IL-6. M2 macrophages are associated with tissue remodeling and inflammation resolution has been published. However, it is important not to look exclusively at the role of macrophages in adipose tissue in the context of insulin resistance and glucose intolerance, as they can also significantly increase the risk of other diseases, including cardiovascular disease and cancer.

Search strategy and selection criteria

Literature used in this article was identified via searching the databases of Medline/PubMed, Scopus and Web of Science. The search phrases used included "adipose tissue macrophages, M1, M2". Only English language papers were reviewed.

Author contributions: JCH: literature search, manuscript writing; VK: concept, critical revision; JD, ZT: critical revision.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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