

Adipose Tissue-Resident Macrophages and Obesity

Allah Nawaz*, Tomonobu Kado, Yoshiko Igarashi, Yagi Kunimasa, Isao Usui, Shiho Fujisaka, Kazuyuki Tobe*

First Department of Internal Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan

ABSTRACT

Type 2 diabetes and insulin resistance are one of the major consequences of obesity as a result of inflammation in adipose tissues. Adipose tissue-resident macrophages (ATMs) have a primary role in tissue remodeling and maintenance of homeostasis within adipose tissue. Two different types of macrophages are present in adipose tissue i.e. M1 and M2-type. Obesity is associated phenotypic transformation of macrophages, from anti-inflammatory M2 to pro-inflammatory M1 macrophages. M1-type macrophages increases in obesity and contribute to the development of type 2 diabetes and insulin resistance. In contrast, M2 ATMs secretes anti-inflammatory cytokines and are involved in maintaining insulin sensitivity. However, little is known about the role of M2 macrophages in adipose tissue. Literature related to the role of M1 and M2 macrophages in metabolism have been reviewed in this article with emphasis on the macrophages associated with adipose tissues. Role of M2 macrophages in adipose tissues have also been highlighted in this article to enhance our knowledge and understanding of macrophages homogeneity.

Keywords: obesity, adipose tissue macrophages, inflammation, insulin resistance

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***Address of Correspondence Author:**
tobe@med.u-toyama.ac.jp
dr.anawaz786@gmail.com
nawaz@med.u-toyama.ac.jp

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INTRODUCTION

Adipokines are the hormones that are responsible for systemic metabolism released by the adipose tissues. Health of adipokines is maintained via macrophages. Activated macrophages (M1) accumulate as the size of adipocytes becomes larger in adipose tissues and trigger systemic and local inflammation by worsening insulin resistance. Alternatively, activated macrophages (M2) possess anti-inflammatory actions. However, the exact mechanism how health of adipose tissues in a lean state is maintained remains elusive. In obesity during pathological expansion, growth of neovasculature fails to catch up with rapid adipose tissue expansion resulting in adipose tissue hypoxia leading to adipose tissue dysfunction and systemic insulin

resistance [1, 2]. In contrast to healthy expansion, pathological expansion is characterized by some prominent features including macrophage infiltration (inflammation), hypoxia and limited vessel growth [2]. Although hypoxia due to insufficient angiogenesis is likely to play a key casual role in the development of pathological expansion, the precise mechanism underlying the inadequate angiogenesis in pathological expansion and adequate angiogenesis in healthy expansion is not yet determined. In obese adipose tissue, hypoxia has been shown to induce the stimulation of hypoxia-inducible factor-1 (HIF-1) [3, 4]. Hypoxic environment causes its HIF-1 α subunit to translocate from cytoplasm to the nucleus, where it dimerizes with its HIF-1 β subunit and certain co-activators interact with its

transactivation domain and results in the transcription of many genes regulating different processes including angiogenesis and glucose metabolism [5].

Hypoxic responses mediated by HIF-1 α affect the adipocytes and macrophages differentially. In adipocytes HIF-1 α has been shown to cause the metabolic dysfunction followed by proinflammatory response as reported in several transgenic mice studies [4, 6-10]. With regard to the role of macrophage HIF-1 α we hypothesize that there are two possibilities about the dual character of macrophage HIF-1 α . The first possibility is that macrophage HIF-1 α is involved in proinflammatory responses [11, 12], thereby causing the adipose tissue inflammation and systemic insulin resistance. The other possibility is that adipose tissue-resident macrophages (ATMs) secrete proangiogenic genes such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, as reported in tumor-associated macrophages (TAMs) [13] under hypoxic conditions, thus providing adipocytes with vascular network to improve adipocyte metabolism.

Adipose vasculature is regulated by various proangiogenic factors released from adipocytes and other cells in the stromal vascular fraction such as macrophages, preadipocytes and endothelial cells. Production of these factors may be impaired in pathological expansion of adipose tissue. There are several pieces of evidence pointing towards the positive role of adipocyte-derived VEGF in inducing the healthy expansion [14]. Under the obese state, the expression of VEGF gene is not sufficiently induced [12], which may be partly due to the fact that adipocyte HIF-1 α is not a strong inducer of VEGF gene expression. In addition, adipocyte HIF-1 α not only failed in inducing the adequate angiogenesis but was involved in the induction of fibrosis through increased formation of extracellular matrix components (ECM) [4]. On the other hand, ATMs express

the VEGF gene *via* HIF-1 α in response to adipose tissue hypoxia. Moreover, differentiating preadipocytes produce high level of a range of proangiogenic factors, including fibroblast growth factor 2 (FGF-2), VEGF, hepatocyte growth factor (HGF) and PDGFs [15]. An early study showed that differentiation from preadipocytes to mature adipocytes was concomitantly linked to elevated production of angiogenic factors [16]. Recently, we identified that macrophage HIF-1 α prevents angiogenesis in preadipocytes [17] (Figure 1).

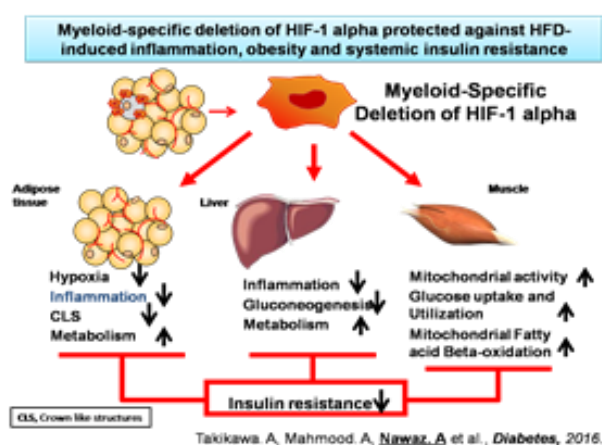


Figure 1. Deletion of HIF-1 α improve systemic insulin resistance.

Deletion of HIF-1 α from myeloid cells causes significant low inflammatory response from adipose tissue-resident macrophages (ATMs), which then turn the expansion of adipose tissue from pathological to healthy one characterized by low fat cell mass, improved metabolism evident by upregulation of PPAR γ gene as well as adiponectin level in adipocytes along with reduction in fibrosis and oxidative stress, but keeping in view that these changes come as secondary changes to reduce proinflammatory responses in macrophages. This improved adipose tissue state was associated with improved insulin signaling in liver and skeletal muscle of KO mice. Of note, increased mitochondrial activity in skeletal muscle

and reduced expression of gluconeogenic genes in liver from HIF-1 α KO mice was observed [17].

Collectively, our results demonstrate that HIF1 α inhibition in macrophages leads to significant metabolic improvements, suggesting that selective HIF1 α inhibition in macrophages may be an effective therapeutic target in the context of metabolic dysfunction.

Adipose tissue macrophage as in other organs play pivotal role in the physiological homeostasis. Much has been known about macrophage role in inflammatory process in obese adipose tissue which is known closely related with the development of insulin resistance both in human and animal [18], however, much less is known about their role in lean condition. Since majority of macrophage in lean adipose tissue is M2 type macrophages, we decided to focus our attention to their physiological role in that condition.

We previously demonstrated that majority of adipose tissue macrophages in lean mice are M2 types which could be identified and separated as distinct macrophage population using surface marker CD206 [19]. So, in subsequent study, we would like to extend our previous data to dissect the role of M2 macrophage using CD206 as marker. To obtain information about M2 macrophage role we used a conditional depletion method using diphtheria toxin-receptor ablation system. Diphtheria toxin receptor-mediated ablation is a specific cell ablation system in which diphtheria toxin receptor (DTR) is transgenically expressed on specific cell targets. Administration of diphtheria toxin (DT) enables the ablation and/or depletion of the targeted cells [20]. This technique has been used effectively in previous studies [20, 21]. This system relies on the fact that the mouse DTR binds DT poorly compared with the human molecule. Here, we elucidated the roles of M2 macrophages in adipose tissue using CD206

as a marker by employing diphtheria toxin receptor-mediated ablation system.

To our surprise, the result of this study shows us that M2 macrophage is pivotal in modulating adipocyte progenitors' proliferation and differentiation into adipocyte through TGF β signaling [22] (Figure 2). We anticipate that CD206 may provide a niche for adipocyte progenitors to keep them in hibernation state.

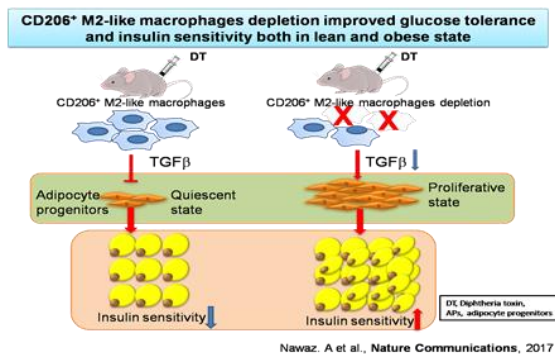


Figure 2. Depletion of CD206 M2 macrophages promote adipocyte progenitor's proliferation and induces insulin sensitivity.

The findings that systemic insulin resistance is ameliorated by M1-macrophage depletion and aggravated by adoptive transfer of M2 to M1 cells strongly suggest that CD206(+) cells are regarded as anti-inflammatory cells and has an impact on systemic metabolism [23-26]. We, however, obtained reverse results showing that depletion of CD206(+) cells resulted in improved glucose tolerance rather than worsening glucose metabolism. We demonstrated that CD206 (M2-like) macrophage may be responsible to regulate insulin resistance and metabolic disorders indicating that M2-like macrophages itself does not furnish insulin sensitivity but just a reflection of favorable environment. This finding led us to look for other homeostatic functions of ATMs than previously reported their anti-inflammatory function. We found that partial depletion of

CD206(+) cells lead to increase the number of smaller adipocytes with expression of metabolic genes characteristics of better metabolic function such as PGC-1 α . In addition, we also found an increased number of PDGFR α + adipocyte progenitors that are differentiated into adipocytes. These data suggested that CD206(+) macrophages in adipose tissue inhibit uncontrolled proliferation of adipocyte progenitors and excessive differentiation into mature adipocytes.

So far how adipocyte progenitor's proliferation and their differentiation into mature adipocytes in vivo is regulated is largely unknown. Our data demonstrated that adipose tissue M2-like macrophage is able to keep adipocyte progenitors in a hibernating state to inhibit excessive proliferation. This is somewhat similar to the previous report that non-myelinating Schwann cells (HSCs) inhibit the growth of hematopoietic stem cells in the bone marrow by activating TGF β pathways [27] in the bone marrow by activating TGF β pathways. Like an HSCs niche in the bone marrow, it may be possible that CD206(+) cells form a niche for adipocyte progenitors, thus maintaining the number of adipocyte progenitors and matured adipocyte by inhibiting the proliferation of progenitors. When the function of CD206(+) cells is abruptly compromised, the progenitor activation and differentiation into adipocytes occur. Recently, Lee and Granneman reported that β 3-agonist treatment early several days resulted in adipocyte cell death, engulfment of all debris by macrophages which express the canonical markers of alternatively activated macrophages (AAMs), and recruitment of PDGFR α (+) adipocyte progenitors [28, 29]. Previous reports have shown that signals or stimuli that activate alternative activation such as extrinsic eosinophil-IL-4/-13 pathway and intrinsic PPAR γ , stat6 pathway improve adipocyte function, thus leading to insulin sensitivity. Most of the experiments were performed under HFD conditions and

demonstrated that AAMs play a role in metabolic adaptations to excessive nutrient intake through anti-inflammatory functions. How should our results be reconciled with preciously reported data? First, most of the experiments were conducted on HFD condition, and adipose tissue resident macrophages may be required for metabolic adaptations to excessive nutrient intake. Second, almost all the previous reports utilized mice with genetic disruption of genes involved in alternative activation pathway. Those mice have some adaptation during both development and post-natal stage.

Our study demonstrated that CD206(+) cells inhibit progenitor proliferation and differentiation into adipocytes in a TGF β -dependent manner. Further research evidences are required to expand and identify the role of TGF β in controlling the progenitor proliferation. It is recently reported that systemic lack of TGF β signal resulted in an insulin-sensitive and metabolically healthy phenotype with increased browning phenomenon in inguinal WAT. However, we observed that browning of WAT was enhanced after depleting CD206(+) cells.

CONCLUSION

The results described here unfold a fresh perspective to relate the role of M2-like macrophages in insulin resistance and metabolic disorders. We identify the novel role of ATMs to inhibit progenitor proliferation and maturation into adipocytes, thus maintaining adiposity and insulin sensitivity of the whole body. This observation will shed a light on not only the mechanism of adipocyte progenitor physiology but also the role of adipose tissue-resident macrophages to regulate systemic glucose metabolism.

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