Adipose tissue inflammation and ectopic lipid accumulation

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Abstract. Obesity may be viewed as a chronic low-grade inflammatory disease as well as a metabolic disease. Indeed, unbalanced production of pro- and anti-inflammatory adipocytokines critically contributes to the obesity-induced insulin resistance. In addition to lipid-laden mature adipocytes, adipose tissue is composed of various stromal cells such as preadipocytes, endothelial cells, fibroblasts, and immune cells that may be involved in adipose tissue functions. Accumulating evidence has suggested that adipocytes and stromal cells in adipose tissue change dramatically in number and cell type during the course of obesity, which is referred to as “adipose tissue remodeling.” Among stromal cells, infiltration of macrophages in obese adipose tissue precedes the development of insulin resistance in animal models, suggesting that they are crucial for adipose tissue inflammation. We have provided evidence suggesting that a paracrine loop involving saturated fatty acids and tumor necrosis factor-α derived from adipocytes and macrophages, respectively, aggravates obesity-induced adipose tissue inflammation. On the other hand, storing excessive energy as triglyceride is also a fundamental function of adipose tissue. Recent evidence suggests that reduced lipid storage in obese adipose tissue contributes to ectopic lipid accumulation in non-adipose tissues such as the liver, skeletal muscle, and pancreas, where lipotoxicity impairs their metabolic functions. Notably, chronic inflammation is capable of inducing insulin resistance, lipolysis, and interstitial fibrosis in adipose tissue, all of which may reduce the lipid-storing function. Understanding the molecular mechanism underlying adipose tissue remodeling may lead to the identification of novel therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation.

Key words: Adipocytes, Adipose tissue inflammation, Obesity, Saturated fatty acids

ADIPOSE TISSUE is an important endocrine organ that secretes a large number of adipocytokines such as leptin, monocyte chemoattractant protein-1 (MCP-1), and adiponectin, which may be involved in a variety of physiologic and pathologic processes [1-5]. Evidence has accumulated indicating that obesity is associated with a state of chronic, low-grade inflammation, which may cause obesity-induced insulin resistance [3-6]. Indeed, unbalanced production of pro- and anti-inflammatory adipocytokines seen in visceral fat obesity critically contributes to the development of many aspects of the metabolic syndrome [1, 3-6]. There is also considerable evidence that macrophages infiltrate into obese adipose tissue to induce inflammatory pathways [7-9]. Notably, macrophage infiltration in adipose tissue precedes or associates with the development of insulin resistance and ectopic lipid accumulation in obese animals and humans [7, 8], suggesting the role of infiltrated macrophages in the pathophysiology of obesity (Fig. 1).

Storing excessive energy as triglyceride is also a fundamental function of adipose tissue. In response to nutritional conditions, lipid metabolism in adipose tissue is tightly regulated by hormones and the sympathetic nervous system. For instance, catecholamines induce lipolysis in the fasted state to provide free fatty acids as fuel to other organs. On the other hand, insulin suppresses lipolysis and facilitates lipogenesis in the fed state. Moreover, adipocytes increase their size (hypertrophy) and number (hyperplasia) during the course of
In advanced stages of obesity, there are a variety of stromal immune cells such as neutrophils, macrophages, and lymphocytes, which infiltrate into obese adipose tissue. For instance, proinflammatory M1 macrophages are recruited, at least partly, via the MCP-1-CCR2 pathway to enhance the inflammatory changes through the crosstalk with mature adipocytes. Thus, adipose tissue inflammation results in dysregulation of adipocytokine production and reduction of adipose tissue lipid-storing capacity, thereby inducing insulin resistance in multiple remote organs and tissues. CCR2, C-C chemokine receptor 2; MCP-1, monocyte chemoattractant protein-1; Mincle, macrophage-inducible C-type lectin; NASH, non-alcoholic steatohepatitis.
and immune cells [6]. Thus, it is important to know the molecular mechanisms underlying adipose tissue remodeling during the course of obesity.

**Heterogeneity of adipose tissue macrophages**

Recent studies have pointed to the heterogeneity of adipose tissue macrophages in obesity; i.e. M1 or “classically activated” (proinflammatory) macrophages and M2 or “alternatively activated” (anti-inflammatory) macrophages [19, 20]. Histological analysis reveals that M1 macrophages are localized to surround dead adipocytes in obese adipose tissue (crown-like structures), where macrophages are considered to scavenge the residual lipid droplet of dead adipocytes and ultimately form multinucleate giant cells, a hallmark of chronic inflammation [21]. On the other hand, M2 macrophages are scattered in interstitial spaces between adipocytes [20]. It is known that M1 macrophages are induced by proinflammatory mediators such as lipopolysaccharide (LPS) and Th1 cytokine interferon-γ (IFN-γ), whereas M2 macrophages are polarized by the stimulation with Th2 cytokines such as interleukin-4 (IL-4) and IL-13 [19, 22]. Recent studies have also shown that epigenetic changes and noncoding RNAs are involved in macrophage polarization [23, 24]. For instance, Jumonji domain containing-3 (Jmjd3) is essential for M2 activation through demethylation of the promoter region of interferon-regulatory factor 4 (IRF4) under infective conditions [23], and miR-155 diminishes the IL-13-induced M2 polarization through reducing IL-13 receptor protein levels [24]. However, the molecular mechanisms underlying macrophage polarization in adipose tissue still remain to be elucidated.

There is substantial evidence that adipose tissue macrophages exhibit the phenotypic change from M2 to M1 polarization during the course of obesity, thereby accelerating adipose tissue inflammation [19, 20]. Indeed, deficiency of Toll-like receptor 4 (TLR4) or TLR4 signaling protects against obesity-induced M1 macrophage polarization and adipose tissue inflammation in vivo [25-28]. On the other hand, peroxisome proliferator-activated receptor γ (PPARγ) and PPARδ stimulate M2 polarization of adipose tissue macrophages and thus improve systemic insulin sensitivity [29-31]. Indeed, activation of PPARγ by pioglitazone, a thiazolidinedione class of insulin sensitizer, improves the unbalanced M1/M2 phenotype of adipose tissue macrophages in diet-induced obese mice [32]. Interestingly, a recent study suggests that antidiabetic effects of thiazolidinediones may be mediated, at least partly, through PPARγ in macrophages [33]. Moreover, both M1 and M2 markers are detected in circulating monocytes [34, 35]. Prior to macrophage infiltration at the site of chronic inflammation, monocytes in obese and/or obese type 2 diabetic patients exhibit higher expression of M1 markers and lower expression of M2 markers than those in normal-weight controls, which is associated with arterial stiffness [34]. Interestingly, pioglitazone treatment significantly improves arterial stiffness along with the unbalanced M1/M2 phenotype of monocytes [34, 35]. Collectively, phenotypic modulation of adipose tissue macrophages may offer a novel therapeutic strategy to prevent or treat the progression of obesity-induced complications such as diabetes and atherosclerosis.

**Paracrine regulation of adipose tissue inflammation**

Adipose tissue macrophages represent a major source of proinflammatory cytokines, which contribute to chronic inflammatory responses during the course of obesity [4, 5]. Using an in vitro co-culture system composed of adipocytes and macrophages, we have demonstrated that a paracrine loop involving saturated fatty acids and TNFα derived from adipocytes and macrophages, respectively, establishes a vicious cycle that augments chronic inflammatory changes [36] (Fig. 1). Because dysregulation of adipocytokine production in the co-culture system in vitro is roughly parallel to that in obese adipose tissue in vivo, there may be an intimate crosstalk between adipocytes and macrophages underlying chronic inflammation in obese adipose tissue. Indeed, macrophage-derived TNFα acts on TNF receptor in hypertrophied adipocytes, thereby inducing proinflammatory cytokine production and adipocyte lipolysis via nuclear factor-κB (NF-κB)-dependent and -independent (possibly MAPK-dependent) mechanisms, respectively [36]. On the other hand, saturated fatty acids thus released from adipocytes, activate TLR4 signaling in macrophages [37, 38]. Since TLR4 is expressed in macrophages more abundantly than in adipocytes, chronic inflammatory responses induced by the interaction between adipocytes and macrophages may be largely mediated via TLR4 in macrophages. This discussion is supported by a recent report by Saberi et al. showing that hematopoietic cell-specific deletion
of TLR4 ameliorates high-fat diet-induced hepatic and adipose tissue insulin resistance [39]. It is, therefore, conceivable that adipose tissue macrophages activated by adipocyte-derived saturated fatty acids may be a unique therapeutic target to prevent or treat obesity-induced adipose tissue inflammation.

In obesity, proinflammatory cytokines such as TNFα and MCP-1 are overproduced and anti-inflammatory cytokines such as adiponectin are decreased. Such dysregulation of adipocytokine production, which is induced, at least partly, by adipose tissue inflammation, may play a critical role in the pathophysiology of the metabolic syndrome and atherosclerosis [3-6]. For instance, deficiency of TNFα, which is mostly derived from macrophages in adipose tissue, protects against obesity-induced insulin resistance [40]. MCP-1 is derived from both adipocytes and macrophages in adipose tissue [7, 8]. MCP-1 and its cognate receptor C-C chemokine receptor 2 (CCR2) play a critical role in the recruitment of macrophages into obese adipose tissue, thereby aggravating adipose tissue inflammation [41-44]. It is also known that MCP-1 directly induces insulin resistance in the skeletal muscle and liver, suggesting a role of MCP-1 as an endocrine hormone [41, 45]. On the other hand, adiponectin, which is exclusively expressed in adipocytes, is markedly down-regulated in obese adipose tissue [46, 47], and supplementation of adiponectin in obese mice effectively reverses insulin resistance in the liver and skeletal muscle [46, 47]. Thus, the paracrine loop by adipocytes and macrophages may be a molecular mechanism underlying obesity-induced dysregulation of adipocytokine production, thereby contributing to the pathogenesis of metabolic derangements of multiple organs.

**Novel regulators of adipose tissue inflammation**

Through a combination of cDNA microarray analysis of saturated fatty acid-stimulated macrophages in vitro and obese adipose tissue in vivo, we have recently screened for novel regulators of adipose tissue inflammation. Activating transcription factor 3 (ATF3), a member of the ATF/cAMP response element-binding protein family of basic leucine zipper-type transcription factors, is induced by the saturated fatty acid-TLR4 pathway in macrophages and markedly upregulated in obese adipose tissue [48]. Although transgenic overexpression of ATF3 in macrophages does not affect the number of infiltrated macrophages in obese adipose tissue, M1 macrophage polarization is significantly inhibited in ATF3 transgenic mice relative to wild-type mice [48]. These findings indicate that ATF3 acts as a transcriptional repressor of the saturated fatty acid-TLR4 pathway in macrophages. Our data also suggest that ATF3 constitutes a negative feedback mechanism that attenuates obesity-induced macrophage activation in adipose tissue. On the other hand, we found that macrophage-inducible C-type lectin (Mincle, Clec4e, or Clecsf9), a pathogen sensor for pathogenic fungi and Mycobacterium tuberculosis [49, 50], is induced in adipose tissue macrophages in obesity, at least partly, through the saturated fatty acid-TLR4 pathway [51]. Yamasaki et al. reported that Mincle senses cell death as well to induce proinflammatory cytokine production [52]. Since dead adipocytes are surrounded by macrophages in the adipose tissue of obese humans and mice (crown-like structures) [15, 21], it is conceivable that Mincle plays a role in sensing adipocyte-derived endogenous ligand(s) during adipocyte death to induce inflammatory changes in obesity.

In addition, accumulating evidence has suggested the involvement of nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome in the pathogenesis of obesity [53]. The NLRP3 inflammasome is a cytosolic protein complex consisting of the regulatory subunit NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC), and the effector subunit caspase-1. It is activated by pathogen-derived components and endogenous damage-associated molecular patterns (DAMPs) or danger signal released from necrotic cells and damaged tissues [53, 54]. NLRP3 deficient mice are protected from obesity-induced adipose tissue inflammation and insulin resistance [55, 56]. In atherogenesis, it is reported that crystalline cholesterol acts as an endogenous danger signal and its deposition in arteries or elsewhere is an early cause rather than a late consequence of inflammation [57].

**Role of other immune cells in adipose tissue inflammation**

In addition to macrophages, there are a variety of immune cells such as neutrophils, eosinophils, lymphocytes, natural killer cells, and mast cells in adipose tissue [11, 58, 59] (Fig. 1). Similar to the sequence of events...
that comprises acute inflammation, neutrophils are transiently infiltrated into adipose tissue, followed by macrophage infiltration in a mouse model of diet-induced obesity [60]. Recent evidence has also revealed a large number of T cells in adipose tissue from both lean and obese mice [61-64]. Among various T cell subsets, the population of CD8+ T cells is significantly increased in the early stages of obesity, followed by macrophage infiltration [61]. CD8+ T cells as well as M1 macrophages localize to crown-like structures in obese adipose tissue. Moreover, depletion of CD8+ T cells results in improvement of obesity-induced adipose tissue inflammation and insulin resistance [61]. In contrast, the population of CD4+ T cells and regulatory T cells is decreased in the advanced stage of obesity [61-63]. These observations suggest that obesity-induced imbalance of T cell subpopulation may contribute to the progression of adipose tissue inflammation. Recently, Moro et al. have reported a new type of lymphocytes, “natural helper cells” in a novel lymphoid structure associated with adipose tissues in the peritoneal cavity, where they may produce large amounts of Th2 cytokines [65]. In addition, Winer et al. reported that B cells may be involved in obesity-induced insulin resistance [66]. It would be, therefore, interesting to elucidate the physiologic and pathophysiologic role of various immune cells and the molecular mechanisms of the intimate crosstalk between the cell types in adipose tissue.

Adipose tissue fibrosis and ectopic lipid accumulation

In addition to the function as an endocrine organ, adipose tissue functions as an energy reservoir that stores excessive fatty acids in the form of triglyceride, which is facilitated by insulin. Adipose tissue inflammation induces insulin resistance in adipose tissue similarly to the liver and skeletal muscle [11]. Moreover, as we discussed above, crosstalk between adipocytes and macrophages in obese adipose tissue may increase lipolysis via overproduction of proinflammatory cytokines such as TNFα [11, 36]. Besides these functional alterations, histological changes are also related to the reduction of lipid-storing function in adipose tissue during the course of obesity [10]. Recent studies have shown overproduction of extracellular matrix components in adipose tissue from obese animals and humans [67-69]. It is also reported that adipose tissue fibrosis is negatively correlated with adipocyte diameters in human adipose tissue [70], suggesting that increased interstitial fibrosis may limit adipose tissue expandability. In this regard, Khan et al. reported that mice lacking collagen VI, which is expressed abundantly in adipose tissue, exhibit the uninhibited adipose tissue expansion and substantial improvements in insulin sensitivity on a high-fat diet [67]. These findings suggest that reduced lipid storage in obese adipose tissue may contribute to ectopic lipid accumulation in non-adipose tissues such as the liver, skeletal muscle, and pancreas, where lipotoxicity impairs their metabolic functions [10, 71-73]. Although the molecular mechanisms underlying adipose tissue fibrosis are still largely unknown, recent evidence suggests that a PPARγ-fibroblast growth factor 1 (FGF1) axis is required for obesity-induced adipose tissue remodeling [74]. Collectively, obesity-induced metabolic problems may become overt, in addition to the dysregulation of adipocytokine production, when adipose tissue cannot fully meet demands for additional lipid storage.

Non-alcoholic steatohepatitis (NASH) is considered a hepatic phenotype of the metabolic syndrome and at high risk for the progression to cirrhosis and hepatocellular carcinoma. Although the “two-hit” hypothesis points to the involvement of excessive hepatic lipid accumulation and chronic inflammation [75, 76], the molecular mechanisms of NASH are still unclear. The melanocortin-4 receptor (MC4R) is a seven-transmembrane G protein-coupled receptor that is expressed in the hypothalamic nuclei implicated in the regulation of food intake and body weight [77]. Recently, we have reported that MC4R deficient (MC4R-KO) mice develop a liver condition similar to human NASH during a high-fat diet, which is associated with obesity, insulin resistance, and dyslipidemia [78]. Moreover, they develop well-differentiated hepatocellular carcinoma, when fed a high-fat diet for a longer time. These findings suggest that MC4R-KO mice would provide a novel rodent model of NASH with which to investigate the sequence of events that comprise diet-induced hepatic steatosis, NASH, and hepatocellular carcinoma (Fig. 2). Notably, MC4R-KO mice show accelerated adipose tissue inflammation, characterized by massive macrophage infiltration, overproduction of proinflammatory cytokines, and intensive interstitial fibrosis. It is, therefore, conceivable that MC4R-KO mice would be a unique model to investigate the role of the inter-organ network in obesity-induced ectopic lipid accumulation.
adipose tissue remodeling is regulated, several lines of evidence suggest that adipocyte death, hypoxia, and chronic inflammation may be attributed to overproduction of extracellular matrix in adipose tissue during the course of obesity [10]. Better understanding the molecular mechanisms underlying adipose tissue remodeling may lead to novel therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation and insulin resistance.

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Concluding Remarks

Obesity may be viewed as a chronic low-grade inflammatory disease as well as a metabolic disease. During the course of obesity, sustained interaction between adipocytes and stromal cells including macrophages induces chronic inflammatory changes in adipose tissue, which may spread to remote organs and tissues such as the liver, skeletal muscle, and pancreas to cause insulin resistance. In this regard, secreted factors such as adipocytokines and free fatty acids are supposed to play an important role in the inter-organ network. Due to the recent progress made in adipocytokine research, it is widely recognized that adipose tissue macrophages give rise to the obesity-induced dysregulation of adipocytokine production. Adipose tissue macrophages may also participate in adipose tissue fibrosis, thereby regulating lipid-storing capacity of adipose tissue. Although it is currently unclear how adipose tissue remodeling is regulated, several lines of evidence suggest that adipocyte death, hypoxia, and chronic inflammation may be attributed to overproduction of extracellular matrix in adipose tissue during the course of obesity [10]. Better understanding the molecular mechanisms underlying adipose tissue remodeling may lead to novel therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation and insulin resistance.

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