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Review

Adipose tissue macrophages: their role in adipose tissue remodeling

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ABSTRACT

The adipose tissue secretes a large number of bioactive substances, adipocytokines, which may be involved in a variety of physiologic and pathologic processes. Unbalanced production of pro- and anti-inflammatory adipocytokines seen in visceral fat obesity contributes critically to the development of the metabolic syndrome. Evidence has accumulated indicating that obesity is associated with a state of chronic, low-grade inflammation, suggesting that inflammation may be a potential mechanism, whereby obesity leads to insulin resistance. Indeed, obese adipose tissue is characterized by adipocyte hypertrophy, followed by increased angiogenesis, immune cell infiltration, extracellular matrix overproduction, and thus, increased production of proinflammatory adipocytokines during the progression of chronic inflammation. The dynamic change found in the adipose tissue can be referred to as "adipose tissue remodeling," in which stromal cells change dramatically in number and cell type during the course of obesity. Among stromal cells, infiltration of macrophages in the adipose tissue precedes the development of insulin resistance in animal models, suggesting that they are crucial for obesity-related adipose tissue inflammation. We have demonstrated that a paracrine loop involving saturated fatty acids and TNF-α derived from adipocytes and macrophages, respectively, aggravates obesity-induced adipose tissue inflammation. Notably, saturated fatty acids, which are released from hypertrophied adipocytes via the macrophage-induced lipolysis, serve as a naturally occurring ligand for TLR4 complex, thereby activating macrophages. 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. *J. Leukoc. Biol.* 88: 33–39; 2010.

Abbreviations: ATF3=activating transcription factor 3, CLS=crown-like structure, DAMP=damage-associated molecular pattern, EPA=eicosapentaenoic acid, ER=endoplasmic reticulum, HMGB1=high-mobility group box-1, MKP-1=MAPK phosphatase-1, PAMP=pathogen-associated molecular pattern , PPARγ/δ=peroxisome proliferator-activated receptor γ/δ, PRR=pattern-recognition receptor, S100A8/A9=S100 calcium-binding protein A8/A9, SVF=stromal vascular fraction

Introduction

The metabolic syndrome is a constellation of visceral fat obesity, impaired glucose metabolism, atherogenic dyslipidemia, and blood pressure elevation, which all increase independently the risk of atherosclerotic diseases, such as ischemic heart disease and cerebral stroke [1–5]. The molecular basis for the clustering of such independent risks of atherosclerosis has not been fully elucidated, and visceral fat obesity is considered most important [1–5]. Evidence has accumulated indicating that obesity is associated with a state of chronic, low-grade inflammation, suggesting that inflammation may be a potential mechanism, whereby obesity leads to insulin resistance [1–4].

Adipose tissue secretes a large number of adipocytokines such as leptin, MCP-1, and adiponectin, which may be involved in a variety of physiologic and pathologic processes [1–3, 5, 6]. 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 [1–5]. There is considerable evidence that obese adipose tissue is markedly infiltrated by macrophages; they may participate in the inflammatory pathways that are activated in the adipose tissue [7–9]. Notably, macrophage infiltration and inflammation-related gene expression in the adipose tissue precede the development of insulin resistance in animal models [7, 8], suggesting that infiltrated macrophages are an important source of inflammation in the adipose tissue. This review summarizes the role of macrophages in adipose tissue inflammation.

ADIPOSE TISSUE REMODELING

In addition to lipid-laden, mature adipocytes, the adipose tissue is composed of various cell types; the remaining SVF includes preadipocytes, endothelial cells, fibroblasts, and immune cells [10]. In contrast to "acute inflammation," which resolves by an active termination program [11], "chronic inflammation" is characterized by sustained interaction between

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parenchymal and stromal cells in response to tissue stress or malfunction, thereby leading to functional maladaptation and tissue remodeling [12]. Recent studies have demonstrated that obese adipose tissue is characterized by adipocyte hypertrophy, followed by increased angiogenesis, immune cell infiltration, extracellular matrix overproduction, and thus, increased production of proinflammatory adipocytokines during the progression of chronic inflammation (**Fig. 1**) [1, 2, 13, 14]. This is reminiscent of the chronic inflammatory responses in atherosclerotic vascular walls, termed vascular remodeling, which is mediated through complex interactions among vascular endothelial cells, vascular smooth muscle cells, lymphocytes, and monocyte-derived macrophages (Fig. 1) [4]. Thus, the dynamic change seen in obese adipose tissue can be referred to as adipose tissue remodeling, in which stromal cells change dramatically in number and cell type during the course of obesity (Fig. 1). Given the multifunctional roles in a variety of biological contexts, among stromal cells, macrophages should play a central role in adipose tissue remodeling. In this regard, adipose tissue remodeling may be viewed as chronic inflammation that involves adipocyte hypertrophy, macrophage infiltration, and adipocyte-macrophage interaction (**Fig. 2**).

ADIPOSE TISSUE MACROPHAGE INFILTRATION

A previous study with bone marrow transplantation demonstrated that most macrophages in the adipose tissue are derived from the bone marrow [7]. In this regard, increased expression of chemokines in obese adipose tissue has been implicated in the control of monocyte recruitment to the adipose tissue. There is considerable evidence for the pathophysiologic role of the MCP-1/CCR2 pathway in macrophage infiltration into obese adipose tissue {Fig. 2, (ii) [15–18]}. Weisberg et al. [15] reported the attenuation of macrophage accumulation and chronic inflammation in the adipose tissue from mice lacking CCR2 ($CCR2^{-/-}$ mice) during a high-fat diet. Moreover, two previous studies with transgenic mice overexpressing MCP-1 in the adipose tissue and MCP-1-deficient mice ($MCP\text{-}1^{-/-}$ mice) showed that MCP-1 plays a role in the recruitment of macrophages into obese adipose tissue [16, 17]. Through a combination of a real-time horizontal chemotaxis assay in vitro and bone marrow transplantation techniques in vivo, we have also demonstrated that CCR2 expressed in bone marrow cells is involved in macrophage infiltration into obese adipose tissue [18]. In addition to the MCP-1/CCR2 pathway, there are several reports suggesting the potential involvement of other chemotactic factors in obesity-induced macrophage infiltration [19, 20]. For instance, recent evidence suggested the role of osteopontin, angiopoietin-like protein 2, and CXCL14 [19–21]. Importantly, inhibition of macrophage infiltration into obese adipose tissue through genetic and/or pharmacologic strategies improved the dysregulation of adipocytokine production, thereby leading to the amelioration of obesity-induced adipose tissue inflammation and insulin resistance. Understanding the molecular mechanisms underlying increased macrophage infiltration into obese adipose tissue may lead to the identification of novel, adipocyte-derived chemokine(s) and even therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation.

ADIPOCYTE HYPERTROPHY AND INFLAMMATORY CHANGES

To understand how macrophages are recruited into obese adipose tissue, it is important to know the molecular mechanism underlying increased production of chemokines in the early stages of obesity. Recent studies have demonstrated that multiple intracellular signaling pathways are activated in adipocytes during the course of adipocyte hypertrophy in vitro and in obese adipose tissue in vivo {Fig. 2, (i) [1–3]}. For instance, MAPKs, such as ERK, p38 MAPK, and JNK, are activated in a variety of cellular processes including adipocyte differentiation and hypertrophy [22–24]. Once activated by the upstream ki-

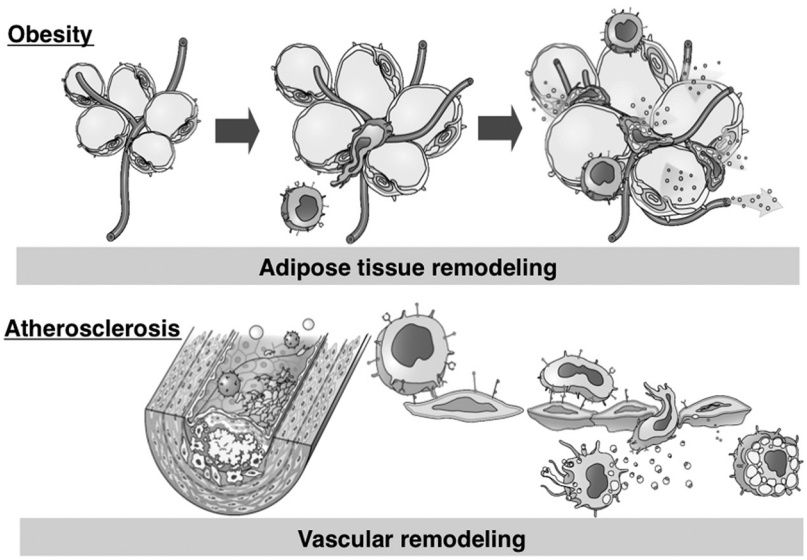

Figure 1. Adipose tissue remodeling. Obesity-induced adipose tissue inflammation is characterized by adipocyte hypertrophy, followed by increases in angiogenesis, immune cell infiltration, extracellular matrix overproduction, and thus, increased production of proinflammatory adipocytokines, which can be referred to as "adipose tissue remodeling." This is similar to chronic inflammatory changes and tissue remodeling in atherosclerotic vascular walls termed "vascular remodeling," which is mediated through complex interactions among vascular endothelial cells, vascular smooth muscle cells, lymphocytes, and monocyte-derived macrophages.

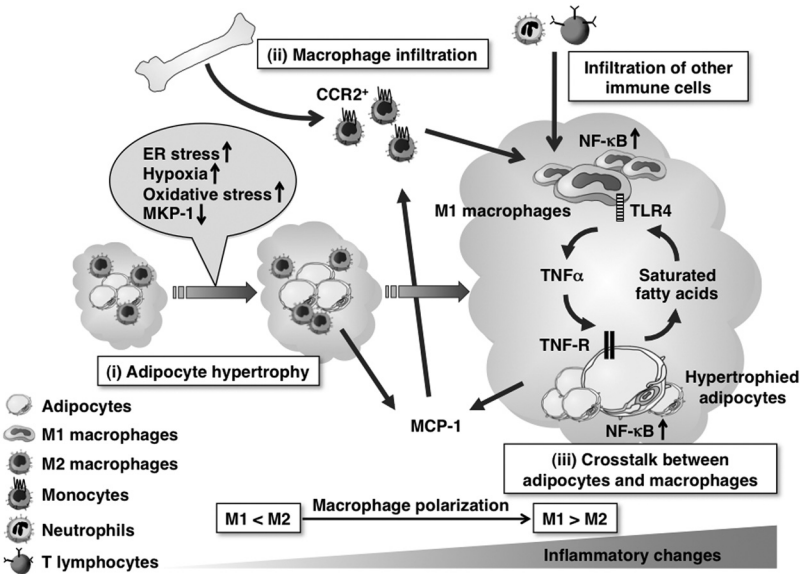

Figure 2. Molecular mechanism underlying adipose tissue inflammation. In the early stages of obesity, adipocytes should be hypertrophied in response to overnutrition (i). Recent evidence suggests that increased metabolic stresses such as ER stress, hypoxia, and oxidative stress and down-regulation of MKP-1 are involved in the induction of inflammatory changes in adipocytes during the course of adipocyte hypertrophy. In the advanced stages of obesity, there are various kinds of stromal immune cells such as neutrophils, T lymphocytes, and macrophages, which infiltrate into obese adipose tissue (ii) and thus, enhance the inflammatory changes through the crosstalk with parenchymal adipocytes (iii). For example, the macrophage-derived TNF-α induces the release of saturated fatty acids from adipocytes via lipolysis, which in turn, induces inflammatory changes in macrophages via TLR4. Such a paracrine loop between adipocytes and macrophages constitutes a vicious cycle, thereby accelerating further adipose tissue inflammation. Recent evidence has also pointed to the heterogeneity of adipose tissue macrophages; i.e., M1 or "classically activated" (proinflammatory) macrophages and M2 or "alternatively activated" (anti-inflammatory) macrophages. Infiltrated macrophages exhibit a phenotypic change from M2 to M1 polarization in obese adipose tissue, thereby accelerating adipose tissue inflammation. TNF-R, TNF-α receptor.

nases, e.g., MEK, MAPKs are inactivated rapidly by a family of protein phosphatases such as MKP-1, an inducible dual-specificity phosphatase [25, 26]. We have demonstrated that down-regulation of MKP-1 is critical for increased production of MCP-1 during the course of adipocyte hypertrophy [27]. On the other hand, Ozcan et al. [28] reported that obesity is associated with the induction of ER stress, predominantly in the adipose tissue and liver, and suggested that ER stress plays a critical role in obesity-induced adipose tissue inflammation. In this regard, Hosogai et al. [29] reported hypoxia-induced ER stress in obese adipose tissue, which is involved in the dysregulation of adipocytokine production. Moreover, Furukawa et al. [30] also showed that reactive oxygen species production is increased in parallel with adipocyte hypertrophy and that oxidative stress induces the dysregulation of adipocytokine production. It is interesting to investigate how such multiple intracellular signaling pathways are integrated during the course of adipocyte hypertrophy and/or in the early stages of obesity.

PARACRINE LOOP BETWEEN ADIPOCTYES AND MACROPHAGES

Once infiltrated into the adipose tissue in the advanced stages of obesity, macrophages participate in the inflammatory pathways that are activated in obese adipose tissue [1, 2]. Using an in vitro coculture system composed of adipocytes and macrophages, we have demonstrated that a paracrine loop involving saturated fatty acids and TNF-α derived from adipocytes and macrophages, respectively, establishes a vicious cycle that augments the inflammatory changes; i.e., marked up-regulation of proinflammatory adipocytokines, such as MCP-1 and TNF-α, and significant down-regulation of anti-inflammatory adiponectin {Fig. 2, (iii) [31]}. As the coculture-induced dysregulation of adipocytokine production is roughly parallel to that in obese adipose tissue in vivo, there may be an intimate crosstalk between adipocytes and macrophages as a potential mechanism that aggravates chronic inflammation in obese adipose tissue. Indeed, TNF-α, which is derived mostly from infiltrated macrophages in obese adipose tissue, acts on TNF-α receptor in hypertrophied adipocytes, thereby inducing proinflammatory cytokine production and adipocyte lipolysis via NF-κB-dependent and -independent (possibly MAPK-dependent) mechanisms, respectively [31]. On the other hand, saturated fatty acids thus released serve as a naturally occurring ligand for the TLR4 complex, which is essential for the recognition of LPS to induce NF-κB activation in macrophages [32, 33].

Evidence has accumulated, suggesting that TLR4 plays an important role in obesity-induced adipose tissue inflammation and systemic glucose and lipid metabolism in vivo [34–37]. As TLR4 is expressed in macrophages more abundantly than in adipocytes, it is likely that chronic inflammatory responses induced by the interaction between adipocytes and macrophages are largely mediated via TLR4 in macrophages. This discussion is supported by a recent report by Saberi et al. [38] showing that hematopoietic cell-specific deletion of TLR4 ameliorates high-fat, diet-induced hepatic and adipose tissue insulin resistance. It is, therefore, likely that inhibition of macrophages activated by adipocyte-derived saturated fatty acids may offer a unique, therapeutic strategy to prevent obesity-induced adipose tissue inflammation. Given the antagonistic relationship between saturated and *n-3* polyunsaturated fatty acids such as EPA [39], we have provided evidence that highly purified EPA increases the otherwise reduced secretion of anti-inflammatory adiponectin in obese adipose tissue, at least partly by interrupting the vicious cycle created by adipocytes and macrophages [40].

The dysregulation of adipocytokine production, which is induced by adipose tissue inflammation, may play a critical

role in the pathophysiology of the metabolic syndrome and atherosclerosis [1–4]. For instance, TNF-α, which is derived mostly from macrophages, is increased in obese adipose tissue [7, 8], and TNF-α-deficient mice are protected from obesity-induced insulin resistance [41]. By contrast, adiponectin, which is expressed exclusively in adipocytes, is markedly downregulated in obese adipose tissue [42, 43], and supplementation of adiponectin in obese mice effectively reverses insulin resistance in the skeletal muscle and liver [42, 43]. On the other hand, MCP-1 is derived from adipocytes and macrophages in obese adipose tissue [7, 8]. Overproduction of MCP-1 in obese adipose tissue induces macrophage infiltration into the adipose tissue, thereby aggravating adipose tissue inflammation [16, 17]. It also induces insulin resistance directly in the skeletal muscle and liver, suggesting a role as an endocrine hormone [17, 44]. Finally, adipocyte-derived leptin acts directly on the hypothalamus, where it regulates food intake and energy expenditure [45, 46]. Several previous reports demonstrated that vascular remodeling and tissue fibrosis are markedly attenuated in leptin-deficient *ob/ob* mice or leptin signaling-deficient *db/db* mice [47–49]. However, the role of leptin in adipose tissue inflammation still remains to be elucidated.

PHENOTYPIC CHANGE OF ADIPOSE TISSUE MACROPHAGES

Recent studies have pointed to the heterogeneity of macrophages infiltrated into obese adipose tissue; i.e., they follow at least two different polarization states: M1 or classically activated (proinflammatory) macrophages, which are induced by proinflammatory mediators such as LPS and Th1 cytokine IFN-γ, and M2 or alternatively activated (anti-inflammatory) macrophages, which are generated in vitro by exposure to Th2 cytokines such as IL-4 and IL-13 [50, 51]. Evidence has accumulated indicating that macrophages exhibit the phenotypic change from M2 to M1 polarization in obese adipose tissue, thereby accelerating adipose tissue inflammation (Fig. 2) [50–54]. Like LPS, saturated fatty acids, as an endogenous ligand for the TLR4 complex, may contribute to the polarization of infiltrated macrophages toward M1 during the interaction between adipocytes and macrophages.

Through a combination of cDNA microarray analysis of saturated fatty acid-stimulated macrophages in vitro and obese adipose tissue in vivo, we have identified recently ATF3, a member of the ATF/CREB family of basic leucine zipper-type transcription factors, as a target gene of saturated fatty acids/TLR4 signaling in macrophages in obese adipose tissue [55]. Transgenic overexpression of ATF3 in macrophages does not affect adipocyte hypertrophy and macrophage infiltration in obese adipose tissue in vivo [55]. Interestingly, mRNA expression of M1 macrophage markers such as CD11c and TNF-α in macrophage-specific ATF3 transgenic mice is reduced significantly relative to wild-type mice, although there is no significant difference in mRNA expression of M2 macrophage markers (mannose receptor and arginase 1) between the genotypes [55]. These findings, taken together, suggest that ATF3 acts as a transcriptional repressor of saturated fatty acids/TLR4 signaling in macrophages, thereby representing a negative-feedback mechanism that attenuates obesity-induced macrophage activation in obese adipose tissue.

Prior to macrophage infiltration at the site of chronic inflammation, M1 and M2 markers are detected in circulating peripheral blood monocytes [56, 57]. Indeed, monocytes in obese and/or obese type 2 diabetic patients show significantly higher expression of M1 markers and lower expression of M2 markers relative to normal-weight controls [56]. The unbalanced M1/M2 phenotype of peripheral blood monocytes is associated with impairment of several metabolic parameters and arterial stiffness [56]. Interestingly, activation of the nuclear receptor, PPARγ by pioglitazone, a thiazolidinedione class of insulin sensitizer, improves the unbalanced M1/M2 phenotype of monocytes [56, 57], which may contribute to its antidiabetic and antiatherogenic effect. The above discussion is consistent with recent observations that PPARγ and PPARδ can stimulate M2 polarization of adipose tissue macrophages and thus, systemic insulin sensitivity [52–54, 58]. On the other hand, pioglitazone treatment improves the unbalanced M1/M2 phenotype of adipose tissue macrophages in diet-induced obese mice [59]. Moreover, a recent study suggests that macrophage PPARγ is required for full antidiabetic effects of thiazolidinediones [60]. Collectively, phenotypic modulation of adipose tissue macrophages may offer a novel, therapeutic strategy to treat or prevent the progression of obesity-induced complications such as diabetes and atherosclerosis.

OTHER IMMUNE CELLS

In addition to macrophages, other immune cells, such as neutrophils and NK cells, are increased in the adipose tissue during the course of obesity (Fig. 2) [61, 62]. Similar to the sequence of events that comprises acute inflammation, a transient increase in neutrophil infiltration precedes macrophage infiltration in a mouse model of diet-induced obesity [62], suggesting the role of neutrophils in the initiation of the inflammatory cascade. Recent evidence has also revealed a large number of T lymphocytes in the adipose tissue from lean and obese mice [63–66]. For instance, the population of $CD8^+$ T cells in the SVF is increased significantly early in the onset of obesity and continues to increase thereafter [63]. Of note, the increase in $CD8^+$ T cells precedes the accumulation of adipose tissue macrophages [63], suggesting the role of $CD8^+$ T cells in the initiation of adipose tissue inflammation. By contrast, the population of $CD4^+$ T cells and regulatory T cells is decreased in the advanced stages of obesity [63–65]. Such imbalance of the T cell subpopulation may play a role in the progression of obesity-induced adipose tissue inflammation. On the other hand, Moro et al. [67] have reported recently a new type of lymphocytes, "natural helper cells" in a novel lymphoid structure associated with adipose tissues in the peritoneal cavity. They also showed that the novel, innate lymphocytes are capable of producing large amounts of Th2 cytokines [67]. It would be interesting to elucidate the physiologic and pathophysiologic role of natural helper cells in visceral fat obesity.

ADIPOSE TISSUE INFLAMMATION AS "HOMEOSTATIC INFLAMMATION"

In addition to exogenous pathogens such as bacteria and viruses, the immune system is capable of sensing endogenous ligands released from damaged and stressed cells and tissues, thereby inducing sterile inflammation (**Fig. 3**) [12, 68, 69]. The endogenous stress signals, which are called DAMPs or "danger signals," include HMGB1, S100A8, and S100A9, modified low-density lipoproteins, and degradation products of extracellular matrices [12, 68, 69]. The danger signals, which are derived from parenchymal cells, are recognized by immune cells such as macrophages through pathogen sensors or PRRs such as TLRs, nucleotide-binding oligomerization domain-like receptors, retinoid-inducible gene-like receptors, scavenger receptors, and C-type lectin receptors [12, 68, 69].

A previous study showed that macrophages in obese adipose tissue are localized to dead adipocytes, where they fuse to scavenge the residual lipid droplet and ultimately, form multinucleate giant cells, a hallmark of chronic inflammation [70]. Indeed, macrophages aggregate to constitute a CLS surrounding dead adipocytes in advanced obesity [13, 14, 70]. Electron microscopic analysis also revealed lipid-laden phagolysosomes in macrophages within CLS [70]. Given that TNF-α induces proapoptotic and/or death signals in a variety of cell types, it is therefore interesting to speculate that hypertrophied adipocytes, which are stimulated and thus, dying by macrophage-derived TNF-α, can release saturated fatty acids as an endogenous danger signal that report their diseased state to macrophages in obese adipose tissue. Indeed, several lines of evidence indicate that adipocyte death and/or the death receptor Fas signaling contribute to obesity-induced adipose tissue inflammation and systemic insulin resistance [71, 72]. On the other hand, free fatty acids are an important energy source mobilized from triglycerides stored in the adipose tissue, particularly during periods of starvation, but recent evidence has suggested the pathophysiologic roles other than the supply of nutrients in times of fasting or increased energy demand. In this regard, free fatty acids, when released physiologically during fasting or starvation via adipocyte lipolysis, may not act as a danger signal. Similar to the relationship between commensal bacteria and pathogen sensors in epithelial cell homeostasis within the intestinal mucosa, activation of the TLR4 complex by saturated fatty acids may be involved in the regulation of metabolic homeostasis within the adipose tissue (Fig. 3). Sustained interaction between endogenous ligands, which are derived from parenchymal cells and pathogen sensors, expressed in stromal immune cells, should lead to chronic/homeostatic inflammatory responses ranging from the basal homeostatic state to diseased tissue remodeling, which may be referred to as homeostatic inflammation (Fig. 3). Dysregulation of this process can result in a variety of chronic inflammatory diseases, such as obesity, diabetes mellitus, atherosclerosis, malignant cancers, autoimmune diseases, and even neurodegenerative diseases. Collectively, adipose tissue inflammation may represent a prototypic example of homeostatic inflammation.

CONCLUDING REMARKS

The adipose tissue communicates with multiple organs or tissues by virtue of a large number of adipocytokines and thus, influences a variety of physiologic and pathophysiologic processes. Obesity may be viewed as a chronic, low-grade inflammatory as well as a metabolic disease; chronic inflammation within the adipose tissue or adipose tissue remodeling results in the dysregulation of adipocytokine production, thereby contributing to the pathophysiology of the metabolic syndrome. Among stromal cells, macrophages should play a critical role in obesity-related adipose tissue inflammation. During the paracrine interaction between adipocytes and macrophages, saturated fatty acids, which are released from hypertrophied

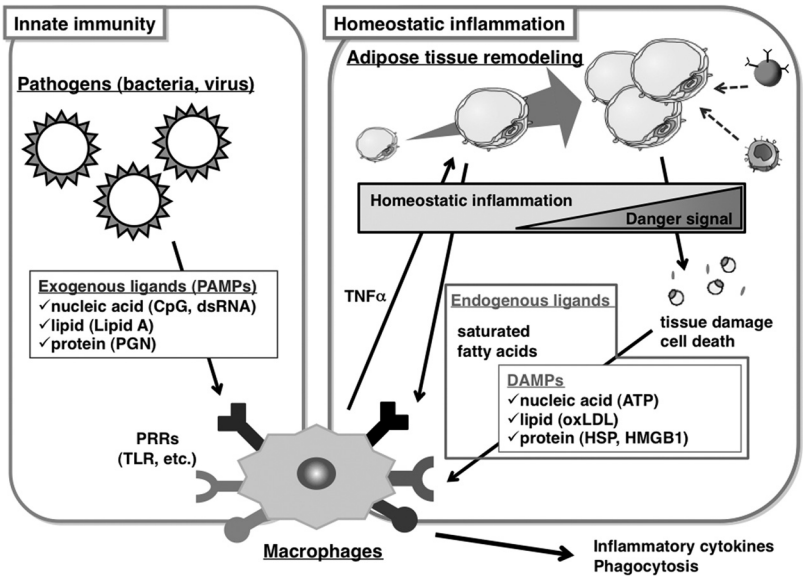

Figure 3. Adipose tissue inflammation as homeostatic inflammation. In "innate immunity," exogenous ligands (PAMPs) are sensed by PRRs, thereby inducing inflammatory changes. On the other hand, DAMPs, released from damaged or stressed cells and tissues, can activate PRRs, thereby inducing homeostatic inflammation ranging from the basal homeostatic state to diseased tissue remodeling. The role of endogenous ligands as a danger signal has been emphasized during the progression of homeostatic inflammation. For instance, free fatty acids, when released as an energy source during fasting or starvation, may not act as a danger signal. However, in adipose tissue inflammation, saturated fatty acids, which are released from hypertrophied adipocytes, can report, as a danger signal, their diseased state to macrophages via the TLR4 complex during the course of obesity. dsRNA, Double-stranded RNA; PGN, peptidoglycan; ATP, adenosine triphosphate; oxLDL, oxidized low-density lipoprotein; HSP, heat shock protein.

adipocytes via the macrophage-induced lipolysis, serve as an endogenous ligand for the TLR4 complex, a major pathogen sensor, to activate macrophages for the regulation of metabolic homeostasis, which is a hallmark of homeostatic inflammation. Understanding the molecular mechanism underlying homeostatic inflammation of obese adipose tissue may lead to novel, therapeutic strategies to prevent or treat obesity-induced adipose tissue inflammation.

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KEY WORDS:
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