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FOCUS: OBESITY

A Dangerous Duo in Adipose Tissue: High-Mobility Group Box 1 Protein and Macrophages

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High-mobility group box 1 (HMGB1†) protein first made headlines 40 years ago as a non-histone nuclear protein that regulates gene expression. Not so long ago, it was also shown that HMGB1 has an additional surprising function. When released into the extracellular milieu, HMGB1 triggers an inflammatory response by serving as an endogenous danger signal. The pro-inflammatory role of HMGB1 is now well-established and has been associated with several diseases, including sepsis, rheumatoid arthritis, and atherosclerosis. Yet very little is known about its role in obesity, wherein adipose tissue is typified by a persistent, smoldering inflammatory response instigated by high macrophage infiltrate that potentiates the risk of obesity-associated comorbidities. This mini-review focuses on the putative causal relationship between HMGB1 and macrophage pro-inflammatory activation in pathologically altered adipose tissue associated with obesity.

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†Abbreviations: IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; IL-1α, interleukin-1α; IL-1β, interleukin-1β; IL-8, interleukin-8; HMGB1, high-mobility group box 1; RAGE, receptor for advanced glycation end products; TLR-2, Toll-like receptor 2; TLR-4, Toll-like receptor 4; ATP, adenosine triphosphate.

Keywords: adipose tissue, adipocyte, obesity, insulin resistance, diabetes mellitus type 2, inflammation, macrophage, phagocytosis, cell death, apoptosis, necrosis, danger signals, damage-associated molecular patterns, pattern-recognition receptors, high-mobility group box 1 protein, crown-like structure, interleukin-6, tumor-necrosis factor-α, lipolysis, lipid droplets, free fatty acids

INTRODUCTION

The worldwide occurrence of obesity has more than doubled over the past 3 decades, according to the World Health Organization [1]. In 2008, more than half a billion adults were classified as obese, thus representing more than 10 percent of the global population [1]. Importantly, obesity-associated diseases have become a leading cause of death. Obesity has long been associated with the development of insulin resistance, diabetes mellitus type 2, and cardiovascular diseases [1]. Additionally, overnutrition has recently been linked with multiple types of cancers, including those of breast, prostate, thyroid, colon, rectum, endometrium, esophagus, and gallbladder [2].

Obesity is defined as an excess of adipose tissue and is linked with a state of chronic, low-grade inflammation that potentiates its associated comorbidities [3,4]. The close relationship between excess adiposity and a persistent, smoldering inflammatory response instigated by high macrophage infiltrate has ignited interest in the relationship between adipocytes and macrophages.

Emerging evidence indicates that obesity is associated with an increased level of high-mobility group box 1 protein (HMGB1), a non-histone nuclear protein that serves as an endogenous danger signal when released into the extracellular milieu [5]. Upon HMGB1 stimulation, macrophages are known to secrete pro-inflammatory cytokines, suggesting that HMGB1 may play a crucial role in the onset of obesity-associated inflammation [6,7]. The importance of this finding is well-established, since neutralizing HMGB1 alleviates severity of several inflammatory and autoimmune diseases [8].

This mini-review focuses on the putative causal relationship between HMGB1 and macrophage pro-inflammatory activation in pathologically altered adipose tissue, the relationship that could offer a novel therapeutic target to prevent or treat obesity-induced adipose tissue inflammation.

ADIPOSE TISSUE MACROPHAGES

Adipose tissue harbors parenchymal (i.e., adipocytes) and stromal cells, including macrophages, which function together to sustain metabolic homeostasis. Thus, the number of macrophages and their activation state reflect the metabolic health of adipocytes [9]. For example, in lean mice and humans, macrophages comprise fewer than 10 percent of all cells in adipose tissue depots. In contrast, macrophage content increases up to 50 percent in extremely obese, leptin-deficient (*op/op*) mice and nearly 40 percent in the most obese humans [3].

The first distinct subpopulation of adipose tissue macrophages was identified by the expression of the integrin CD11c [4,10]. In lean subjects, adipose tissue macrophages that lack expression of CD11c predominate, whereas in obesity, CD11c-expressing macrophages prevail. CD11c-expressing macrophages are thought to contribute to the inflammatory phenotype and are classified as M1 polarized or classically activated as opposed to M2 polarized or alternatively activated. According to this classification, M1 macrophages secrete a slew of pro-inflammatory cytokines, produce abundant amounts of oxygen and nitrogen intermediates, and express higher levels of major histocompatibility complex (MHC) class II molecules following stimulation with interferon gamma alone or in combination with microbial products (e.g., lipopolysaccharide) and selected cytokines (e.g., tumor necrosis factor-α) [11]. In contrast, M2 macrophages upon stimulation with IL-4, IL-13, IL-10, IL-1 receptor antagonist (IL-1ra), transforming growth factor-β, immune complexes, macrophage colony stimulating factor (also referred to as CSF-1), or glucocorticoids secrete anti-inflammatory cytokines and express high levels of scavenger, mannose, and galactose-type receptors [11]. Additionally, M2 macrophages produce ornithine and polyamines via arginase 1 (ARG1) as opposed to the nitric oxide and citrulline via inducible nitric oxide synthase (iNOS, also referred to as NOS2) that is observed in M1 macrophages [11].

In lean subjects, macrophages sustain insulin sensitivity by secretion of IL-10, which potentiates insulin action in adipocytes [9]. Although acute changes in energy intake are easily handled by adipocytes in lean subjects, chronic increase in energy intake imposes

substantial metabolic stress on adipocytes. Interestingly, more than 90 percent of macrophages within adipose tissue in obese mice and humans were found forming crown-like structures around dead or dying adipocytes, as judged by the absence of the lipid storage mediator, perilipin [12]. Additionally, adipocyte death was positively correlated with adipocyte hypertrophy coincident with an increase in the number of macrophages [12,13].

In obese subjects, as macrophages scavenge dead or dying adipocytes and free lipid droplets, they secrete pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor-α (TNF-α) [4,14]. Importantly, macrophage infiltration into adipose tissue precedes or coincides with an increase in the plasma insulin level [15]. Therefore, it has been suggested that macrophage-triggered inflammatory response promotes insulin resistance, a condition in which cells fail to respond to insulin that regulates glucose level and, if sustained, leads to the development of diabetes mellitus type 2 characterized by a hyperglycemia [15]. Indeed, adipocytes treated with TNF-α were found to down-regulate expression of glucose transporter-4 protein, thus explaining a compensatory increase in plasma insulin level following macrophage infiltration [4,16].

Given that obesity is associated with an increase in the number of macrophages, weight loss would be expected to reduce their amount and ultimately quench an inflammatory response. On the contrary, weight loss has been associated with a rapid, although short-lived, increase in the number of macrophages within adipose tissue [17]. However, macrophage recruitment, rather than exacerbating inflammatory response, has been attributed to the regulation of adipose tissue lipolysis with release of free fatty acids acting as a general signal for macrophage recruitment [17,18].

In adipose tissue from lean subjects, macrophages are found interspersed among adipocytes, whereas in obesity, they form crown-like structures around dead or dying cells. Noteworthy, dead cells differently affect macrophage polarization with apoptotic and necrotic cells inducing anti- and pro-inflammatory phenotype, respectively [19]. For example, phagocytosis of apoptotic neutrophils by human monocyte-derived macrophages inhibited production of several pro-inflammatory mediators, including IL-1β, IL-8, TNF-α, and granulocyte-macrophage colony stimulating factor [20]. Instead, increased production of anti-inflammatory factors, including transforming growth factor-β1, has been observed [20].

These seemingly paradoxical properties of cell death are due to differences in the mode of death and morphologic, biochemical, and molecular characteristics [19,21]. Apoptotic cell death usually occurs as a part of physiological events (e.g., developmental processes) and is characterized by the shrinkage of apoptotic cells, which maintain integrity of their plasma membrane [21]. Conversely, necrotic cell death occurs as a part of pathological events (e.g., physical trauma) and is associated with cell swelling and loss of membrane integrity [21]. Because necrotic cells lose membrane integrity, they rapidly discharge their intracellular components, some of which may serve as danger signals that trigger an inflammatory response [22,23].

Apart from exogenous signals associated with a group of pathogens such as bacteria and viruses, collectively termed pathogen-associated molecular patterns, the immune system is capable of sensing endogenous signals released from necrotic cells and thereby inducing sterile inflammation. One nuclear protein, HMGB1, has received particular attention as fulfilling the functions of the endogenous danger signal (or damage-associated molecular pattern) by being involved in several autoimmune and inflammatory conditions, including sepsis, rheumatoid arthritis and atherosclerosis [7,8,24-26]. Recently, an increased serum level of HMGB1 has been observed in obese children and has strictly been connected with an increased level of pro-inflammatory cytokines, such as IL-6 and TNF-α [5].

HIGH-MOBILITY GROUP BOX 1 PROTEIN

HMGB1 protein was first described 40 years ago as a non-histone nuclear protein that

is present in almost all eukaryotic cells, and it acts as an architectural chromatin-binding factor that stabilizes nucleosome formation and regulates transcription of several genes [8,27]. HMGB1 is composed of 215 amino acids organized in two DNA-binding domains, box A and box B, and an acidic C-terminal tail. Due to its high content of positively charged amino acids, HMGB1 (~25 kDa) positions itself at a 30 kDa in SDS-PAGE gels, hence the name "high mobility" [25].

However, its nuclear role is not the sole function of HMGB1. When released into the extracellular milieu, HMGB1 signals through the receptor for advanced glycation end products (RAGE), Toll-like receptor 2 (TLR2), and TLR4 in macrophages to trigger an inflammatory response [27]. For example, human monocytes were found to express pro-inflammatory mediators such as TNF-α, IL-1α, IL-1β, IL-6, IL-8, macrophage inflammatory protein-1α, and macrophage inflammatory protein-1β upon stimulation with recombinant HMGB1 [7]. Interestingly, apoptotic cells retain HMGB1 within nuclear remnants due to deacetylation of histones and chromatin condensation and, thus, do not trigger inflammation [28].

Well-characterized signaling pathways of HMGB1 are NF-κB and ERK1/2 activation by RAGE, and NF-κB and IKKa/b activation by TLRs [29]. Additionally, HMGB1 and RAGE mediate transendothelial migration of monocytes [30].

Noteworthy, adipose tissue macrophages forming crown-like structures were found expressing TNF-α and IL-6 [13]. Furthermore, electron microscopy analysis revealed necrotic features of obesity-associated adipocyte death, including ruptured membranes, cellular debris within extracellular milieu, and small cytoplasmic lipid droplets [12]. None of the classical features of apoptosis were observed [12]. Additionally, adipocyte apoptosis through targeted activation of caspase-8 in an inducible transgenic mouse model of lipodystrophy led to the recruitment of anti-inflammatory macrophages [31].

Given that necrotic death is usually triggered by physical trauma, it has been suggested that adipocyte hypertrophy constitutes a risk for membrane rupture of the fragile adipocytes in the abdominal cavity when submitted to common physical forces and certain intra-abdominal pathologies, collectively associated with the formation of an increased intra-abdominal pressure [32]. Additionally, correlation between higher body mass index and an increased intra-abdominal pressure has been described [32,33].

Adipocyte death may also be caused by hypoxic conditions that occur during rapid expansion of adipose tissue [34]. Hypoxia occurs when oxygen supply does not meet the demand of the surrounding tissue. In obesity, adipocyte size increases up to 180 μm in diameter, whereas the diffusion distance for oxygen is at most 100 μm [34,35]. Indeed, hypoxia has been demonstrated by both pimonidazole staining and increased lactate concentration in adipose tissue of obese mice [36]. Additionally, excess adiposity led to an increased expression of hypoxia-inducible genes such as leptin, plasminogen activator inhibitor type-1, matrix metalloproteinase-2, and adrenomedullin [36]. Interestingly, hypoxic environment devoid of nutrients has been shown to skew cells from undergoing energy (i.e., ATP) dependent apoptosis into necrosis [37].

In addition, adipocytes subjected to increased TNF-α, reactive oxygen species, and free fatty acids activate inflammatory signaling pathways that regulate stress-induced cell death, down-regulate adipocyte insulin signaling, and peroxisome proliferator-activated receptor-γ-regulated gene expression [38]. Noteworthy, selective ablation of peroxisome proliferator-activated receptor-γ in adipocytes using the tamoxifen-dependent Cre-ER (T2) recombination system led to necrotic death of mature adipocytes and subsequent infiltration of inflammatory cells [38].

Finally, adipose tissue macrophages themselves may contribute to a feed forward mechanism of adipocyte death by releasing TNF-α and reactive oxygen species. It has been established that reactive oxygen species play a critical role in TNF-α-stimulated necrotic death. Increased necrosis is partially mediated by a

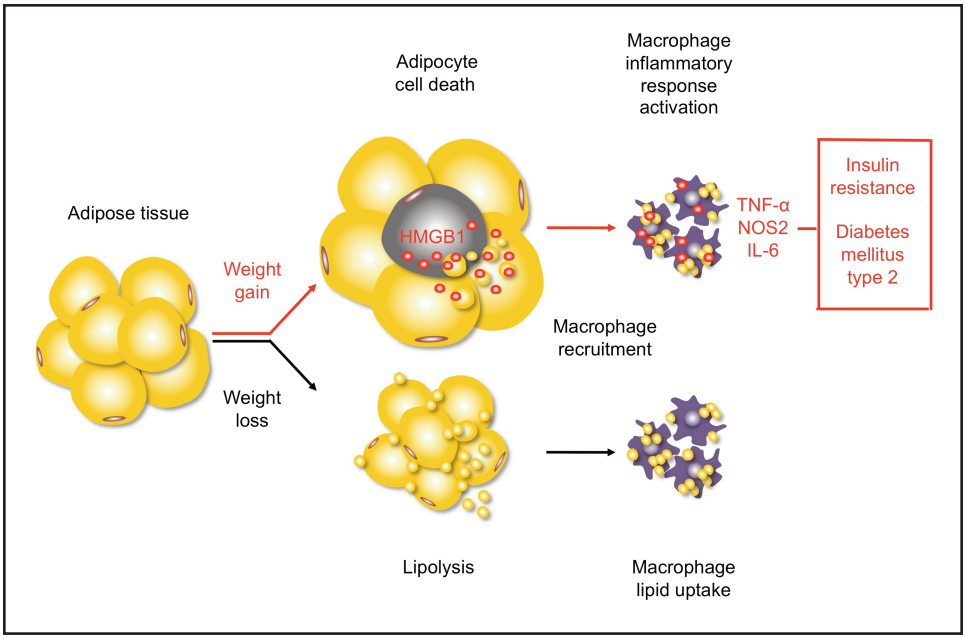

Figure 1. Inflammatory responses to weight gain and weight loss. Early weight loss leads to an increase in the local release of lipids and to the recruitment of anti-inflammatory macrophages that regulate adipose tissue lipolysis. In contrast, weight gain results in the recruitment of pro-inflammatory macrophages. Necrotic adipocytes lose membrane integrity and release their intracellular contents, including HMGB1, and may stimulate macrophages to trigger an inflammatory response, which further contributes to obesity-associated comorbidities (e.g., insulin resistance, diabetes mellitus type 2).

requirement of c-Jun NH2-terminal kinase for TNF-induced production of reactive oxygen species [39]. Importantly, it has been demonstrated that c-Jun NH2-terminal kinase in macrophages is indispensible for the establishment of insulin resistance and inflammation [40].

Based on the observation that necrotic adipocytes define localization of pro-inflammatory macrophages, a model in which endogenous signals released from dead or dying cells trigger an inflammatory response has been proposed (Figure 1). In contrast to macrophages transiently recruited to adipose tissue in weight loss, the macrophages detected in weight gain reside in expanded adipose tissue. Endogenous danger signals released from dead or dying cells, such as HMGB1, may impose the pro-inflammatory phenotype on macrophages, which further impairs adipocyte function (e.g., insulin signaling, adipogenesis) and contributes to insulin resistance and diabetes mellitus type 2.

CONCLUSION

It is now well established that M2 macrophages predominate in insulin-sensitive adipose tissue in lean subjects, whereas M1 macrophages accumulate commensurate with the adipose tissue mass in the obese state [4,17,41]. The close relationship between obesity and the pro-inflammatory phenotype in adipose tissue macrophages highlights the role of macrophages in the development of metabolic disorders such as insulin resistance and diabetes mellitus type 2. This raises the question of the identity of molecules that impose the pro-inflammatory phenotype on macrophages. The observation that the majority of macrophages surround necrotic adipocytes in obese mice and humans suggests that endogenous danger signals released from dysfunctional cells may directly activate macrophages [8,12,13]. Evidence has accumulated indicating that obesity is associated with an increased serum level of HMGB1 in obese children, suggesting that necrotic

adipocytes could have an impact, both locally and systemically [5]. Therefore, it would be interesting to determine whether HMGB1 could offer a novel, therapeutic target to prevent or treat obesity-induced adipose tissue inflammation.

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