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Review Article

The Role of Osteopontin in Adipose Tissue Inflammation and Macrophages Proliferation

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Abstract

Obesity is pathology associated with chronic low-grade inflammation, characterized by infiltration of adipose tissue with activated macrophages (ATMs). ATMs represent the main producers of cytokines involved in the onset of insulin resistance and type 2 diabetes. Osteopontin (OPN) is a cytokine expressed in the site of inflammation known to be strongly upregulated in obese adipose tissue. Recent studies point towards a critical role of local macrophage proliferation in adipose tissue inflammation suggesting that in obesity viability of macrophages is enhanced and apoptosis diminished. This review focuses on inflamed adipose tissue, ATMs infiltration and proliferation in the background of obesity and insulin resistance.

Keywords: *OPN; Adipose Tissue Macrophage; Inflammation; T2DM*

Introduction

The obesity epidemic problem has been rising with no signs of decline since the early eighties to nowadays. The World Health Organisation (WHO) has described obesity as one of the most serious global health issue in adults, which has become recently as serious as under nutrition [1]. In a recent report, it was discovered that about 13% of the world's adult population was obese in 2014, with prevalence more than doubled in the last 35 years. Overweight and obesity are defined by a body mass index (BMI) greater than 25 kg/m2 for overweight and greater than 30 kg/m2 for obesity. BMI is a widely used, although not necessarily precise, measurement to classify the amount of fat accumulation. Obesity determines a variety of medical and socio-economic issues, bearing heavily on the public healthcare systems. In fact, it is well established that overabundance of body weight leads to many comorbidities such as diabetes, stroke, hypertension, liver disease and cancer. This risk is proportional to the amount of weight in excess, escalating dramatically when the overweight becomes severe [2,3].

The etiology of obesity is multifactorial and extremely complex. Energy imbalance is one of the triggering factors especially of early overweight, in which energy intake exceeds energy expenditure. This translates in excess of nutrients, which are stored in the form of triglycerides mainly in adipose tissue and liver. Also endocrine disorders, though rare, could cause obesity, as well as genetic factors, which appear pivotal in the development of the disease [4]. However, the primary triggering factors of obesity and overweight still lie in environmental and lifestyle changes after the industrial revolution. Indeed, in recent years the level of physical activity in the population has dramatically decreased, whereas food intake increased, a phenomenon driven by the introduction of fast food and low priced/high energy foods [5]. This so called 'obesogenic' environment, characterized by low sport activity, TV viewing and cheap-high-fat-foods, is sadly overcoming healthy food choices and active lifestyles [6,7].

A crucial factor, which resides in the clinical presentation of obese patients and may trigger consequent comorbidity, is the distribution of excess adipose tissue. In fact, It has been demonstrated in many studies [8-10], that the localization of body fat is a predictor of metabolic and cardiovascular disease. For instance, the 'male-type' of obesity in which the fat distribution mainly localizes in the upper body, particularly in the visceral areas, was associated to increased risk of mortality and development of cardiovascular disease and diabetes. And these are the most dangerous comorbidities that significantly impact life expectancy and quality of life. Obese adipose tissue promotes inflammation, insulin resistance, type 2 diabetes (T2DM), atherosclerosis and cardiovascular disease, with a direct connection with liver pathologies, cancer and dyslipidemia [11,12].

Macrophage infiltration and insulin resistance in obese adipose tissue

The adipose tissue (AT) is a dynamic endocrine organ, which undergo several changes during obesity and weight gain. It has been shown in mice, how after 16 weeks of high fat diet their AT went through a complete remodelling, in which AT infiltration with activated macrophages took place [13]. Activated macrophages dramatically contribute to adipocytes´ remodelling engulfing necrotic-like cells [14,15]. This might stimulate other resident immune cells and adipocytes themselves, triggering the onset of an inflammatory milieu locally in obese AT. Indeed, in the AT reside not just adipocytes, but also preadipocytes, pericytes (both capable to become mature adipocytes), fibroblast, mast cells and immune cells. Adipocytes are specialised in lipid accumulation, characterised by a single lipid vacuole occupying most of the cytoplasm. They are able to secrete a variety of cytokines such as interleukin 6 (IL6), monocyte chemoattractant protein (MCP-1), adiponectin and leptin, which may act locally or systemically. Pre-adipocytes instead are fibroblasts-like cells, which can differentiate into mature adipocytes, and upon fat accumulation they were shown to increase in number and size. Adipose tissue macrophages (ATMs) are locally resident immune cells, of which number and characteristics can change in response to diet and AT expansion. ATMs are classified as anti- inflammatory (M2), which secrete for instance interleukin (IL)-10 and contribute to AT homeostasis. The pro-inflammatory type is known instead as M1; M1 macrophages are able to secrete cytokine such as TNF-α, IL-1β and IL6, the most dangerous pro-inflammatory cytokines which worsen inflammation in AT and were proven to promote cardiovascular risk. Interestingly, those phenotypes are interchangeable in response to the local inflammatory milieu, meaning that M2 macrophage will switch to a M1 pro-inflammatory type and vice versa [16]. Other immune cells such as T cells are also present in AT. In fact, different subsets have been found: Regulatory T cells (which mainly maintain immune homeostasis), T helper 1 cells (Th1 - pro-inflammatory) and Th2. Dendritic cells are responsible to initiate T cells response, secreting different cytokines. Natural killer cells and eosinophils are also present however in a lesser amount [17].

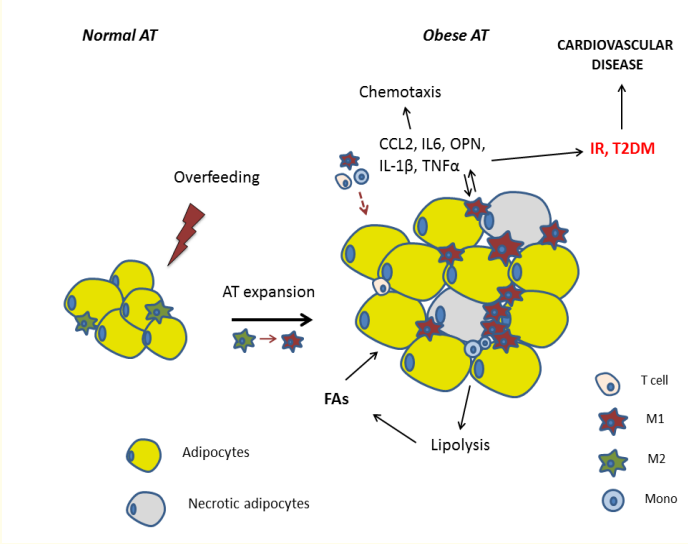
The cross-talk and interaction among these different cell types during obesity determines an established state of low-grade inflammation, driving insulin resistance [18].

Macrophages infiltrate obese adipose tissue in a decisive manner, increasing from 10-15% in lean mice to 50 - 60% in obese counterparts [19]. Moreover, the type and localization change significantly along AT expansion. In fact, it was shown that whereas in lean mice mainly alternative activated macrophages (M2 type) were present, in obese animals a huge amount of classical activated macrophages (or M1 type) were found disposed in crown-like structures around the adipocytes. These classically activated M1 macrophages allegedly induce insulin resistance via a chronic production of TNF and IL-1 β [20-22].

ATMs can undergo activation via different molecular mechanisms, one of which is triggered by fatty acids (FAs) released during lipolysis by adipocytes. A disruption in the FA homeostasis locally in the AT, may classically activate macrophages via direct binding with Toll-like receptor 4 (TLR4) [23]. This finding was confirmed in a study in which a knock out mouse model was employed, showing that the lack of TLR4 was sufficient to block the inflammation induced by fatty acids [24]. Cytokines are produced locally by activated macrophages and may attract other immune cells into AT, as for instance CC- chemokine ligand 2 (CCL2) does. It is known that especially CCL2 but also osteopontin (OPN) induce AT infiltration and inflammation therefore worsening insulin resistance states [25,26].

Osteopontin

OPN is a 44 kDa secreted protein, expressed in many tissues and cells, which undergo several post-translational modifications (such as glycosylation, phosphorylation, sulfation and cleavage) according to its origin. It also exists in an intracellular variant called iOPN, which is present in nucleus and cytoplasm and is mainly involved in cytoskeletal rearrangements and may contribute to cell cycle progression [27]. The secreted form was found as a soluble cytokine but also as an immobilized protein in, e.g., calcified matrix. OPN acts as a factor driving cell adhesion, migration and survival in a paracrine and autocrine fashion, interacting with many cell receptors such as integrins and CD44. Its expression was found to be up regulated in many pathological states such as cancer, infection, inflammation and ischemia [28-30]. The two classical cell binding sites of OPN are: the RGD sequence which binds preferentially to αv integrins (such as αvβ5, αvβ1, αvβ3) and the SVVYGLR region, binding α9β1, α4β1 (Figure 1). Other integrins and receptors have been reported to bind OPN, including some isoforms of CD44, αvβ6, α5β1, α8β1 and αxβ2 [31].



Many authors have demonstrated the link between OPN and AT inflammation and consequently insulin resistance. In fact, its expression in mice was found to be augmented in recruited macrophages just after 2 weeks of high fat diet treatment [25,32,33]. This was further demonstrated in an OPN knock out mouse model (SPP1KO), in which OPN deficiency ameliorated insulin resistance and glucose tolerance, reducing simultaneously AT inflammation [26,27,34]. OPN can undergo a proteolytic cleavage by thrombin and matrix metalloproteases locally in AT, which take place especially in inflammation status. Cleaved OPN behaves differently from the full length and it is likely to gain diverse biological functions due to the exposure of the cryptic region to integrin binding [35]. Thus, via binding to diverse integrins OPN acts as a mediator of inflammatory processes, playing a key role not only in AT but also in development of liver fibrosis and cholangiopathies.

Local macrophage proliferation and its role in adipose tissue inflammation

The accumulation of ATMs in crown like structures around dead adipocytes is critical step in determining obesity development [15,19,36,37]. What is thought traditionally is that ATMs accumulation is a consequence of monocytes recruitment under inflammatory condition, rather than an independent mechanism which takes place in parallel to cell migration [38]. This has been shown recently, highlighting the fact that many tissue-resident immune cells such as Kupffer cells in liver are indeed capable of proliferation, independently of tissue replenishment after inflammatory stimuli [39]. In chronic inflammation, macrophages do proliferate locally in adipose tissue and this triggers the first in situ accumulation in early stages of obesity [38]. Then recruited monocytes contribute to the number of local

ATMs at a relatively later time point [40,41]. Different studies in the field found two cytokines contributing to this mechanism: MCP-1 and IL-4 [38,40]. Interestingly, macrophage local proliferation was also found in arterial walls during atherosclerosis, another inflammation-driven disorder [42].

It was shown that cytokines such as MCP-1 and IL-4 are critical players in obesity-induced ATM proliferation [40,43]; also another study [44] confirmed these data in human AT. In fact, ATMs do strongly proliferate in response to IL-4, preferably in visceral AT depots rather than subcutaneous, forming crown-like structures around adipocytes. ATMs proliferation is thought to be independent of the amount of circulating monocytes and extremely organ specific, since for instance liver and spleen do not encounter an increase in macrophage numbers in obesity [38]. Indeed, the results showed in the fore mentioned studies undermined the classical vision of immunologists, in which monocytes' recruitment was seen as the main reason of the increased number of immune cells in AT. Therefore, what many authors tried to discover in recent years, was whether or not migration is the most important mechanism augmenting ATM accumulation in obesity [38].

On one hand, some works showed in mice that the majority of ATMs are actually bone marrow derived, performing elegant bone marrow transplantation or destroying its biological functions with irradiation [19]. On the other hand, others insisted on the greater importance of recruitment-independent mechanisms such as diminished apoptosis and augmented proliferation, which could well contribute to increase local ATMs' number [45]. As mentioned in the present article, it is known that in the AT a local population of anti-inflammatory macrophages take part into adipocytes homeostasis and shift into an inflammatory one in response to AT expansion, adipocyte apoptosis and cytokine production. This finding was also addressed in a recent publication [38], which shows that ATM proliferation is independent of monocyte recruitment. In fact, it is suggested that resident macrophages tend to proliferate in the first stages of obesity, whilst monocytes recruitment happens only at a later time point. In mice, this heterogenic population of local ATMs and recruited monocytes has been proved to reside and proliferate locally in AT [38].

It has been shown from our group and others [46], that another cytokine such as OPN plays a role in this scenario, as it was found highly upregulated in mice after high fat diet, also activating the inflammatory cascade in monocytes and macrophages. Although many different theories were postulated in these regards, not many studies to our knowledge systematically addressed a role of OPN in driving ATM proliferation in obese AT. This makes this cytokine a good candidate to address its play in the context of local macrophage proliferation in obese AT.

Conclusions

In conclusion, OPN seems to be a substantial driver of ATM proliferation in obese AT; macrophage accumulation appears to be aided by in situ proliferation of local ATMs, enhanced and promoted by monocytes migration [41]. Interestingly, OPN is an important factor for all of these processes. Still to be explored is the contribution of cytokines like MCP-1 and IL-4 in this scenario. A potential cross-talk of the OPN-effect described here and other cytokines needs to be clarified in future research. The molecular mechanism in AT infiltration needs also to be refined in regards to OPN, for instance by looking at which integrins are involved in cell to cell interaction, and therefore recruitment and activation. Targeting macrophage proliferation via neutralizing OPN or its receptors may well be a future therapeutic approach in treatment and prevention of obesity-associated chronic inflammation.

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