Obesity, Inflammation, and Macrophages

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**Abstract**

The World Health Organization estimates that since 1980 the prevalence of obesity has increased more than threefold throughout much of the world, and this increase is not limited to developed nations. Indeed, the incidence of obesity is increasing most rapidly among rapidly industrializing countries raising the specter of a burgeoning epidemic in obesity-associated diseases, including diabetes, dyslipidemia, non-alcoholic fatty liver disease and atherosclerosis. Reducing the rates of obesity and its attendant complications will require both coordinated public health policy and a better understanding of the pathophysiology of obesity. Obesity is associated with low grade chronic inflammation, a common feature of many complications of obesity that appears to emanate in part from adipose tissue. In obese individuals and rodents adipose tissue macrophage accumulation is a critical component in the development of obesity-induced inflammation. The macrophages in adipose tissue are bone marrow-derived and their number is strongly correlated with bodyweight, body mass index and total body fat. The recruited macrophages in adipose tissue express high levels of inflammatory factors that contribute to systemic inflammation and insulin resistance. Interventions aimed at either reducing macrophage numbers or decreasing their inflammatory characteristics improves insulin sensitivity and decreases inflammation. Macrophage accumulation and adipose tissue inflammation are dynamic processes under the control of multiple mechanisms. Investigating the role of macrophages in adipose tissue biology and the mechanisms involved in their recruitment and activation in obesity will provide useful insights for developing therapeutic approaches to treating obesity-induced complications.

**Introduction**

Obesity is caused by a chronic imbalance between caloric intake and energy expenditure, leading to the storage of excess calories as body fat. The
incidence of obesity has dramatically increased worldwide in the last quarter century. Once considered a disease of the affluent countries, obesity is now on the rise in developing nations. The World Health Organization estimates that approximately 1.6 billion adults worldwide are overweight and about 400 million adults are obese [1, 2]. This increased prevalence has translated to a disturbing increase in the incidence of obesity-related diseases and an associated increase in morbidity and mortality. Obesity contributes significantly to the development of insulin resistance and type 2 diabetes mellitus, dyslipidemia, atherosclerosis, hypertension, osteoarthritis, non-alcoholic fatty liver disease, and certain forms of cancer [2]. The increase in prevalence of obesity is not restricted to adults, but is increasing rapidly among children and adolescents as well [3]. Worldwide, approximately 20 million children are overweight [1]. This increased prevalence of obesity has lead to a disturbing rate of obesity-associated complications in children that predicts future morbidity not seen in previous generations [3]. If the challenge of obesity is unmet by the scientific and clinical community, the human and economic toll on the world will continue to grow exponentially.

**Obesity-Induced Inflammation**

Over that last dozen years, research has revealed that a chronic state of low-grade systemic inflammation is a common feature of obesity and that this inflammatory state mechanistically links obesity to many of its complications [4]. Originally recognized as the response to invading pathogens, inflammation is now broadly recognized as the complex biological response to noxious stimuli such as infectious agents, damaged cells or foreign bodies. Inflammation can either be acute or chronic in nature [5]. Acute inflammation is a short-term response, usually lasting a few days, in which the body rapidly removes the eliciting stimulus. Chronic inflammation occurs when there is no resolution and the stimulus is not removed. Classically, acute inflammation is characterized by swelling, redness, heat, and pain at the site of the insult [5]. Most acute inflammatory responses are characterized by resolution, which is critical for repair, regeneration and ultimately survival of the organism. Acute inflammation that does not resolve can either escalate to a lethal state typified by sepsis or can develop into a chronic state. While the signs and symptoms of acute inflammation are often apparent, the effects of chronic inflammation are often, at least initially, more subtle. However, with time the effects of chronic inflammation are often manifested with deterioration of one or more specific tissues or systems. Obesity induces a state of chronic low-grade inflammation as measured by activation of inflammatory signaling pathways, production of increased cytokines and alterations in immune cell function.

Epidemiological and clinical studies, some dating from the 1960s, revealed an association of inflammatory markers, e.g. circulating concentration of
fibronectin, with both obesity and diabetes. In the mid-1990s Hotamisligil et al. [6] observed that adipose tissue expression of tumor necrosis factor-α (TNF-α), the prototypical inflammatory cytokine, is increased by obesity and argued it to be responsible for obesity-induced diabetes in some rodent models. They showed that the expression and secretion of the classic inflammatory cytokine TNF-α from adipose tissue was significantly increased in rodent models of obesity [6]. In some studies, neutralizing TNF-α in obese mice with antibodies improved insulin sensitivity [7]. In humans, a similar correlation of TNF-α expression with adiposity was observed, although attempts to improve insulin sensitivity by neutralizing TNF-α have not been successful [8, 9]. Nonetheless, these initial studies of TNF-α revealed that the production of an inflammatory molecule can play a significant role in the development of obesity-induced complications. Since this initial observation, many studies have shown that obesity increases adipose tissue expression and secretion of other inflammatory markers including, interleukin-6 (IL-6), C-reactive protein (CRP), resistin, monocyte chemoattractant protein-1 (MCP-1), and plasminogen activator inhibitor-1 (PAI-1) [4]. Studies in animal models demonstrated that genetic deletion of individual inflammatory factors can modestly improve insulin sensitivity in mice on a high fat diet suggesting a role for inflammation in the development of obesity-induced insulin resistance. Furthermore, an increased concentration of circulating proinflammatory proteins, e.g. IL-6, predicts the development of insulin resistance, type 2 diabetes, and cardiovascular disease in humans. That no single inflammatory factor fully explains the inflammatory changes associated with obesity suggests that there is a complex interplay between multiple pathways and systems.

**Adipose Tissue Macrophages**

Hotamisligil et al. [6] originally postulated that adipose tissue production of TNF-α and other inflammatory molecules is derived from adipocytes. However, adipose tissue is a heterogeneous organ that in addition to adipocytes contains pre-adipocytes, fibroblasts, endothelial cells, and immune cells including macrophages and lymphocytes. Fain et al. [10, 11] separated adipose tissue into adipocytes and non-adipocyte populations and found that while adipocytes express some of the inflammatory cytokines induced by obesity, the majority of these cytokines are derived from the stromal vascular fraction. This observation was initially puzzling and some suggested an artifact of isolation and in vitro culture. However, two recent reports demonstrated that in obesity, adipose tissue is infiltrated by macrophages and that these cells indeed express a substantial portion of inflammatory factors that are linked to obesity-related complications [12, 13].

Macrophages belong to the mononuclear phagocyte system and are derived largely from circulating monocytes. Macrophages are present in most tissues
Adipose tissue macrophages in obesity-induced inflammation. 

**a** In lean animals the macrophage content is low in adipose tissue. These macrophages express low amounts of inflammatory factors including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1) and high levels of anti-inflammatory factors including interleukin-10 (IL-10). **b** Obesity increases monocyte adhesion and recruitment to adipose tissue, consequently increasing macrophage number in adipose tissue. The macrophages in obese adipose tissue express increased amounts of inflammatory factors including TNF-α, IL-6, and PAI-1 and decreased amounts of IL-10. The inflammatory molecules act locally in a paracrine fashion to alter adipocyte function and adipokine production.
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and have distinct phenotypes and functions specific to their local microenvironment. They are the primary effectors of the innate immune system, and therefore, form a first line of defense against foreign pathogens. Resident tissue macrophages also are critical for maintaining normal tissue function by clearing apoptotic and dead cells and cellular debris especially following tissue injuries [5], and in response to injury play a central role in coordinating the reparative response.

Studies of adipose tissue macrophages (ATMs) in both humans and rodents demonstrate that increases in adiposity are closely associated with increases in the macrophage content of adipose tissue. Consistent with ATMs being authentic macrophages, bone marrow transplant studies in rodents demonstrate that almost all of the macrophages in adipose tissue are bone marrow-derived and are dependent upon the key regulator of macrophage differentiation and development, macrophage colony-stimulating factor (M-CSF or CSF-1) [12].

In obesity, the initial instigator of adipose tissue inflammation is not clear. Data suggest that dying cells, hypoxia and excess local concentrations of free fatty acids can contribute to the inflammatory signal that attract and sustain ATMs. However, once initiated it is clear that ATMs play a critical role in the inflammatory response seen in obesity. In humans and rodents, the number of macrophages in fat depots correlates positively with adipocyte size, body mass index, and percent body fat [12]. In obese mice [12] and severely obese individuals [14], visceral adipose tissue, which is strongly correlated with the development of insulin resistance and metabolic syndrome, contains higher numbers of macrophages than subcutaneous adipose tissue. The higher macrophage content of visceral adipose tissue depots is also reflected in the high expression of inflammatory molecules from these depots compared to subcutaneous ones. Defining the mechanisms that lead to differences in ATM content between visceral and subcutaneous depots will likely provide insights into the functional differences between these depots.

Recruitment of Monocytes to Adipose Tissue

The recruitment of bone marrow-derived circulating monocytes to tissues is a complex process under the control of numerous chemokines, cytokines, and other local factors [15]. Under homeostatic conditions peripheral monocytes cross the endothelium into the tissue and replenish any loss of resident macrophages, thus maintaining the resident tissue macrophage populations. However, the presence of local inflammation causes activation of endothelial cells and production of chemoattractant proteins. A necessary step in the accumulation of macrophages is the adhesion of circulating monocytes to activated endothelial cells and transmigration into the tissue. Cytokines like TNF-\(\alpha\) secreted from adipose tissue are capable of activating endothelial cells.
In obesity, adipose tissue expression of adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) is increased [12]. However, there are conflicting data as to the metabolic effects of ICAM-1 deficiency [16, 17]. An initial analysis of macrophage content in the adipose tissue of ICAM-1-deficient mice did not reveal a significant effect. Further studies are therefore required to identify the adhesion molecules critical for recruitment of monocytes to adipose tissue in obesity.

Chemokines are a large, structurally related family of proteins that regulate immune cell trafficking and activation. Profiling of adipose tissue expression has revealed that obesity increases expression of MCPs, members of the C-C chemokine ligand family. MCP-1 (CCL2) is the prototypical MCP and its expression is increased in obesity. Studies in mice have demonstrated that overexpression of MCP-1 within adipocytes induces macrophage accumulation and inflammation in adipose tissue, and reduces insulin sensitivity [18, 19]. There are conflicting data as to whether a genetic deficiency of MCP-1 is sufficient to reduce macrophage accumulation in adipose tissue and improve glucose homeostasis in obese rodents [19, 20]. This may in part be due to the redundancy of ligands that can bind a key MCP-1 receptor, C-C chemokine receptor 2 (CCR2). Obesity increases adipose tissue expression of MCP-1, MCP-3, MCP-5, all of which bind the CCR2. Genetic deletion or pharmacological antagonism of CCR2 reduces macrophage accumulation in adipose tissue of high fat fed mice by ~30%. This reduction is accompanied by decreased adipose tissue inflammation, increased systemic insulin sensitivity, and reduced hepatic steatosis [21]. These studies suggest that MCPs through CCR2 are important for the recruitment of monocytes to adipose tissue and the development of obesity-induced inflammation and metabolic syndrome. However, CCR2 alone does not account for obesity-induced accumulation of ATMs.

Once recruited to the tissue, monocyte differentiation to macrophages is governed by local signals. CSF-1 is the primary regulator of macrophage differentiation, survival and proliferation. This is supported by the observation that mice deficient in either CSF-1 (Csf1op/op) or the CSF-1 receptor have markedly reduced macrophage content in almost all tissues [22, 23]. Consistent with a role for CSF-1 in ATM function, adipose tissue from mice lacking CSF-1 has significantly reduced the number of macrophages compared to those from control mice [12].

The population of macrophages in adipose tissue is diverse. Broadly, macrophage populations in most tissues can be divided into resident and recruited macrophages. Resident macrophages are a stable population of cellular sentries that reside in tissue under non-pathological states. By contrast, recruited macrophages hone to tissues in response to pathological or immunological stimuli [24]. The stable populations of resident macrophages in various tissues have distinct phenotypes and functions specific to the local microenvironment in the tissue. In the lean state resident macrophages in adipose tissue produce low levels of classic inflammatory molecules (alter-
nately activated macrophages). In contrast, macrophages actively recruited
to sites of injury or infection express high levels of inflammatory molecules
[24]. The majority of macrophages in adipose tissue from obese individuals
has a distinctly inflammatory character typical of classically activated macro-
phages, and thus, expresses high levels of inflammatory molecules, including
TNF-α, MCP-1 and inducible nitric oxide synthase [12]. Alternative activa-
tion of macrophages is the typical response seen to some parasitic infections
and important in the resolution of inflammation. Peroxisome proliferator-acti-
vated receptor-γ (PPAR-γ) is an important regulator of macrophage alter-
native activation, and deletion of PPAR-γ impairs alternative activation. In
high fat diet-induced obesity, deletion of PPAR-γ specifically in myeloid cells
increases adipose tissue inflammation and further impairs glucose homeosta-
sis [25, 26].

Role of Macrophages in Metabolic Diseases

Adipose tissue inflammation and macrophage content are dynamic, regu-
lated by weight changes and correlated with insulin resistance. Intervention
studies aimed at either reducing macrophage numbers or manipulating their
inflammatory state in murine models have improved insulin sensitivity and
decreased overall adipose tissue inflammation. In humans, weight loss fol-
lowing gastric bypass is associated with a significant decrease in macro-
phage number in subcutaneous adipose tissue [27]. Concomitantly, there was
also a significant decrease in circulating levels of proinflammatory factors.
Thiazolidinediones are potent insulin-sensitizing compounds that also exhibit
powerful anti-inflammatory effects [28]. Treating obese mice [13] and humans
[29, 30] with thiazolidinediones reduces ATM content and improves insulin
sensitivity.

Conclusion

Obesity is associated with a state of chronic low grade inflammation.
Adipose tissue is an important source of systemic inflammation. The mac-
rophage population within adipose tissue and their activation state are
increased in obesity, contributing to the systemic low grade inflammation
associated with weight gain. Furthermore, these immune cells have been
implicated in the development of obesity-associated complications, including
insulin resistance, cardiovascular disease and nonalcoholic fatty liver disease.
However, there are still numerous questions that will likely be answered in
the coming years as to the role of ATMs in systemic and local inflammation.
The understanding of the interaction between immune cells and adipocytes
is still incomplete. Understanding the role of immune cells in adipose tissue
physiology, and the molecular signals needed and the kinetics of recruitment, survival, and activation of these cells in obesity will help identify new therapeu- tic candidates for treating obesity-related complications.

References

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Discussion

Dr. Chowdhury: What is the role of n-3 fatty acids in manipulating obesity-induced inflammation? In particular have you found in your laboratory or in any other report that it can reduce the hepatic expression of MCP-1?

Dr. Subramanian: Actually there is a lot of work on the type of fatty acids in n-3 versus n-6, saturated versus unsaturated. At least in in vitro models it seems that saturated fatty acids activate the macrophages and result in more proinflammatory states whereas n-3 fatty acids actually do not induce inflammation. So the type of fatty acid seems to play an important role in the extent of the inflammatory process. I don’t know if anybody has actually measured MCP-1 in the liver, but I think MCP-1 automatically decreases as inflammation and macrophages decrease. So yes, the various types of fatty acids have different inflammatory effects. We have looked at the various fats in rodents: visceral, subcutaneous, and mesenteric fat which surrounds the intestine. So as I showed before, in all depots there is accumulation of macrophages and it correlates with the degree of adiposity or the adipocyte site, but there is a difference in absolute numbers of macrophages. So it seems in rodents that the perigonadal adipose tissue has more macrophages than subcutaneous. You are very right, the secretion of cytokines is completely different in the various adipose tissues. So there are differences in terms of accumulation. But all the macrophage depots that are accumulating are proinflammatory. In humans with weight loss, subcutaneous adipose tissue could actually be sampled and a decrease in macrophages and in the expression of the proinflammatory cytokines could be seen. But I don’t think anybody has actually very clearly or very thoroughly dissected the different adipose tissues. We know that the adipose tissues are different so there are going to be differences in terms of accumulation of macrophages and the extent of inflammation just by the fact that is the depots are different, but the macrophages of all depots are inflammatory.
**Dr. Mathur:** What are your views on some papers available on incriminating infective etiology in triggering the inflammatory processes for obesity [1, 2]? In addition, How well does your hypothesis apply to the immune-compromised state of obesity?

**Dr. Subramanian:** Let me answer the second question first. In the immune-compromised state the processes are different. In obesity there is no immune compromise. The immune system is activated but it is fully functional and capable of mounting an immune response. It is very difficult to compare the inflammatory process in obesity to immunosuppressor or other states because in those conditions you have the complication that the immune system is actually not primed or cannot handle whatever the underlying disease condition is. Regarding your first question, I actually don’t know the literature you are talking about in terms of viruses causing inflammatory processes. I am sure any infective agent can cause an inflammatory response, but in obesity we think that this whole process is triggered by the adipose tissue or the adipocytes that are expanding. I know that, at least in the last year, literature has been published on gut microflora and their role in obesity and inflammation. It is a very new topic, but for most part we think that this inflammatory process is not started by a pathogen but it is the response of the body to the expanding adipocytes.

**Dr. Prentice:** You suggested that the initial driver of this process is a metabolic perturbation without any detail to that, probably because we don’t know about it. I just wanted to try the possibility that there could be a physical perturbation because adipose tissue obviously has to be perfused. There is a large growth of the capillary bed, I would imagine. Intuitively to me it seems that if there is a big mass of adipose tissue there may be problems in perfusing that bed. Is there any evidence as to whether that is part of the story or not?

**Dr. Subramanian:** Metabolic perturbation is again only part of the story. There is a hypothesis out there that the dimension, the expanding adipose tissue, is very hard to study because a lot of neovascularization is needed to get blood flow to this expanding adipose tissue, and hypoxia is another big factor in the recruitment of macrophages to adipose tissue. Actually there is some evidence showing that it is actually the inability of the fat cell to expand continuously to recruit these macrophages, and if you were able to just keep on expanding the fat cell you probably would not have this accumulation or some of the complications of obesity because the fat would be where it should be. So yes, in addition to metabolic factors the physical expansion of the adipocyte and hypoxia induced by it play a role in recruiting macrophages to the tissue.

**Dr. Ganapathy:** You just mentioned inflammatory markers and obesity. One of the mechanisms of macrophage activation is TNF-a endotoxin and macrophages have now been subdivided into three categories: one for TH1, one for TH2, and one for the immune complex. Could it be that the inflammatory markers themselves are activating the macrophage system, and have we done anything on the inducible NOS system because that could go a long way to control the inflammatory side effects of obesity?

**Dr. Subramanian:** The inflammatory cytokines actually do feed back and activate the macrophages and these macrophages that you see in obese adipose tissue are proinflammatory type 1, the TH1 response macrophages. About the iNOS, we see increases in iNOS expression in obese adipose tissue and I think that mice lacking iNOS are actually protected from obesity-induced macrophages as well as systemic insulin resistance. We haven’t specifically looked at iNOS, but we are looking at all of the mixtures of these proinflammatory cytokines.

**Dr. Ganapathy:** You told us a little bit about the PPAR system. Could you add something more about PPAR and its role? Are you trying to produce drugs through the TNF-b receptors? You mentioned a slide about the PPAR ligand and it was also mentioned in the first talk about the inheritance and genetic basis for PPAR. Does it play a role?
Dr. Subramanian: I don’t know about the genetic basis of PPAR, I definitely can’t answer. But PPAR seems to play a role because knocking out the PPAR-g actually primes the macrophage to be more proinflammatory and these macrophages actually secrete more inflammatory proteins compared to wild-type macrophages that have PPAR-g, and this is also true for the other isoforms as well PPAR-d. So these PPARs seem to play a role in priming the macrophages or in controlling the activation of macrophages type 1 versus the TH2 macrophages. So at this point the data are very preliminary, but we think that the normal function of the PPARs at macrophages is to clamp down on the proinflammatory state.

Dr. Ganapathy: Could it also be possible that if there is a parasitic infection and macrophages of the TH2 type that this is an anti-inflammatory state, just as in inflammatory bowel disease? We have tried to use hepatocytes to get the TH2 changed since we are talking about inflammation.

Dr. Subramanian: I don’t know about bowel disease but there is evidence showing that in obesity the resident macrophages in the adipose tissue are actually TH2, they are more anti-inflammatory. As weight is gained and macrophages are recruited, these macrophages are more TH1, so they are more proinflammatory.

Dr. Ravussin: You have shown the infiltration of macrophages in the adipose tissue and then in the liver. Systemic insulin resistance, in humans at least, as measured by clamp is in the muscle. Do you have data showing that there would be some infiltration by macrophages in the muscle, or is the increase in cytokines, TNF-a, IL-6 enough to explain the decrease in glucose disposal into the muscle?

Dr. Subramanian: With regard to the muscle, in obesity there is intramyocellular fat and there is macrophage accumulation in intramyocellular fat, but the role of those macrophages in muscle insulin resistance is really not known. It could be that those macrophages locally produce inflammatory cytokines that play a role in impairing myocyte glucose uptake, and systemically it could be TNF, IL-6. So it could be both, macrophages in the fat within muscle as well as systemic inflammatory markers could both contribute to muscle insulin resistance.

Dr. Jaigirdar: You mentioned the CCR2 receptor protein. Is there any way, any drug or any physiological way to decrease it?

Dr. Subramanian: No, we really don’t want to mess with immune systems without really understanding what is going on in obesity-induced inflammation. But there is a company in the US that has a small antagonist for CCR2 and I think they are looking at it in terms of using it in arthritis and multiple sclerosis. Actually I did not present the data but if the CCR2 antagonist is given to rodents, a fairly similar effect is seen; there is decreased macrophage accumulation in adipose tissue and increased insulin sensitivity. But to advocate the use of that drug in large scale human studies, more data and more research are needed to actually see what other systems are being perturbed by knocking out CCR2.

Dr. Jatana: Did you look at calorie restriction diets or increased activity in these rodents to decrease macrophages, or do we only need to reduce the amount of adipose tissue in order to reduce the macrophages?

Dr. Subramanian: The studies about caloric restriction are actually currently underway in the laboratory, and the results are actually not that clear-cut. In terms of physical activity I don’t think we have looked at it. We have looked at physical activity in terms of a genetically obese model, the agouti and the obese strain, but I cannot recall the data in terms of macrophage accumulation and inflammation.

Dr. Arora: In severe malnutrition there is a lot of fat in the liver and there is no fat elsewhere. What is the inflammatory status in the fatty liver of the severely malnourished individual? Are there situations in which there is adipose tissue and obe-
inity without evidence of ongoing inflammation? In other words I am asking whether inflammation triggers adiposity or adiposity triggers inflammation?

**Dr. Subramanian:** To answer your first question on malnutrition, fatty liver and inflammatory state; this is just a speculation I actually have no data or expertise in that area. The inflammation or the immune response in malnutrition is again slightly different from that in obesity because in malnutrition and undernutrition the immune system is immunocompromised, it is not able to function normally, while in obesity the functions are very normal. So the immune response or the inflammation in malnutrition and obesity may be completely different. To answer to your second question, we really don't know whether adiposity triggers macrophage accumulation or macrophage accumulation triggers adiposity.

**Dr. Giovannini:** Is the inflammatory process in obesity linked to the development of atherosclerosis?

**Dr. Subramanian:** There is some evidence in rodents but I cannot recall the exact data. Obesity-induced inflammation results in an inflammatory cascade in the endothelial cells which play a major role in atherosclerosis plaque formation, so we think that obesity-induced inflammation plays a major role in atherosclerosis, but I can't recall the exact data.

**Dr. Jatana:** Is there a difference between childhood obesity and age-onset obesity and the number of years a person was obese, and whether it was reversible? Are there any studies?

**Dr. Subramanian:** The accumulation of macrophages in adipose tissue was actually first reported in 2003 from our laboratory. There have not been a lot of studies looking at childhood obesity versus age-onset obesity and how much this reversal occurs during weight loss. These are studies that are probably ongoing right now, but there are no results so far.

### References