Interplay between the immune system and adipose tissue in obesity

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Abstract

Obesity is a major risk factor for metabolic disease, with white adipose tissue (WAT) inflammation emerging as a key underlying pathology. Alongside its major role in energy storage, WAT is an important endocrine organ, producing many bioactive molecules, termed adipokines, which not only serve as regulators of systemic metabolism, but also possess immunoregulatory properties. Furthermore, WAT contains a unique immune cell repertoire, including an accumulation of leukocytes that are rare in other locations. These include alternatively activated macrophages, invariant natural killer T cells, and regulatory T cells. Disruption of resident adipose leukocyte homeostasis contributes to obesity-associated inflammation and consequent metabolic disorder. Despite many recent advances in this new field of immuno-metabolism, fundamental questions of why and how inflammation arises as obesity develops are not yet fully understood. Exploring the distinct immune system of adipose tissue is fundamental to our understanding of the endocrine as well as immune systems. In this review, we discuss the roles of adipose tissue leukocytes in the transition to obesity and progression of inflammation and highlight potential anti-inflammatory therapies for combating obesity-related pathology.

Key Words
- CD1d
- NKT cells
- adipocytes
- lipids

Adipose tissue as an immune organ

Immune organs represent sites of exclusive immunological function; however, the definition of an immune tissue has been extended to organs such as the liver (Doherty & O'Farrell 2000), uterus (Lynch et al. 2007), and small intestine (Camerini et al. 1993). The primary function of these organs is of course not immunological, but each has specialized immune mechanisms mediating their physiological roles, as well as providing immune surveillance against pathogens and tumors. Similarly, white adipose tissue (WAT) can now be defined as an immune organ, with important roles in anti-microbial defense, wound healing, and inflammation.

Immune aggregates in human adipose tissue were first described in 1874 and termed ‘milky spots’ (Ranvier 1874). For a century, surgeons have used the wound healing properties of the omentum to their advantage during surgery; the omentum can adhere to intra-abdominal foreign bodies, including drains and catheters. It also acts as the first defense against peritonitis and isolates sites of inflammation and injury to localize inflammation (Platell et al. 2000). Despite the discovery of milky spots and the
healing characteristics of the omentum, the complex interactions between the adipose immune network and the stromal and adipocyte components of this tissue are now only unfolding.

The two main WAT depots are the visceral (including the omentum; ~10% of body total) and subcutaneous (~85%) adipose tissue beds, although smaller depots are scattered throughout the body, surrounding organs, such as the heart and kidneys, and lymph nodes. Compared with subcutaneous adipose tissue, visceral WAT has enhanced metabolic activity and is more strongly associated with adverse metabolic risk factors, which will be discussed in the sections below (Kershaw & Flier 2004).

Visceral adipose tissue is immunologically dynamic, with a remarkable concentration of resident leukocytes. This population includes CD4 (Winer et al. 2009) and CD8 T cells (Nishimura et al. 2009), T regulatory (Treg) cells (Feuerer et al. 2009), invariant natural killer T (iNKT) cells (Lynch et al. 2009, 2012), B cells (Winer et al. 2011), mast cells (Liu et al. 2009), eosinophils (Wu et al. 2011), and macrophages (Weisberg et al. 2003, Lumeng et al. 2007, Wentworth et al. 2010). Resident adipose leukocyte populations represent distinct subsets, with particular functions compared with their equivalent populations elsewhere in the body. Striking examples include iNKT and Tregs, which are specifically enriched in human and murine adipose tissue (Lynch et al. 2009, 2012, Brennan et al. 2010). Resident adipose leukocyte populations represent distinct subsets, with particular functions compared with their equivalent populations elsewhere in the body. Striking examples include iNKT and Tregs, which are specifically enriched in human and murine adipose tissue (Lynch et al. 2009, 2012, Brennan et al. 2010) and express unique combinations of surface receptors and cytokine profiles compared with resident populations present at other locations (Feuerer et al. 2009).

Obesity-related inflammation

Crosstalk between adipocytes and resident leukocytes allows these systems to coordinate available energy stores for survival during times of starvation and pathogen challenge. However, over the last century, the major threat of energy deficit to living organisms has been replaced by overnutrition of humans in the developed (and increasingly in the developing) world. Obesity is now a major public health issue, with the principal cause of morbidity due to metabolic dysfunction (insulin resistance, type 2 diabetes, dyslipidemia, hepatic steatosis, and cardiovascular disease). Progression of clinical pathology associated metabolic dysfunction.

Increased tissue inflammation through adipocyte release of cytokines (e.g. tumor necrosis factor a (TNFa; Hotamisligil et al. 1993, Kern et al. 1995)), chemokines (e.g. monocyte chemoattractant protein (MCPI/CCL2; Kanda et al. 2006)), and pro-inflammatory fatty acids (Nguyen et al. 2007) drives alterations in leukocyte number and phenotype, thereby expanding the inflammatory environment within adipose tissue beds. Altered expression of pro- and anti-inflammatory factors produced by leukocytes acts reciprocally on adipocytes, perpetuating WAT inflammation and dysfunction.

How WAT inflammation is triggered is not completely understood, but it is suggested that lipotoxicity (Unger & Scherer 2010), endoplasmic reticulum (ER) stress due to excess lipid burden (Ozcan et al. 2004), hypoxia due to decreased oxygen diffusion into enlarged adipocytes (Halberg et al. 2009), and Toll-like receptor activation through free fatty acid sensing (Shi et al. 2006) are involved. Each immune cell population present in WAT has been shown to participate in the progression of obesity-related inflammation (Fig. 1), via different mechanisms that will be explained in the subsequent sections. In 2013, the Council on Science and Public Health of the American Medical Association (AMA) ‘recognize(s) obesity as a disease state with multiple pathophysiological aspects requiring a range of interventions to advance obesity treatment and prevention’. One potential therapeutic avenue is the immunometabolic interface in adipose tissue.

Macrophages

Macrophages are the most abundant leukocyte in adipose tissue and appear to be at the center of obesity-related inflammation (Chawla et al. 2011). In lean animals, adipose tissue macrophages are dispersed throughout WAT and display an alternatively activated (M2) anti-inflammatory phenotype (Lumeng et al. 2007), promoting insulin sensitivity in adipocytes by secreting interleukin 10 (IL10). Impairment of M2 macrophage activation using genetic models (Weisberg et al. 2003, Patsouris et al. 2008) enhances susceptibility of rodents to diet-induced obesity and insulin resistance, while potentiation of M2 macrophage activation by pharmacological inhibition (Odegaard et al. 2007) confers protection from obesity-associated metabolic dysfunction.

In obese subjects, adipose tissue macrophage numbers increase remarkably, and this population shifts toward the
classical pro-inflammatory (M1) state. M1 macrophages aggregate around necrotic adipocytes in inflamed tissue, forming ‘crown-like structures’, and produce substantial amounts of pro-inflammatory cytokines such as IL6 and TNFα (Lumeng et al. 2007, Wentworth et al. 2010), contributing directly to local and systemic inflammation and insulin resistance. Blockade of inflammatory macrophage trafficking into adipose protects mice from obesity-induced inflammation and loss of insulin sensitivity (Arkan et al. 2005, Weisberg et al. 2006) and, similarly, selective depletion of M1 macrophages in obese animal models reduces WAT inflammation without affecting diet-induced obesity (Patsouris et al. 2008).

Of course, separation of macrophages into these defined M1/M2 phenotypes has its limitations; in vivo, they exhibit plasticity across the entire spectrum of activation states encompassed by the M1 and M2 nomenclature. Nonetheless, sustained weight loss results in reduced total numbers of adipose tissue macrophages, which is accompanied by a decrease in pro-inflammatory profiles of obese individuals (Cancello et al. 2005). This, and the genetic manipulations referred to above, provides strong support for the causative role of macrophage-mediated inflammation in insulin resistance. The triggers for the trafficking and inflammatory activation of macrophages in obesity are still being uncovered, and we refer the reader to recent reviews (Chawla et al. 2011, Osborn & Olefsky 2012), which summarize the latest findings.

Eosinophils

Eosinophils have recently been added to the immune sentinels in WAT, which, similar to the other immune populations, influence metabolic regulation. Eosinophils are innate leukocytes with an important role in allergy development and parasitic infection. They are the primary source of adipose IL4, a cytokine that mediates M2 activation of macrophages (Wu et al. 2011). In line with this, adipose eosinophil numbers are decreased in obesity, and mice lacking eosinophils exhibit enhanced adipose M1 macrophage activity, weight gain, and systemic insulin resistance (Wu et al. 2011). The production of eosinophils in bone marrow and their recruitment into WAT is largely controlled by IL5 (Mould et al. 1997, Molofsky et al. 2013); mice with tissue eosinophilia (which occurs in IL5 transgenic mice) demonstrate decreased adiposity and improved insulin sensitivity when maintained on a high-fat diet (Wu et al. 2011). The source of adipose IL5 is a newly recognized population, innate lymphoid type 2 cells (ILC2s; Molofsky et al. 2013). Thus, accumulation of eosinophils and maintenance of adipose M2 macrophages has been shown to depend on ILC2s, and loss of this population in mouse models exacerbates diet-induced obesity and metabolic dysfunction. This latest example adds further complexity to the network of immune cells in adipose tissue, demonstrating how perturbations at any of these levels result in whole-adipose dysfunction and subsequent metabolic sequelae.
B cells

In lean animals, resident adipose B cells provide immunity against infection by mounting initial responses against local peritoneal antigens, including bacteria from intestinal perforations, or antigens associated with abdominal injuries (Rangel-Moreno et al. 2009). Herein, B cells produce antibodies, undergo isotype switching, and somatic hyper-mutation, and can regulate immune function. However, two independent studies have shown that during obesity, B cells undergo functional changes, leading them to play a pathogenic role in inflammation and insulin resistance (Winer et al. 2011, DeFuria et al. 2013). Obesity is associated with increased IgG production, and infiltration of IgG⁺ B cells into adipose tissue (Winer et al. 2011). Serum IgG or major histocompatibility complex (MHC) class II-expressing B cells isolated from obese mice and transferred into B cell-deficient lean mice induce insulin resistance in these animals (Winer et al. 2011). Both of these events compound differentiation of M1 macrophages: B cell pathogenic antibody production directly activates macrophages, and B cells from obese mice promote inflammatory responses by T cells and affect Treg survival negatively (effect of which is discussed in further detail below; DeFuria et al. 2013). In agreement, diet-induced obese mice lacking B cells exhibit decreased inflammation, and an increase in adipose Tregs, associated with improved metabolism, despite typical weight gain (Winer et al. 2011).

Treg cells

Tregs are critical to the maintenance of peripheral immunological tolerance and immune homeostasis, as evidenced by the catastrophic consequences of Treg ablation. Treg loss causes spontaneous development of severe autoimmune disease, allergy, and immunopathology in humans and rodents (Gavin et al. 2007). Tregs are highly enriched in adipose of lean mice (Feuerer et al. 2009), compared with their frequencies in other lymphoid and non-lymphoid tissue. Their number declines in obese mouse models and obese human patients (Feuerer et al. 2009) and modulation of WAT Treg number to selectively increase or decrease this population, improves or worsens inflammation and metabolic dysfunction respectively (Feuerer et al. 2009). These data suggest that Tregs are required for maintenance of an anti-inflammatory environment at steady state in lean adipose tissue. Indeed, adipose Tregs have a distinct cytokine profile, expressing large amounts of IL10, which maintains macrophages in the alternatively activated M2 state (Feuerer et al. 2009). IL10 may also directly affect adipocyte function, as adipocytes also express IL10 receptor. When adipocytes are treated with IL10, they phosphorylate Akt, decrease their expression of macrophage chemoattractant CCL2, and importantly, insulin-stimulated glucose uptake is dramatically enhanced (Lumeng et al. 2007). Thus, Tregs are a key population that prevents self-destructive immune responses in adipose tissue, and their loss during obesity aggravates the inflammatory milieu, through effects on their neighboring immune cells and on adipocytes.

Interestingly, adipose Tregs also express lipid receptors, and most surprisingly, the adipogenic transcription factor peroxisome proliferator-activated receptor gamma (PPARγ; Cipolletta et al. 2012), which is crucial for Treg WAT anti-inflammatory function. Initially PPARγ was thought to be unique to adipocytes, but it has later been shown as an important receptor of macrophages and myeloid cells, facilitating their uptake of oxidized lipids (Tontonoz et al. 1998). Its expression by adipose Tregs is the first description of PPARγ in T cells, and it appears to control Treg accumulation in adipose tissue (Cipolletta et al. 2012). Ligands for PPARγ include naturally occurring fatty acids and the thiazolidinedione (TZD) class of anti-diabetic drugs. Tregs, through their expression of PPARγ seem to be necessary for the optimal effect of thiazolidinedione treatment in obese diabetic mice (Cipolletta et al. 2012).

iNKT cells

iNKT cells are innate-type T cells, so called because they express an invariant T cell receptor α-chain, Vz24Jz18, paired with Vβ11 in humans and Vz14Jz18 coupled with either Vβ7, Vβ8.2, or Vβ2 in mice (Matsuda et al. 2000, Brigl & Brenner 2004, Gumperz 2006, Bendelac et al. 2007, Berzins et al. 2011). Unlike adaptive T cells, that recognize peptide antigens presented by MHC class molecules, iNKT cells recognize lipids presented by CD1d antigen-presenting molecules (Matsuda et al. 2000, Brigl & Brenner 2004, Gumperz 2006). Despite advances in identifying endogenous lipids recognized by iNKT cells (Brutkiewicz 2006), the most studied lipid antigen is alpha-galactosylceramide (αGC) (Matsuda et al. 2000, Gumperz 2006), which was originally isolated from a marine sponge and is a potent activator of iNKT cells.

CD1d is expressed by professional antigen-presenting cells, including dendritic cells and macrophages, as well as B and T cells. It is also found on the surface of non-hematopoietic cells including some epithelial cells,
hepatocytes, and adipocytes (Bendelac et al. 2007, Schipper et al. 2012). Therefore, in adipose tissue, several cell types can directly interact with iNKTs. A striking feature of iNKT cells is their rapid production of both Th1 and Th2 cytokines upon activation with CD1d-presented αGC (Bendelac et al. 2007, Matsuda et al. 2008, Berzins et al. 2011), equipping them with considerable immunological potential. Indeed, iNKT are involved in a multitude of disease states including cancer, type 1 diabetes, atherosclerosis, and rheumatoid arthritis (Simoni et al. 2013).

Human and murine adipose tissues are highly enriched for iNKT cells (Lynch et al. 2009, 2012). Analogous to adipose Tregs, iNKTs not only accumulate in adipose tissue, but also represent a unique subset of iNKT cells compared with elsewhere in the body. Microarray analysis revealed that adipose iNKT cells express a distinct genetic profile (L Lynch & MB Brenner, unpublished observations). This includes increased anti-inflammatory cytokine expression, such as IL10, which as discussed previously, promotes alternative M2 macrophage polarization to an anti-inflammatory state. Accumulation of IL10 production by iNKT cells, and their enhancement of M2 macrophages, which produce further IL10, suggests that iNKT cells can directly and indirectly enhance the ability of adipocytes to control metabolism (Fig. 2).

Loading of CD1d with lipid antigen occurs in the ER, and is essential for the correct assembly and surface expression of CD1d (Gumperz 2006, Bendelac et al. 2007). Obesity is associated with increased ER stress in WAT, due to activation of the unfolded protein response (UPR). Importantly, the UPR has been linked to the loss of CD1d in obese liver (Li et al. 2004), which in turn affects iNKT cell levels.

As adipose expands in obesity, iNKT cells become reversibly depleted, with numbers restored following weight loss (Lynch et al. 2009, 2012). Adoptive transfer of iNKT cells into obese mice or in vivo activation of iNKTs with αGC administration causes weight loss, improvement of glucose handling, and insulin sensitivity (Lynch et al. 2012). However, there remains some controversy concerning the beneficial effects of iNKT cells in controlling adipose weight as well as metabolic syndrome. The consensus that restoring iNKT activity (adoptive transfer; iNKT stimulation) is clear, and although a number of reports observe that genetic ablation of iNKT is deleterious in all obesity measures taken, others find no change, or in
some cases, a level of protection (Table 1). A number of technical differences partly account for these varying results, including employment of different strategies to manipulate NKT levels (Cd1d<sup>−/−</sup> vs Jα<sub>18</sub><sup>−/−</sup>), and use of variable high-fat diet compositions and durations. Moreover, other potential disparities that could lead to a dramatic shift in experimental outcome. For example, there are well-established links between the circadian clockwork and inflammatory pathways (Bechtold et al. 2010). Thus, time-of-day differences in tissue collection/experimental challenge might affect the results. Additionally, recent studies have highlighted an interesting relationship between the microbiome and host immune functions. Specifically, iNKT homeostasis is modified by early life changes in the microbiome (Olszak et al. 2012), and thus subtle alterations in the microbiota of the models used (and use of non-littermate controls) in the aforementioned studies could alter the data obtained.

### Conclusions

It has become increasingly clear that endocrine–immune interactions are crucially involved in a reciprocal regulatory system, which in the lean state adopts a ‘virtuous cycle’ with an anti-inflammatory ‘flavor’, but which in obesity develops an increasingly vicious circle promoting chronic adipose inflammation and metabolic dysfunction. Loss- and gain-of-function studies implicate multiple leukocyte subpopulations in the pathology of obesity-related WAT inflammation, underlining the complexity of this system. Additional investigations are necessary to address the primary functions of the individual leukocyte groups in adipose and the mechanisms underlying their alterations in WAT during obesity. Therapeutic interventions that target the molecular components of this phenomenon could prove effective in reducing inflammation, inducing weight loss, and improving metabolic disorder in obese type 2 diabetic patients. One possible target could be iNKT cells. Lipid antigens that target iNKTs have already been used clinically to treat patients with melanoma (Richter et al. 2013). Furthermore, parenteral administration of lipid can activate iNKT cells, making the idea of targeting adipose iNKT cells in obesity a promising and viable strategy. Whether shifting the balance of obese adipose in favor of a tolerogenic immune environment will have meaningful clinical effects remains yet to be determined.

### Declaration of interest

M A E has equity in NKT Therapeutics, Inc.

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