Review doi: 10.5146/tjpath.2015.01323

Immunopathology of Adipose Tissue during Metabolic Syndrome

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ABSTRACT

Excess energy intake and a sedentary lifestyle have led to increasing incidence of obesity which is a major risk factor for the development of insulin resistance. Research in the last two decades has revealed that chronic-low grade inflammation in adipose tissue is a key link between obesity and insulin resistance. As a result, adipose tissue is now considered an active immune organ with a key role in metabolic homeostasis. In the course of obesity, cells of the immune system infiltrate visceral adipose tissue (VAT) in an active process that promotes local and systemic inflammation. This inflammatory process in VAT is driven by various subsets of immune cells and is a central mechanism connecting obesity with its metabolic complications. One key event of adipose tissue inflammation is the switching of macrophages towards a pro-inflammatory phenotype. In addition, recent research has discovered an expanding list of immune cells contributing to this inflammatory process. Pro-inflammatory immune cells are crucial to obese VAT inflammation because of their production of cytokines, which can interfere with insulin signaling in peripheral tissues. This review summarizes our current knowledge of the pathology of innate and adaptive immune cells in obese adipose tissue, with emphasis in the immunological mechanisms mediating obesity-associated insulin resistance.

Key Words: Obesity, Visceral adipose tissue, Inflammation, Immunology, Diabetes mellitus

INTRODUCTION

Obesity and type 2 diabetes (T2D) are growing epidemic concerns with the rates of obesity having more than doubled between 1980 and 2014 (1). The World Health Organization (WHO), estimates that 39% of adults worldwide are overweight and over half a billion are obese (1). It has been estimated that diabetes will become the 7th leading cause of death by 2030 (2). In addition to T2D, obesity and metabolic syndrome are highly associated with other comorbidities such as cardiovascular disease, non-alcoholic fatty liver disease, and cancer (3-6). Over the last decade, researchers have turned to the adipose tissue as a key modulator of obesity and T2D. In addition to its function as a major energy storage depot, adipose tissue is a critical organ for metabolic and thermoregulatory processes. Obesity-related insulin resistance (IR) precedes T2D and is largely linked to chronic low-grade inflammation and altered immunity in the visceral adipose tissue (VAT) in both mice and humans. Here we discuss highlights of the immunopathological processes, both innate and adaptive, in adipose tissue inflammation during obesity-related IR.

COMPONENTS AND REMODELING OF VAT DURING OBESITY

White adipose tissue can be divided into two major stores: subcutaneous and visceral. Subcutaneous fat is located in the hypodermis beneath the skin, whereas visceral fat connects inner organs and accumulates within the abdominal cavity and mediastinum (7). VAT is supported by a loose connective tissue that is predominately populated with tightly packed adipocytes that are vascularized by a dense network of capillaries. A second component of VAT, which excludes adipocytes, is termed the stromal vascular fraction, and includes pre-adipocytes, multi-potent stem cells, fibroblasts, vascular endothelial cells, and immune cells surrounded by the extracellular matrix (ECM). The ECM contains a variety of structural proteins and collagen networks that anchor adipocytes to maintain the structural and functional integrity of the tissue (8).

During obesity, excessive nutrient intake results in an expansion of VAT that alters both adipocyte and stromal vascular compartments (9). Although the number of adipocytes and their turnover rates seem to be consistent between lean and obese states, accumulation of triglycerides causes adipocytes to become hypertrophic (10). This growth

(Turk Patoloji Derg 2015, 31(Suppl):172-180)

Received : 29.05.2015 **Accepted :** 01.06.2015

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in cell size has been associated with increased adipokine and pro-inflammatory cytokine production that are linked to IR (11). Hypertrophic adipocytes are supported by the formation of new vascular networks, or angiogenesis, which can be a source for new therapeutic targets (12, 13). Progressive hypertrophy is accompanied by hypoxia, which likely induces adipocyte cell death via apoptosis or pyroptosis (14, 15). Obese adipose tissue also undergoes fibrotic changes and enhanced expression of different ECM components that can impact adiposity and glucose homeostasis (8, 16, 17). For instance, obese VAT displays increased expression of collagen VI that is associated with greater metabolic risk in humans (16). It is believed that progressive fibrosis in VAT may also limit the amount of fat stored in adipocytes, which promotes the deposition of ectopic fat in the liver and muscle (18). Local inflammation of the growing adipose tissue is believed to drive the remodeling process (19), as well as contribute to underlying IR. Below, we discuss the roles of distinct immune cells in governing inflammation in VAT.

INNATE IMMUNE CELLS

Macrophages

Macrophages are innate immune cells that clear invading foreign pathogens and dying cells via phagocytosis. Diet-induced obese inflammation in adipose tissue is largely mediated by macrophages (20). Early on in obesity, macrophages accumulate in VAT as a result of chemokines and cytokines released by adipocytes and other immune cells (21, 22). Approximately 5% of cells in the VAT of lean individuals are composed of macrophages while this proportion rises to 50% in obese VAT (23, 24). Infiltrating macrophages in VAT of obese mice and humans surround dying adipocytes in an arrangement pathologically manifested as "crown-like structures" (CLS) (25). CLS are a morphological hallmark of obese VAT inflammation (Figure 1). Two major subtypes of macrophages are found in adipose tissue: "alternatively activated" M2 macrophages and "classically activated" M1 macrophages. The proportions of these cell populations in VAT are dependent on the tissue microenvironment. M2 macrophages maintain VAT homeostasis in lean individuals through the secretion of anti-inflammatory cytokines such as IL-10 (26). During obesity, pro-inflammatory M1 polarized macrophages heavily outweigh M2 macrophages (26, 27). M1 macrophages secrete pro-inflammatory cytokines including TNFα, IL-1β and IL-6, some of which can directly alter insulin receptor signaling in adipocytes, leading to IR (26-28). In the CLS, M1 macrophages and adipocytes work closely to promote chronic inflammation and proliferation of other inflammatory immune cells (29).

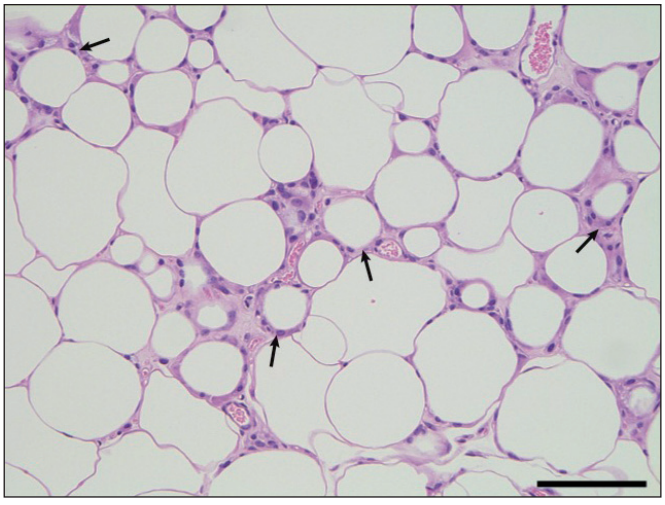

Figure 1: Obese visceral adipose tissue (VAT) exhibits increased accumulation of immune cells that localize around dying adipocytes forming crown like structures (CLS). Histological stain of VAT extracted from C57BL/6 mice fed a high fat diet (HFD) for 12 weeks and stained with hematoxylin and eosin. Arrows indicate representative CLS. Scale bar is set at 100μm.

The mechanisms leading to increased infiltration of macrophages into VAT during diet-induced obesity are not entirely clear. However, during obesity, adipocytes increase their expression of monocyte chemoattractant protein 1 (MCP-1) to recruit macrophages (30). More recently, IL-6 *trans*-signaling, involving the soluble form of the IL-6 receptor (sIL-6R) which binds IL-6 and together then bind gp130 expressing cells, has been shown to act as a chemotactic signal for bone marrow (BM)-derived monocyte migration into VAT (31). Once in the VAT, macrophage production of IL-1β binds IL-1 receptors on BM myeloid progenitors to stimulate monocyte and neutrophil proliferation (32). Further experimentation is required to test whether reducing adipose tissue macrophages or refining the balance of the M1 to M2 macrophage ratio in VAT is a viable treatment option for obesity-induced IR. Nonetheless, macrophages are a dominant immune cell type in mediating adipose tissue inflammation and IR.

Neutrophils

Neutrophils are among the first immune cells to respond at sites of inflammation and are known to fight infections through phagocytosis and the release of antimicrobial contents (33). Higher numbers of circulating activated neutrophils as measured by levels of myeloperoxidase (MPO), an antimicrobial protein, have been found in severely obese patients (34). Neutrophils are believed to be the first immune cell to infiltrate VAT during obesity and

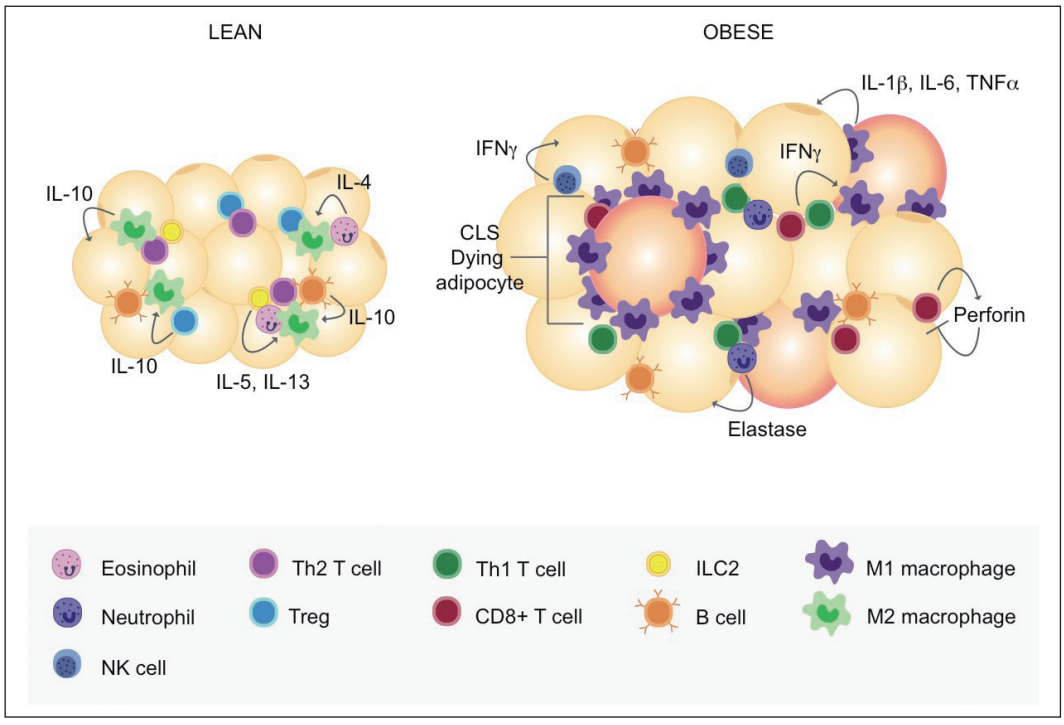

Figure 2: Changes of immune cell populations in adipose tissue during obesity. In lean VAT, regulatory B cells (Bregs), regulatory T cells (Tregs), Th2 T cells, eosinophils and type 2 innate lymphoid cells (ILC2s) maintain an anti-inflammatory environment through the production of IL-10, IL-4, IL-5, and IL-13. These anti-inflammatory cytokines promote anti-inflammatory M2 polarized macrophages in VAT. During obesity, there is an expansion of VAT, which leads to adipocyte hypertrophy, the secretion of adipokines and free fatty acids (FFA), the formation of crown-like structures (CLS) and adipocyte cell death. The immune cell profile in obese VAT is altered to a pro-inflammatory state. This includes increased elastase-secreting neutrophils and IFNγ-secreting CD8 T cells, Th1 T cells and NK cells. Inflammatory mediators promote pro-inflammatory M1 macrophage polarization and their release of IL-1β, IL-6, and TNF-α cytokines. Perforin regulates T cell turnover and inflammatory activity of CD8 T cells in VAT.

initiate adipose tissue inflammation. In mice fed a high fat diet (HFD), an increased proportion of neutrophils were seen in VAT just after 3 days of feeding and maintained up to 12 weeks compared to normal chow diet (NCD)-fed mice (35, 36). Adipose tissue neutrophils in HFD-fed mice have increased secretion of serine proteases, such as elastase, which promote inflammation in a TLR4-dependent manner (35). Addition of elastase to hepatocytes *in vitro* decreased protein levels of important insulin signaling molecules including, IRS-1 and Akt, suggesting that elastase can also directly interfere with insulin signaling (35). Further investigation is needed to elucidate neutrophil function in initiating HFD-induced inflammation in VAT and if blocking the early arrival of these cells in this tissue can improve downstream IR.

Eosinophils

Eosinophils are innate immune cells involved in asthma and allergy, both of which are complicated by obesity (37, 38). Eosinophils are more numerous in lean VAT, and are major producers of IL-4 and IL-13, which help to promote M2 macrophage polarization to maintain a 'lean phenotype'(39). During a HFD feeding, mice deficient in eosinophils showed worsened IR and increased adipose tissue weight highlighting the importance of eosinophils in preventing obesity-related IR (39).

Cold temperatures and exercise can up-regulate meteorin-like hormone (Metrnl) expression in adipocytes and myocytes to stimulate eosinophils to trigger events that increase heat production and energy expenditure (40, 41). Eosinophils can activate M2 macrophages to convert white adipocytes into beige adipocytes in a process termed "browning", due to similar thermogenic properties of beige and brown fat. M2 macrophages induce tyrosine hydroxylase expression and catecholamine production to activate this browning process in adipocytes (40). Administration of IL-4, highly expressed by eosinophils, can improve diet-induced obesity. However, increasing eosinophil numbers may worsen asthma or allergies in obese individuals (37, 38). Thus, the implications of eosinophils in obesity, IR and its associated complications, such as asthma and allergy await further elucidation.

Type 2 Innate Lymphoid Cells (ILC2s)

Innate lymphoid cells (ILCs), originally known to play a role in lymphoid tissue development, have recently been found to regulate inflammatory processes during obesity (42). Along with reduced numbers of eosinophils and M2 macrophages, obese humans and mice reportedly have decreased numbers of type 2 ILCs (ILC2s) in VAT (43). ILC2s produce IL-5 and IL-13 to promote eosinophil and M2 macrophage accumulation in VAT (44). The action of ILC2s is dependent on IL-33 stimulation to increase uncoupling protein 1 (UCP-1) expression in adipocytes, which increases caloric expenditure and promotes the biogenesis of beige adipose tissue (43, 45). Interestingly, ILC2s are able to increase UCP-1 expression in adipocytes directly through the production of methionine-enkephalin peptides (43). Thus, ILC2s may work independently or synergistically with eosinophils to drive the conversion of white to beige fat. Together with eosinophils, ILC2s may be exciting targets for white-beige adipose tissue conversion in future therapeutics for obesity-related IR.

Mast Cells

Mast cells are important inflammatory immune cells during allergy and inflammation but have not been well studied in adipose tissue and obesity. Similar to M1 macrophages and neutrophils, obese individuals have increased mast cells within VAT compared to lean individuals (46, 47). In a hyperglycemia model, high doses of glucose *in vitro* induce the activation of human mast cells to highly express pro-inflammatory cytokines including TNFα, IL-1β, and IL-6 (48). Consistently, diet-induced obese mice deficient in mast cells are protected from weight gain and IR, possibly as a result of reduced VAT inflammation, as demonstrated by decreased amounts of IL-6, TNFα, IFNγ, MCP-1 and matrix metalloprotease-9 in VAT and serum (46). However, recent work challenges the notion of mast cell involvement in IR, as the phenotypes may be more dependent on the Kit mutation used in the mouse models, rather than a deficiency of mast cells themselves (49).

Natural Killer (NK) Cells

Natural killer (NK) cells are cytotoxic innate immune cells that have only been recently investigated in obesity and IR. NK cells are increased in the VAT of obese humans and mice and produce IFNγ, which promotes M1 macrophage polarization (22, 50, 51). Induced ablation or genetic loss of NK cells has been found to improve IR and glucose tolerance (22, 52, 53). HFD in mice induces adipocytes to increase expression of NK cell-activating receptor (NCR1) ligands to stimulate NK cells to proliferate and secrete IFNγ (22). Thus, NK cells may be another important immune cell present in the inflammatory environment of obese VAT contributing to IR.

ADAPTIVE IMMUNE CELLS

T Cells

Numerous studies have supported a role for T cells in the inflammatory process of obese adipose tissue (54). During obesity, the composition of T cells in adipose tissue is altered favoring inflammatory subsets that promote IR. HFD feeding increases the amount of VAT resident T cells, which can be found dispersed in between adipocytes and within the CLS (21). T cells expressing either CD4 or CD8 likely contribute to inflammation in VAT through the release of pro-inflammatory cytokines, such as IFNγ (55). Treatment of HFD-fed mice with an anti-CD3 T cell depleting antibody improved insulin sensitivity and limited adipose tissue inflammation (55). Furthermore, the majority of CD4 and CD8 T cells in VAT are effector memory T cells and express biased T cell receptor (TCR) repertoires, suggesting that the T cell responses may be antigen-specific (56). Although it is unclear whether these responses target specific self-reactive antigens or are merely the result of bystander immune activation, these findings raise the possibility of a potential autoimmune component to obesity-related IR (57).

IFNγ-producing CD8 T cells concentrate in the VAT of obese mice as early as 2 weeks after HFD feeding (58). In addition, subcutaneous adipose tissue display elevated CD8a expression in obese patients (58). Despite no changes in body weight, CD8 T cell deficient mice, or mice treated with anti-CD8 depleting antibody displayed improved glucose tolerance and insulin sensitivity, suggesting a pathogenic role for CD8 T cells in this process (58). Furthermore, depletion of CD8 T cells reduced the number of M1 macrophages in VAT, whereas adoptive transfer of CD8 T cells into CD8 null mice was sufficient to promote M1 macrophage infiltration in VAT, glucose intolerance and IR. A recent study has implicated a role for the cytolytic protein, perforin, which is produced by CD8 T cells and other immune cell types, in mediating T cell turnover in VAT which protects from IR (53).

CD4 T cells can mature into different subsets with distinct effector cytokine profiles including IFNγ-producing Th1 cells, IL-4-producing Th2 cells, IL-17-producing Th17 cells, and IL-10-producing regulatory T (Treg) cells. Th1 cells likely play an important role in VAT inflammation, as several studies have indicated that these cells accumulate in adipose tissue during obesity and outweigh Th2 cells

(55). In humans, weight gain is correlated with increases in Th1 cells, and accordingly, VAT of obese humans has increased expression of the Th1 transcriptional regulator, T-bet (55, 59). IL-12p35 deficient mice, which have reduced Th1 cells, show improved insulin sensitivity during HFD-feeding. In addition, the dominant Th1 effector cytokine IFNγ has been shown to directly impair insulin signaling in human adipocytes (55, 60). Interestingly, IFNγ can also act on adipose tissue macrophages to enhance expression of MHC II, which promotes antigen presentation to Th1 cells resulting in their activation, proliferation and cytokine production (29).

Th17 cells are found in adipose tissue, but at much lower frequencies than Th1 cells (55). In humans, T cell IL-17 production seems to correlate with glucose intolerance, as measured by HBA1c in type 2 diabetics (61). IL-17 deficient HFD-fed mice display increased weight gain, but are more glucose tolerant and insulin sensitive, suggesting a pathogenic role for IL-17 in glucose homeostasis (62). Interestingly, much of the IL-17 produced in VAT is derived from γδ T cells (62) and thus, more work is needed to better clarify roles for Th17 cells in adipose tissue inflammation.

In contrast to pro-inflammatory T cells, T regulatory cells (Tregs) counteract the development of chronic inflammation in VAT. Tregs express the transcription factor Foxp3 and are associated with potent anti-inflammatory capabilities. Tregs are highly enriched in lean adipose tissue and are believed to maintain insulin sensitivity by limiting inflammation and producing insulin-sensitizing cytokines, such as IL-10 (55). In lean adipose tissue, Tregs are also believed to promote the development of anti-inflammatory M2 macrophages (63). Interestingly, compared with other tissues, VAT Tregs express a unique gene signature with enhanced expression of PPARγ, and IL-10 (64). A recent study determined that the accumulation of VAT Tregs is dependent on antigens presented in the context of MHC II molecules and the release of soluble mediators, including IL-33 (65). Over the course of HFD-feeding, Treg numbers decrease in VAT as they are diluted by increasing pro-inflammatory Th1 and CD8 T cells, which intensifies inflammation and adipose dysfunction (55, 64). Human studies measuring Foxp3 in adipose tissue during obesity also report a decrease in the number of Tregs (55). More recently, studies have determined that thiazolidinediones, which are well known PPARγ agonists used in the treatment of T2D, or gut-specific anti-inflammatory agents carry out a part of their insulin sensitizing effects by acting on Tregs to enhance their anti-inflammatory properties (66, 67).

Natural Killer T (NKT) Cells

NKT cells are innate-like T cells that share properties of both T cells and NK cells. They express the natural killer receptor 1.1 (NK1.1) and recognize CD1d associated lipid and glycolipid antigens (68). Based on their TCR diversity, they can be subdivided into variant (vNKT) or invariant (iNKT) cells. In general, VAT NKT cell numbers decrease during obesity in mice and humans (69-71). Whether NKT cells display pro- or anti-inflammatory effects in adipose tissue remains controversial. Studies have reported that the presence of NKT cells in adipose tissue improves glucose tolerance by promoting anti-inflammatory M2 macrophage polarization (69). Other studies have shown that iNKT cells play a role in limiting inflammation in adipose tissue by their release of IL-10 and IL-2, which helps sustain Treg activity (71). In contrast, transgenic mice that develop iNKT cells in excess exhibit dyslipidemia, enhanced M1 macrophages in VAT and impaired insulin sensitivity (72). Overall, the contribution of NKT cells in adipose tissue inflammation and IR remains inconclusive.

B Cells

Several studies have investigated the role of B cells and their different subsets in VAT inflammation. Upon HFD-feeding, pro-inflammatory B cells infiltrate adipose tissue prior to T cells, and promote the release of Th1-polarizing cytokines, such as IFNγ and TNFα that modulate T cell and macrophage function (73, 74). Human B cells, but not monocytes, have been indicated to promote pro-inflammatory cytokine production by Th17 cells during obesity (74). HFD-fed B cell deficient mice are protected from IR, and transfer of IgG antibody from obese wild type mice into obese mice lacking B cells can worsen glucose intolerance and IR (73). Indeed, treatment of obese mice with depleting anti-CD20 antibody reverses IR and reduces VAT inflammation (73). One study reported that B cell inflammation during obesity might not be restricted to adipose tissue, as splenic B cells displayed a unique pro-inflammatory cytokine profile (74). Interestingly, more recent studies have identified the presence of B regulatory cells and B-1a cells in adipose tissue and are more prominent during the lean state (75, 76). Adipose tissue Bregs (defined as IgM+IgD+CD22+), as well as B-1a cells, function to limit inflammation and protect from IR primarily through the release of anti-inflammatory cytokines such as IL-10. HFD-feeding reduces the frequency of these cells in VAT, and the transfer of B-1a cells into HFD-fed B cell deficient mice improves glucose tolerance and insulin sensitivity (76).

CONCLUDING REMARKS

Low-grade chronic inflammation of VAT appears to be one driving link between obesity and IR. A great deal of complexity exists in the crosstalk between adipocytes and VAT-associated immune cells. Although many questions remain unanswered, it is likely that the balance between pro- and anti-inflammatory immune cells in VAT govern the outcome of metabolic disease (Figure 2). Lean VAT is concentrated by a variety of immune cells with potent anti-inflammatory and insulin sensitizing capabilities. During excess food intake, hypertrophic and dying adipocytes lead to fibrotic changes and pro-inflammatory immune cell recruitment in VAT. Activated immune cells and their release of pro-inflammatory cytokines impair insulin signaling, leading to metabolic complications. Targeting VAT inflammation has shown success in the treatment of obesity-related IR in rodent models. Although further work is required, these findings have the potential to translate into new and innovative approaches in human patients to help manage this growing epidemic.

ACKNOWLEDGMENTS

This work was supported in part by CIHR grant 119414 (DW), and CDA grants OG-3-12-3844 (DW) and CS-5-12-3886 (DW). MG and HL received Canada Graduate Scholarships. XR received BBDC Fellowship in Diabetes Care (Eli Lilly Canada).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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