Macrophages, Immunity, and Metabolic Disease

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Chronic, low-grade adipose tissue inflammation is a key etiological mechanism linking the increasing incidence of type 2 diabetes (T2D) and obesity. It is well recognized that the immune system and metabolism are highly integrated, and macrophages, in particular, have been identified as critical effector cells in the initiation of inflammation and insulin resistance. Recent advances have been made in the understanding of macrophage recruitment and retention to adipose tissue and the participation of other immune cell populations in the regulation of this inflammatory process. Here we discuss the pathophysiological link between macrophages, obesity, and insulin resistance, highlighting the dynamic immune cell regulation of adipose tissue inflammation. We also describe the mechanisms by which inflammation causes insulin resistance and the new therapeutic targets that have emerged.

Introduction
Type 2 diabetes (T2D) has become a global epidemic, with huge social and economic costs. The World Health Organization estimates that 3.4 million deaths per year worldwide are attributable to T2D, a number that is predicted to increase in the next decade (http://www.who.int/mediacentre/factsheets/fs312/en/). Approximately $175 billion is spent on diabetes-related healthcare annually in the United States alone (Centers for Disease Control and Prevention, 2011). The majority of cases of diabetes (80%) are attributable to the parallel increasing rates of obesity (http://www.diabetes.org.uk/About_us/News_Landing_Page/Diabetes-and-obesity-rates-soar/) and thus extensive research efforts have been made to elucidate the mechanistic links between these two conditions. Nutrient excess and adiposity activate several metabolic pathways implicated in the development of insulin resistance, including inflammatory signaling, lipotoxicity, aberrant adipokine secretion (Sartipy and Loskutoff, 2003; Steppan et al., 2001; Yamauchi et al., 2001), adipose tissue hypoxia (Cramer et al., 2003), endoplasmic reticulum (ER) stress (Ozcan et al., 2004; Urano et al., 2000), and mitochondrial dysfunction (Furukawa et al., 2004). A detailed description of all of these processes is beyond the scope of this piece and there are excellent recent reviews on these subjects (Samuel and Shulman, 2012; Hotamisligil, 2010; Johnson and Olefsky, 2013; Lee and Ozcan, 2014). Here, we will focus on obesity-associated chronic inflammation, which we believe is a key, unifying component of insulin resistance. Indeed, several of the metabolic processes mentioned above, such as ER stress, hypoxia, and lipotoxicity, can all converge on the development of metabolic inflammation.

Obesity-associated metabolic inflammation is unlike the paradigm of classical inflammation—an acute inflammatory response, defined by the characteristic signs of redness, swelling, and pain. Instead, it is a form of “sterile inflammation” produced in response to metabolic (rather than infectious) stimuli and is chronically sustained at a subacute level without adequate resolution.

The first evidence for a pathophysiological link between obesity, inflammation, and insulin resistance was provided more than a century ago, when it was observed that the anti-inflammatory drug salicylate, the principle metabolite in aspirin, had beneficial effects on glucose control in diabetic patients (Williamson, 1901). This concept was revisited in 1993 when Hotamisligil et al. (1993) demonstrated that tumor necrosis factor-α (TNF-α) (a proinflammatory cytokine) is secreted, in increased amounts, from the adipose tissue of obese rodents and is a potent negative regulator of insulin signaling. The complexity of this inflammatory response was realized, some 10 years later, when two groups independently demonstrated that obesity is associated with the accumulation of macrophages in adipose tissue, which were found to be the principal source of inflammatory mediators, including TNF-α, expressed by this metabolic tissue (Weisberg et al., 2003; Xu et al., 2003). A number of reports have now demonstrated the key importance of macrophage-elicited metabolic inflammation in insulin resistance. During obesity this immune cell population differs, not only in number, but also in inflammatory phenotype and tissue localization. In this review we will focus on the pathophysiological connections between obesity, macrophages, and insulin resistance. In particular, we will describe the mechanisms by which macrophages are recruited to metabolic tissues, mediate inflammation, and impact insulin signaling. We will also discuss current anti-inflammatory therapeutic strategies for the treatment of type 2 diabetes (T2D).

Inflammatory Signaling
The secretion of inflammatory cytokines and chemokines by adipose tissue macrophages (ATMs) extends beyond TNF-α and includes interleukin-6 (IL-6), IL-1β, monocyte chemotactic protein 1 (MCP-1, CCL2), and macrophage inhibitory factor (MIF) (Olefsky and Glass, 2010). Production of these inflammatory factors is under the transcriptional control of two key intracellular inflammatory pathways, c-Jun N-terminal kinase (JNK)-activator protein 1 (AP1) and IKappa B kinase beta (IKK)-nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which differ in their upstream signaling components but converge on the induction of overlapping inflammatory genes. These two inflammatory pathways are initiated by almost
Inflammation plays a significant role in the pathogenesis of disease (Solinas and Karin, 2010). Acids, and inflammatory cytokines, highlighting their importance in the development of insulin resistance, including oxidative and ER stress, saturated fatty acids (Nguyen et al., 2007), ATP, and heat shock proteins. Of the TLRs, TLR4 has been shown to play a particularly important role in initiating saturated fatty acid-mediated macrophage inflammation. Indeed, hematopoietic cell-specific deletion of TLR4 protects mice from high fat diet (HFD)-induced insulin resistance (Orr et al., 2012; Saberi et al., 2009).

Obesity-associated PAMPs and DAMPs have also been shown to activate the nucleotide-binding domain and leucine-rich-repeat-containing (NLR) protein NLRP3 inflammasome, a multiprotein complex comprised of a PRR (NLRP3), a protease (caspase 1), and an adaptor protein. Several studies have shown that obesity is associated with the activation of the inflammasome in adipose tissue (Stienstra et al., 2012; Vandannmagsar et al., 2011). Upon activation of the inflammasome, caspase 1 initiates the maturation of pro-IL-1β and pro-IL-18. Consistent with the proinflammatory effects of these cytokines, genetic ablation of components of the NLRP3 inflammasome ameliorates insulin resistance (Stienstra et al., 2011; Vandannmagsar et al., 2011). In addition to receptor-mediated pathways, inflammatory signaling can be stimulated by cellular stresses such as reactive oxygen species (ROS), ER stress, hypoxia, and lipotoxicity, which can all be enhanced in the obese insulin-resistant state (Cramer et al., 2003; Furukawa et al., 2004; Samuel and Shulman, 2012; Urano et al., 2000; Lee et al., 2014).

Mechanisms of Insulin Resistance in Inflammation

Numerous studies have shown that metabolic inflammation mediates insulin resistance through the inhibition of insulin signaling. Insulin (I) binding to its receptor (R) initiates a complicated signaling cascade (Figure 1). In brief, IR activation stimulates the recruitment and phosphorylation of several IR substrates including insulin receptor substrate 1-4 (IRS-1-4), src homology 2 containing protein (SHC), and growth factor

**Figure 1. Inflammatory Signaling Pathways Implicated in the Development of Insulin Resistance**

Activation of TLR2, TLR4, and/or tumor necrosis factor receptor (TNFR) leads to the activation of NF-κB and JNK signaling. The serine kinases IKKα and IKKβ phosphorylate IRS-1 and IRS-2, inhibiting downstream insulin signaling. In addition, the activation of IKKα leads to the phosphorylation and degradation of the inhibitor of NF-κB, iκB, which permits the translocation of NF-κB to the nucleus. Similarly, the activation of JNK leads to the formation of the AP-1 transcription factor. Nuclear NF-κB and AP-1 transactivate inflammatory genes, which can contribute to insulin resistance in a paracrine manner. Abbreviations are as follows: PI3K, phosphoinositide 3-kinase; RIP, receptor interacting protein; Mydd88, myeloid differentiation primary response gene-88; SFA, saturated fatty acid; TRADD, TNF receptor-associated death domain; TRAF2, TNF receptor-associated factor-2; TRIF, TIR domain containing adaptor protein inducing IFN-γ. Adapted from Osborn and Olefsky (2012).
Several transcriptional and posttranscriptional mechanisms. The phosphatidylinositol 3-kinase pathway (PI3K)-protein kinase B (PKB) pathway plays a major role in eliciting the effects of insulin on metabolism, increasing skeletal muscle and adipocyte glucose uptake, glycogen synthesis, and lipogenesis, while suppressing hepatic glucose production. Activation of the Ras-mitogen activated protein kinase (MAPK) pathway mediates the effect of insulin on mitogenesis and cell growth.

Inflammatory signaling can interfere with insulin action through several transcriptional and posttranscriptional mechanisms. First, stress-activated serine kinases, such as JNK and IKKβ, phosphorylate IRS and IRS proteins at inhibitory sites, attenuating downstream insulin signaling (Gao et al., 2002; Ozes et al., 2001). Accordingly, abrogation of inflammatory signaling with salicylates, which inhibit IKKβ, prevents inhibitory IRS-1 phosphorylation, restoring insulin sensitivity (Gao et al., 2003). Second, the transcription factors NF-κB and AP-1 regulate the expression of several metabolic genes that influence insulin sensitivity. For example, inflammatory mediators induce the expression of suppressor of cytokine signaling (SOCS) proteins which bind to the insulin receptor and impair its ability to phosphorylate IRS-1 and IRS-2 proteins (Emanuelli et al., 2000; Kawazoe et al., 2001; Ueki et al., 2004). Conversely, NF-κB represses the expression of several components of the insulin signaling pathway including glucose transporter type 4 (GLUT4) (Stephens and Pekala, 1991), IRS-1, and AKT (Ruan et al., 2002). Third, JNK signaling can regulate cytokine expression posttranscriptionally by causing stabilization of miRNAs that encode inflammatory cytokines (Chen et al., 2000). Finally, a relatively recent discovery is that inflammatory signals may also influence insulin sensitivity by regulating microRNA (miRNA) expression. For example, TLR4 signaling represses the expression of miR-223, which negatively regulates inflammatory gene expression (Chen et al., 2012; Haneklaus et al., 2013). Several miRNAs are dysregulated in obesity and this topic has been the subject of several recent reviews (Haneklaus et al., 2013; Quijat and Olson, 2013).

Inflammation can also affect insulin action indirectly by modulating various metabolic pathways, resulting in the production of “second messengers,” such as fatty acids, that promote insulin resistance. For example, TNF-α stimulates adipocyte lipolysis contributing to elevated serum free fatty acid (FFA) concentrations, which can lead to decreased insulin sensitivity. Additionally, inflammatory signaling induces the expression of genes involved in lipid processing, including the enzymes that synthesize ceramide, a sphingolipid that inhibits insulin activation of AKT (Holland et al., 2011; Schubert et al., 2000). Indeed, mice lacking TLR4 are protected from ceramide accumulation and insulin resistance after the infusion of saturated fatty acids (Holland et al., 2011), and treating HFD mice with myricin, an inhibitor of ceramide production, improves glucose tolerance (Ussher et al., 2010). Inflammatory mediators also stimulate de novo hepatic lipogenesis, contributing to steatosis and elevated serum lipid levels. Treatment of mice with TNF-α or IL-1β increases the activity of acetyl-CoA carboxylase, the rate-limiting step in lipid synthesis (Feingold and Grunfeld, 1992). Similarly, transgenic overexpression of IKK-β in hepatocytes stimulates de novo hepatic lipogenesis (van Diepen et al., 2011). NF-κB and AP-1 also induce the expression of inflammatory cytokines, which can then act in an autocrine or paracrine manner, initiating a feed-forward loop to exacerbate insulin resistance. In addition, it is thought that if the magnitude of cytokine production is great enough, they can “leak” out of the adipose tissue and potentiate insulin resistance in an endocrine fashion in peripheral tissues such as muscle and liver (Osborn and Olefsky, 2012). In line with this concept, elevated concentrations of TNF-α, IL-6, and MCP-1 have been observed in the serum of individuals with diabetes and prospective studies have shown that circulating inflammatory markers are indicative of future disease risk. However, further studies are required to determine whether circulating cytokines are sufficient to induce insulin resistance or whether they are merely a marker of tissue inflammation.

**Obesity and Adipose Tissue Macrophages**

Adipose tissue macrophages (ATMs) can span the spectrum from an anti-inflammatory to a proinflammatory phenotype. The nomenclature to define different macrophage populations is variable and somewhat confusing, as described in the accompanying review (Murray et al., 2014, this issue). Here we refer to anti-inflammatory macrophages as M2-like or alternatively activated macrophages (AAMs), and proinflammatory macrophages as M1-like or classically activated macrophages (CAMs) (Olefsky and Glass, 2010). AAMs predominantly make up the tissue-resident macrophages dispersed throughout lean adipose tissue and support adipose homeostasis (Odegaaard et al., 2007). Conversely, during obesity, the balance is tilted toward the recruitment of CAMs, which are primarily found in a ring-like configuration around large dying adipocytes, termed crown-like structures (CLSs) (Luceng et al., 2008). These two macrophage populations are phenotypically and functionally distinct. M2 macrophages express CD11b, F4/80, CD301, and CD206 and promote local insulin sensitivity through production of anti-inflammatory cytokines, such as IL-10 (Olefsky and Glass, 2010). In contrast, M1 macrophages express CD11c in addition to CD11b and F4/80 and secrete inflammatory factors including TNF-α, IL-1β, IL-6, leukotriene B4 (LTB4), and nitric oxide (NO) (Luceng et al., 2007).

The recruitment, differentiation, and/or survival of these macrophage subpopulations are contingent on the local signals produced within adipose tissue. The alternative activation of tissue-resident macrophages is mediated by the type 2 cytokine IL-4, which is expressed at high amounts in lean adipose tissue (Wu et al., 2011). IL-4 induces the expression of peroxisome proliferator activated receptor gamma (PPARγ) (Huang et al., 1999) and peroxisome proliferator activated receptor delta (PPARδ) (Kang et al., 2008), which are required for maintenance of the alternatively activated state (Desvergne, 2008; Odegaaard et al., 2007). Conversely, in the obese state, inflammatory mediators released from adipose tissue, such as saturated fatty acids, cytokines, LTB4, and interferon-γ (IFN-γ), induce the recruitment of monocytes and/or their differentiation into M1-like macrophages.

Macrophage polarization states are also associated with differential activation of intrinsic biochemical pathways, including those of glucose, lipid, amino acid, and iron metabolism. For example, M1 macrophages rely on glycolysis and oxidative...
phosphorylation of pyruvate, whereas M2 macrophages exhibit high rates of fatty acid oxidation (Biswas and Mantovani, 2012). Modifications to macrophage metabolic homeostasis result in altered energy supply and the production of lipid- and amino acid-derived mediators, which enable the macrophage to promote or resolve inflammation and contribute to the maintenance of the polarization state. Excellent reviews on this topic have recently been published (Biswas and Mantovani, 2012; Recalcati et al., 2012).

Although the classification of these two distinct ATM populations is useful for experimental purposes, it is important to appreciate that it is an oversimplification. In vivo, macrophages are a heterogeneous population and can display phenotypes across the spectrum from anti- to proinflammatory. Furthermore, ATMs display plasticity and can alter or “switch” phenotypes in response to changes in the local microenvironment (Li et al., 2010).

Mechanisms of Inflammation-Induced Insulin Resistance: Lessons from Animal Models
The most compelling evidence for a mechanistic link between inflammation and insulin resistance has been provided by murine studies that, by a variety of models, have repeatedly demonstrated the etiological role of M1 macrophages in insulin resistance (see Table S1 available online). Although murine models strongly suggest a role for inflammation in the pathogenesis of insulin resistance in human obesity, the fidelity with which these mouse models translate to man is not proven and there are several differences in immune response mechanisms between mice and men. Definitive anti-inflammatory pharmacological studies will be needed to solidify the applicability of mouse to human disease and this is described in more detail later in this review (see Anti-inflammatory Therapeutic Strategies). Nevertheless, several lines of evidence indicate that inflammation is causally linked to insulin resistance in mice. First, the ablation of inflammatory CD11c+ myeloid cells (Pat-sours et al., 2008) or depletion of ATMs by intraperitoneal administration of clodronate liposomes (Bu et al., 2013; Feng et al., 2011) improves glucose tolerance in obese insulin-resistant mice, confirming the requirement of this immune cell population in the etiology of insulin resistance. In addition, studies have shown that the polarization state of ATMs is a key determinant of the adipose tissue inflammatory milieu and insulin sensitivity. Accordingly, mice with a myeloid-specific deletion of the transcriptional regulators PPARγ (Hevener et al., 2007; Odegaard et al., 2007) and PPARα (Desvergne, 2008; Kang et al., 2008; Odegaard et al., 2008), which are critical for the maintenance of the AAM state, display reduced adipose AAMs and are predisposed to HFD-induced adipose tissue inflammation, glucose intolerance, and insulin resistance. Finally, ablation of JNK (Han et al., 2013; Sabio et al., 2008; Solinas et al., 2007; Vallerie et al., 2008; Zhang et al., 2011) or IKKβ (Arkan et al., 2005) protects mice from HFD-induced adipose tissue inflammation, confirming the importance of these inflammatory signaling pathways. In these studies, the gene-targeted mice retained systemic insulin sensitivity, demonstrating that inhibition of inflammatory signals in macrophages is sufficient to mitigate obesity-induced insulin resistance not only in adipose tissue, but also in muscle and liver.

ATM Recruitment
Although macrophages are a key effector cell in the propagation of inflammation, it is clear that adipocytes are an important initiator of the inflammatory response. Adipocytes are not simply a storage depot for excess energy but are dynamic endocrine cells that produce and secrete both proinflammatory and anti-inflammatory bioactive molecules, depending on microenvironmental cues. Secretion of these factors can regulate the recruitment and activation of immune cell populations. During the development of obesity, nutrient excess tips the balance toward the development of a more inflammatory adipocyte state, including the secretion of potent chemoattractants such as MCP-1 and LTB4. These chemoattractants provide a chemotactic gradient for the recruitment of monocytes to adipose tissue, where they subsequently mature into ATMs. In addition, once recruited, proinflammatory macrophages themselves secrete additional chemokines, initiating a feed-forward loop and potentiating the inflammatory response.

Of the known adipocyte-derived chemokines, MCP-1 and its receptor chemokine (C-C motif) receptor 2 (CCR2) have been intensively studied. Several reports have shown that MCP-1 is secreted in parallel with increasing adiposity in both mice and humans (Chen et al., 2005; Christiansen et al., 2005; Kim et al., 2006). In murine models of obesity, adipose tissue expression of MCP-1 is rapidly induced after the initiation of HFD feeding and serum MCP-1 concentrations are significantly elevated after 4 weeks of this regime (Chen et al., 2005). In support of the MCP-1-CCR2 system playing a role in ATM recruitment, CCR2- and MCP-1-deficient mice exhibit reduced ATM content, insulin resistance, and hyperinsulinemia (Gutierrez et al., 2011; Weisberg et al., 2008), and overexpression of adipocyte MCP-1 was sufficient to induce adipose inflammation and insulin resistance in lean mice (Kamei et al., 2006). Furthermore, treatment of mice with a pharmacological antagonist of CCR2 lowered ATM content and improved insulin sensitivity without altering body mass (Sullivan et al., 2013; Tamura et al., 2010). However, other studies have shown that CCR2-deficient mice are not protected from HFD-induced insulin resistance and macrophage accumulation (Chen et al., 2005; Gutierrez et al., 2011). The reasons for these discordant findings are unclear, but the complexity and redundancy of chemokine signaling in different genetic backgrounds may play a role.

The chemoktractant LTB4 and its specific receptor BLT1 have also been implicated in macrophage recruitment to inflamed adipose tissue. LTB4 is synthesized from arachidonic acid by the 5-lipoxygenase (5-LOX) pathway (Spite et al., 2011). The expression and activity of key components of this pathway are increased in adipocytes and M1 macrophages in obesity (Mothe-Satney et al., 2012). Consistent with this, LTB4 concentration is elevated in the adipose tissue and serum of murine models of obesity, in correlation with adipocyte size (Mothe-Satney et al., 2012). Supporting a pathological role for this increase, genetic deletion or pharmacological inhibition of 5-LOX (Mothe-Satney et al., 2012) or 5-LO activating protein (FLAP) (Horillo et al., 2010) protects mice from HFD-induced macrophage accumulation and associated insulin resistance. Targeting the LTB4-BLT1 axis more specifically, recent studies show that genetic depletion of BLT1 protects mice from obesity-induced inflammation and insulin resistance (Spite et al., 2012).
et al., 2011), making this receptor an attractive potential target for drug discovery.

Neuronal guidance molecules, factors typically studied for their role in embryonic axon development, were recently found to participate in the regulation of immune cell function. So far, four families of neural guidance cues have been implicated in the regulation of immune cell migration: the netrins, slits, ephrins, and semaphorins (Funk and Orr, 2013; Wanschel et al., 2013). One such molecule, Semaphorin 3E (Sema3E), can act as an adipocyte-derived chemokine to induce macrophage recruitment to adipose tissue via its receptor PlexinD1, expressed on ATMs. Shimizu et al. (2013) observed that HFD feeding selectively increased Sema3E expression in visceral adipose tissue, accompanied by a parallel increase in serum Sema3E levels. Overexpression of Sema3E in adipocytes induced adipose tissue inflammation and insulin resistance in chow-fed mice, whereas genetic deletion of Sema3E or the sequestration of serum Sema3E with a soluble form of PlexinD1 markedly improved these parameters. Sema3E is also elevated in the serum of diabetic humans, suggesting that this pathway may play a role in human disease (Schmidt and Moore, 2013).

ATM Retention

The majority of studies on ATM accumulation have focused on the recruitment of monocytes to inflamed adipocytes, but macrophage emigration from adipose tissue might also be impaired in the obese state. The resolution of inflammation is a highly orchestrated process involving several cell types and mediators. The egress of macrophages out of inflamed tissue to local lymphoid tissues is an integral part of this process and is due to the concerted effect of chemo-repulsive forces from inflamed tissue and chemo-attractive signals from local lymph nodes (Bellingan et al., 1996; Randolph, 2008). In addition to classical chemokines, neural guidance molecules also regulate this process (van Gils et al., 2012).

The concept that macrophage emigration might be impaired in obese adipose tissue stems from the study of macrophage retention in atherosclerotic plaques. In murine models of atherosclerosis, lowering of serum cholesterol concentrations or transplantation of the aortic arch from atherosclerotic LDL receptor KO mice to WT mice reestablishes macrophage egress to lymph nodes, reducing artery wall inflammation and plaque instability (Feig et al., 2011). These studies have led to the identification of several key pathways that regulate this process. For example, the chemokine receptor CCR7, which is expressed on macrophages, promotes the recruitment of inflammatory macrophages toward chemokine (C-C motif) ligand 19 (CCL19) and CCL21, secreted from lymphoid tissues. Upregulation of CCR7 by atheroma macrophages is necessary for the resolution of inflammation induced by the correction of dyslipidemia (Wan et al., 2013).

There may also be signals that emanate from adipose tissue that prevent macrophage egress. For example, Netrin-1, secreted by macrophages in mouse atheroma, acts in an autocrine/paracrine manner to retard the egress of macrophages that express the Netrin-1 receptor UNC5b. Netrin-1 is particularly interesting because, unlike other chemokines, it blocks macrophage movement by inhibiting actin reorganization, making cells refractory to further chemokine stimuli. It is likely that expression of Netrin-1 by adipocytes or ATMs potentiates the inflammatory phenotype of obese adipose and ATMs potentiates the inflammatory phenotype of obese adipose tissue by inhibiting the process of resolution.

Inflammation in Other Tissue Types

Given the obvious connection between obesity and adiposity, studies have naturally focused on obesity-driven inflammation in adipose tissue. However, obesity can also cause inflammation in other metabolic tissues such as liver, pancreatic islets, and perhaps also muscle.

The liver is the major source of endogenous glucose production, which in the normal state is inhibited by the postprandial rise in insulin elevations. When the liver is insulin resistant, this inhibitory effect is impaired while the stimulatory effect of insulin on lipogenesis remains intact, contributing to the development of hyperglycemia and hepatic steatosis. Many studies have shown that obesity induces hepatic inflammation (Lanthier et al., 2011; Osborn and Olefsky, 2012) associated with a substantial increase in liver macrophages (Johnson and Olefsky, 2013; Ostfield et al., 2010). As in adipose, liver macrophages comprise two populations-resident macrophages, termed Kupffer cells (KCs) and recruited hepatic macrophages (RHMs). KCs are long lived and relatively abundant in the liver, representing about 20%–25% of nonparenchymal cell population, in the noninflamed state (Tang et al., 2013). KCs play an important role in tissue homeostasis, clearing foreign and harmful particles, for which their location in the liver sinusoids makes them well positioned. In contrast, recruited macrophages are short lived and enter the liver in increased numbers during obesity, due to the secretion of chemokines, particularly MCP-1 (Ostfield et al., 2010; Oh et al., 2012). Chemical ablation of phagocytic cells in the liver (including KCs and RHMs) protects mice from HFD-induced insulin resistance, demonstrating the importance of these cells in the development of metabolic dysfunction (Lanthier et al., 2011; Neyrinck et al., 2009). In addition, genetic models have been used to establish a role for hepatic inflammation in insulin sensitivity. Depletion or overexpression of IKK, specifically within hepatocytes, has shown that hepatic inflammation can regulate local insulin sensitivity, but not peripheral insulin sensitivity, in muscle and fat (Arkan et al., 2005; Cai et al., 2005). In obesity, the situation in liver is similar to that in adipose tissue with increased recruitment and activation of liver macrophages, increased inflammatory signaling, and local production of inflammatory cytokines and chemokines. It is likely that the inflammatory cytokines exert paracrine effects to cause hepatic insulin resistance, similar to the situation in adipose tissue (see Figure 2).

Skeletal muscle is the primary site of glucose uptake, accounting for around 80% of insulin-stimulated glucose disposal (Osborn and Olefsky, 2012). Therefore, decreased muscle insulin sensitivity in obesity has a profound effect on hyperglycemia in insulin-resistant individuals. Several studies have shown that obesity is associated with increased muscle inflammatory gene expression, along with macrophage infiltration in both mice and humans (Fink et al., 2013, 2014; Hevener et al., 2007; Nguyen et al., 2007). These macrophages are largely localized to the small intermuscular adipose depots (termed marbling) that arise within skeletal muscle in obesity (Fink et al., 2014).
However, other reports show no increase in macrophage number (Bruun et al., 2006; Tam et al., 2012). It is possible that inflammatory factors released from these intramuscular macrophages can exert paracrine effects to cause local insulin resistance, but this remains to be demonstrated (Figure 2).

**Other Immune Cell Populations**

Besides macrophages, the representation of several other immune cell populations is altered in obese adipose tissue and their influence on insulin resistance has been intensively studied. Several of these immune populations elicit their effects on the potentiation or repression of inflammation by altering the recruitment or activation state of ATMs.

**Innate Immune Cells**

Along with M2-like macrophages, eosinophils serve as a negative regulator of adipose tissue inflammation. Eosinophils are present in the stromal vascular fraction (SVF) of lean adipose tissue, but their representation at this site declines rapidly with adiposity. In the lean state, eosinophils contribute to the repression of adipose tissue inflammation through the production of IL-4, a key driver of alternative macrophage polarization (Goh et al., 2013; Wu et al., 2011). Accordingly, mice deficient of eosinophils exhibit reduced visceral adipose tissue (VAT) AAM content, together with increased weight gain, impaired glucose tolerance, and insulin resistance. Conversely, IL-5 transgenic mice that display relative eosinophilia have elevated VAT AAM adipose tissue content and are protected from HFD-induced obesity and insulin resistance (Wu et al., 2011). Recently a subset of innate lymphoid cells, termed innate lymphoid type 2 (ILC2) cells, have been shown to be key regulators of eosinophil recruitment (Nussbaum et al., 2013). In parallel to eosinophils, ILC2 cells are resident in lean adipose tissue but their content declines with adiposity. These cells secrete the Th2 cytokines IL-5 and IL-13, which induce eosinophil maturation and adipocyte expression of the eotaxin chemokines (CCL11 and CCL24), potent attractors of eosinophils (Molofsky et al., 2013; Nussbaum et al., 2013). Consistent with this, ILC2-deficient mice display reduced adipose eosinophil and M2 macrophage content (Molofsky et al., 2013) and impaired glucose tolerance (Hams et al., 2013), mirroring the phenotype of eosinophil-deficient mice.

Mature mast cells contribute to microbial defense by the secretion of granules, rich in histamine, serine proteases, and cytokines (notably TNF-α and IL-1β). Although best known for their role in allergy and anaphylaxis, one study has shown that mast cells are rapidly recruited to adipose tissue after HFD feeding and might contribute to inflammation and insulin resistance via the secretion of inflammatory cytokines (Liu et al., 2009).

Neutrophils are one of the first responders recruited to adipose tissue after the initiation of HFD feeding, an increase that is maintained in the chronic obese state, thus representing another feature of the proinflammatory response to obesity. Neutrophils circulate in the resting state until they are recruited to sites of infection or tissue damage by the chemokines IL-8, complement 5a (C5a), formyl-methionyl-leucyl-phenylalanine (fMLP), and the chemotactant LTβ4. Upon activation, neutrophils secrete antimicrobial factors such as proinflammatory cytokines and serine proteases. One of these proteases, neutrophil elastase, has been shown to impair insulin signaling by promoting IRS-1 degradation (Talukdar et al., 2012). Mice genetically or pharmacologically deficient in neutrophil elastase are protected from HFD-induced adipose tissue inflammation, glucose tolerance, and insulin resistance. Conversely, injection of recombinant neutrophil elastase into lean mice provokes insulin resistance (Talukdar et al., 2012). In addition to increased elastase secretion, the regulation of elastase activity appears to be
impaired in obesity. The circulating concentration of the endogenous inhibitor of elastase, α1-antitrypsin, is reduced in the obese state, which could potentiate the detrimental effects of neutrophils on insulin sensitivity (Mansuy-Aubert et al., 2013).

**Adaptive Immune Cells**

After ATMs, T cells comprise the next largest immune cell component of adipose tissue, constituting around 10% of the stromal vascular fraction (SVF) in lean mice, and further increasing approximately 3-fold in HFD mice. CD3+ T cells can be classified into two groups dependent on their phenotypic expression of the surface coreceptors CD4 or CD8. CD4+ T cells can be further subcategorized based on their functional profile. T helper (Th) 1 cells and Th17 cells are proinflammatory whereas Th2 cells and T regulatory (Treg) cells are anti-inflammatory in the context of obesity. The number of adipose tissue Th1 cells increases in obesity, whereas the abundance of Th2 and Treg cells, which elicit immunomodulatory suppressive effects, is decreased.

Several recent studies have shown that obesity initiates pathogenic adaptive T cell responses that contribute to HFD-induced insulin resistance. Nishimura et al. (2009) showed that CD8+ T cells interact with ATMs to participate in the development of obesity-driven adipose tissue inflammation. Genetic ablation or antibody-mediated neutralization of CD8+ T cells protected mice from HFD-induced M1 ATM recruitment, adipose tissue inflammation, and insulin resistance. In contrast, adoptive transfer of CD8+ T cells worsened adipose tissue inflammation and insulin sensitivity (Nishimura et al., 2009). In vitro, coculture experiments demonstrated a complex interplay between CD8+ T cells, adipocytes, and M1 macrophages. Adipocytes from obese, but not from lean, mice stimulated CD8+ T cell proliferation. Furthermore, coculture of adipocyte derived CD8+ T cells with peripheral monocytes stimulated chemotaxis and M1 activation, demonstrating that CD8+ T cells can elicit inflammatory effects in vivo via the regulation of CAMs.

CD3+CD4+ Th1 cells have also been implicated as positive regulators of adipose tissue inflammation, via production of IFN-γ, which contributes to the activation of CAMs. Winer et al. (2009) showed that depletion of Th1 cells with CD3-specific antibodies protected mice from HFD-induced adipose tissue inflammation and insulin resistance. In contrast, CD3+CD4+ Th2 cells dampen adipose tissue inflammatory responses. In support of this, reconstitution of lymphocyte-deficient Rag1-null mice with CD4+ cells, which predominantly expand to Th2 cells, reduced adipose tissue inflammation and improved glucose tolerance.

An unusual finding of adipose tissue T cells is their limited T cell receptor (TCR) repertoire (Nishimura et al., 2009; Yang et al., 2010). It has been hypothesized that this is a consequence of local antigen presentation by adipocytes and/or macrophages to CD8+ T cells or CD4+ T cells, in a MHCI- or MHCIIL-dependent manner (see Figure 3; Deng et al., 2013; Morris et al., 2013). This is postulated to promote the clonal expansion of CD4+ T cells (Morris et al., 2013) and CD8+ T cells (Nishimura et al., 2009). Indeed, in vitro primary murine adipocytes (Deng et al., 2013) or ATMs (Morris et al., 2013) promote the proliferation and activation of CD4+ T cells in a contact- and MHCIIL-dependent manner. Furthermore, the ability of adipocytes and ATMs to stimulate T cells was greater when they were isolated from obese compared to lean mice, possibly because of the higher amount of MHCIIL expression by these cells (Deng et al., 2013; Morris et al., 2013). In line with these findings, mice deficient in MHCIIL are protected from obesity-induced adipose tissue inflammation (Deng et al., 2013).

Along with M2-like macrophages, CD3+CD4+FOXP3+ Treg cells are important negative regulators of VAT inflammation. Treg cells are highly enriched in adipose tissue, compared to lymphoid tissues, but their abundance is significantly reduced at this site with obesity, creating an elevated Th1:Treg cell ratio (Feuerer et al., 2009). Interestingly, adipose tissue Treg cells have a unique TCR repertoire and gene expression compared to their counterparts in the spleen and lymph nodes, including greater expression of the PPARγ and anti-inflammatory IL-10. Consistent with their immunosuppressive role, ablation of Treg cells results in an increase in adipose tissue inflammation, associated with increased expression of TNF-α, IL-6, and serum amyloid A-3 (Feuerer et al., 2009). Conversely, the in situ expansion of Treg cells, or adoptive transfer of Treg cells, increased adipose tissue IL-10 expression, reduced adipose tissue inflammation, and improved glucose tolerance (Eller et al., 2011; Feuerer et al., 2009; Ilan et al., 2010; Zhong et al., 2014).

Cipolletta et al. (2012) have shown that the unique profile of adipose tissue Treg cells is driven by their unusual expression of PPARγ (Mathis, 2013). In vitro experiments demonstrated that PPARγ can cooperate with FOXP3 to promote the characteristic adipose Treg cell gene expression profile in naive CD4+ T cells. Consistent with this, the treatment of HFD mice with Pioglitazone, a PPARγ agonist, increased the number of adipose tissue Treg cells and reduced inflammation. Strikingly, much of the well-known insulin-sensitizing effect of Pioglitazone disappeared in mice specifically lacking PPARγ in Treg cells.

B cells, which participate in antigen presentation to T cells, have also been implicated in obesity-induced adipose tissue inflammation. During obesity, IgG2c B cells accumulate in VAT and this is associated with an increase in circulating IgG2c autoantibodies that display a unique antigenic profile (Winer et al., 2011). Depletion of mature B cells results in reduced adipose tissue inflammation and CAM content, by a T-cell-dependent mechanism, reflecting the contribution of antigen presentation by B cells to CD4+ and CD8+ T cell activation (see Figure 3; DeFuria et al., 2013; Winer et al., 2011). In line with this, the introduction of B cells to lymphocyte-deficient Rag1−/− mice failed to impair insulin tolerance, as was observed in T-cell-competent recipient mice. Strikingly, adoptive transfer of serum IgG from HFD, but not NC, mice was sufficient to induce insulin resistance in B-cell-deficient mice, suggesting that pathogenic autoantibodies contribute to obesity-induced metabolic disease (Winer et al., 2011).

Taken together these findings demonstrate that both the innate and adaptive arms of the immune system actively participate in the highly complex regulation of the adipose tissue and systemic inflammatory environment. Due to the nature of innate immune cells, they appear to be the initial responders to cellular stress and can contribute to the further recruitment and activation of immune cells. However, recent findings suggest that adipocyte-derived antigens can activate the adaptive immune system, inducing the local clonal expansion of T cells.
Anti-inflammatory Therapeutic Strategies

Because chronic tissue inflammation is a key etiologic factor in insulin-resistant states, therapeutic attempts to interfere with proinflammatory processes have potential importance. Several clinical approaches have already been tried with varied degrees of success. For example, salicylates have been shown to have anti-inflammatory effects, possibly by inhibition of IKK through poorly defined mechanisms. Indeed, in a mechanistic-based clinical study, Hundal et al. (2002) demonstrated that high-dose salsalate treatment of type 2 diabetic patients led to improved systemic insulin sensitivity, as measured by euglycemic hyperinsulinemic glucose clamp studies, as well as substantial glucose lowering. This proof-of-concept study led to a larger clinical trial, TINSAL-T2D (targeting diabetes using salicylate in T2D) study, which has shown that treatment of type 2 diabetic patients with salsalate for 48 weeks reduced hemoglobin A1C by 0.46%, indicating the therapeutic potential of these kinds of approaches (Goldfine et al., 2013).

Although the TINSAL trial results suggest the potential benefit of anti-inflammatory strategies in metabolic disease, the other approaches that have been tested in clinical development have been unsuccessful or, at best, of only modest benefit. Because the blood levels of TNF-α and IL-1β are elevated in obesity, anti-TNF-α and anti-IL-1β therapies have been studied in the clinic. Although anti-TNF-α antibodies have been effective in rodent models of insulin resistance and diabetes (Hotamisligil et al., 1993), they have produced only marginal beneficial effects in human T2D (Bernstein et al., 2006; Dominguez et al., 2005). The reasons for this are unclear but may relate to penetration of the therapeutic agents to tissues sites of TNF-α action. On the encouraging side, one recent study has shown that patients treated with etanercept, a TNF-α inhibitor, for...
inflammatory conditions such as rheumatoid arthritis and psoriasis demonstrated a decreased risk of developing T2D diabetes (Solomon et al., 2011). Thus, it would seem that future clinical trials are still needed to determine whether TNF-α is a useful target for treatment or prevention of insulin resistance and T2D. Therapies directed against IL-1β have also been explored, using anti-IL-1β antibodies, IL-1β receptor antagonists, or the natural inhibitor of IL-1β, IL-1RA. In general, all these approaches resulted in only small reductions in circulating glucose concentrations with no real evidence that they improve insulin sensitivity (Larsen et al., 2007; van Asseldonk et al., 2011). The glucose-lowering effects of these agents seem to involve modest improvements in β-cell function, potentially due to decreased islet inflammation, because IL-1β might have a deleterious effect on β-cell activity. Other anti-inflammatory strategies have been employed to inhibit recruitment of inflammatory macrophages to sites of inflammation. This is a mechanistically logical approach, because it targets one of the etiologic factors in obesity-induced inflammation, namely recruitment of ATMs to adipose tissue and liver. Indeed, a recently completed phase II clinical trial showed that when adding a CCR2 antagonist to a stable metformin treatment regimen, modest but positive glucose-lowering effects were achieved.

The marketed antidiabetic thiazolidinediones (TZDs, e.g., rosiglitazone and pioglitazone) are PPARγ agonists that are well known to produce insulin-sensitizing effects. Although a full understanding of how these agents induce insulin sensitization is lacking, the beneficial actions can be partially explained by the anti-inflammatory effects of TZDs. PPARγ activation broadly inhibits proinflammatory pathways, leading to decreased ATN content, increased adipose tissue eicosanophils number, and increased differentiation of anti-inflammatory Treg cells (Ahmadian et al., 2013; Hamaguchi and Sakaguchi, 2012). TZDs have many other potential mechanisms for insulin sensitization, such as induction of adiponectin, FGF21, redistribution of fat stores, etc., so the exact contribution of the anti-inflammatory effects to overall systemic insulin sensitization is unknown. Although still early, these studies point to the potential for more targeted and specific anti-inflammatory therapeutics for the treatment of insulin resistance and T2D.

Concluding Remarks and Future Directions
Dramatic progress has been made in recent years in our understanding of the unifying mechanisms that regulate inflammation and insulin resistance. In particular, we now have a much greater appreciation of the complex interactions that occur between macrophages and other immune cell populations that can direct ATM polarization. This development has uncovered several processes that can potentially be exploited therapeutically to dampen the inflammatory response of effector ATMs. For example, inducing the polarization of ATMs toward an M2 phenotype, by direct effects on macrophages, or indirectly targeting other immune cells, is likely to have potent insulin-sensitizing effects. In addition, omega 3 fatty acids are well-known anti-inflammatory agents, and GPR120 has recently been identified as the omega 3 fatty acid receptor/sensor (Oh et al., 2010). Pharmacologic activation of this receptor leads to potent anti-inflammatory (Hudson et al., 2013), insulin-sensitizing effects, which appear targeted toward the mechanisms specifically related to insulin resistance. Therefore, this receptor has emerged as a drug discovery target for these purposes. Another key development is the identification of chemokines, such as LTB4, Sema3E, CCR7, and Netrin-1, that contribute to ATM recruitment and retention at sites of metabolic inflammation (Shimizu et al., 2013; Spite et al., 2011; van Gils et al., 2012). These findings suggest that treatment with a combination of chemokine inhibitors may have insulin-sensitizing effects.

New diabetes therapies are needed because the current therapeutic options to target inflammation have failed to provide a clinically significant improvement in insulin sensitivity. The reasons for this are unclear, but might be due to redundancy in inflammatory signaling. For example, targeting TNF-α or IL-1β alone leaves several other inflammatory pathways that can still activate TLR4, TNFR, and IL-1R. A more effective approach might be to target the steps in inflammatory pathways that are more specific to inflammation-induced insulin resistance. Such approaches might be more efficacious and avoid unwanted side effects. This latter point is of key clinical importance, because one must always be concerned that an anti-inflammatory therapy could potentially cause a drug-induced state of relative immunodeficiency leading to susceptibility to infections, as well as other unwanted side effects.

Despite recent advances, there are still a number of unanswered questions in the field of immunometabolism. The relative contribution of inflammation to systemic insulin sensitivity in man is still unproven. Do macrophages that are recruited to muscle and pancreatic islets upon HFD or obesity induce local skeletal muscle insulin resistance or β-cell dysfunction, or are they simply an indicator of late-stage disease? What are the inflammatory mediators that directly cause insulin resistance and through what mechanism can reigning in what has been termed “metainflammation” at its earliest stages prevent disease onset? A striking feature about many of the genetic studies in mice is that disruption of adipose tissue inflammation is sufficient to cause systemic insulin sensitization. Therefore, how do events in adipose tissue lead to beneficial changes in liver and muscle, with respect to improved insulin sensitivity? Finally, although obesity-induced ATM accumulation and inflammation in adipose tissue causes metabolic disease, there must be a homeostatic purpose for these events in the first place. Answering these questions would enable the development of more effective, appropriately targeted therapies.

It is likely that in the next few years rapid developments in the field of immunometabolism will continue, accompanied by the discovery of new targets, eventually leading to better therapies for insulin-resistant states in man.

SUPPLEMENTAL INFORMATION
Supplemental Information includes one table and Supplemental References and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2014.05.010.

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complex plays an essential role in obesity-induced adipose inflammation. Cell Metab. 17, 411–422.


