CellPress

Immunity
Review

Macrophages, Immunity, and Metabolic Disease

Joanne C. McNelis[1] and Jerrold M. Olefsky[1,*]
[1]Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA
*Correspondence: jolefsky@ucsd.edu
http://dx.doi.org/10.1016/j.immuni.2014.05.010

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

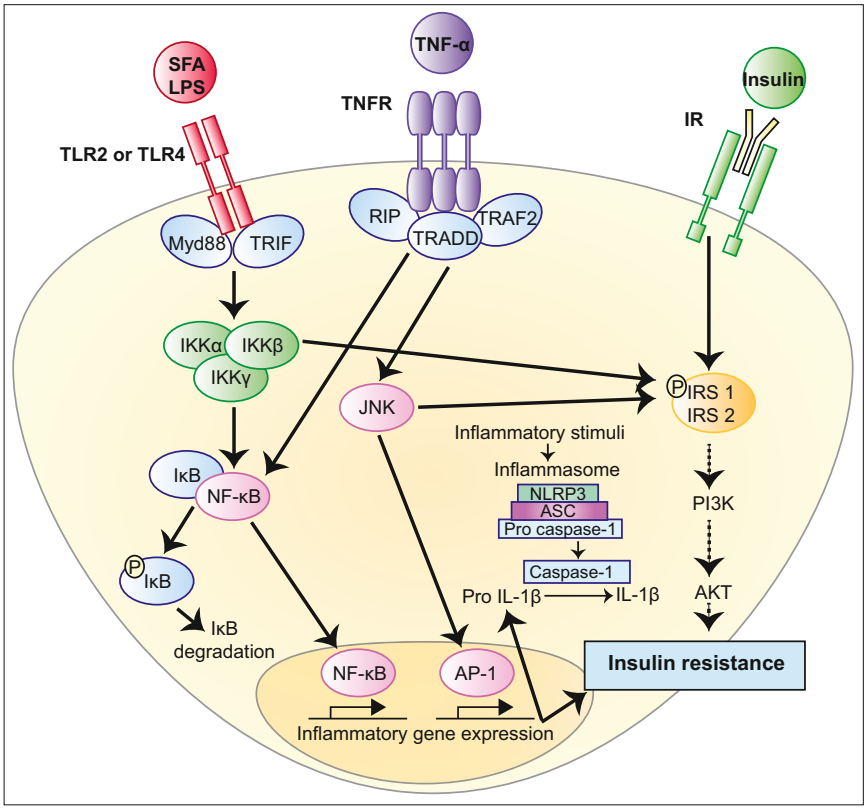

Figure 1. Inflammatory Signaling Pathways Implicated in the Development of Insulin Resistance

Activation of TLR2, TLR4, and/or tumor necrosis factor receptor (TNFR) leads to the activation of NF-κB and JNK signaling. The serine kinases IKKβ and JNK phosphorylate IRS-1 and IRS-2, inhibiting downstream insulin signaling. In addition, the activation of IKKβ 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; Myd88, 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).

all of the mediators implicated in the development of insulin resistance, including oxidative and ER stress, saturated fatty acids, and inflammatory cytokines, highlighting their importance in the pathogenesis of disease (Solinas and Karin, 2010). IKKβ-NF-κB signaling is initiated by activation of IKKβ and subsequent phosphorylation of the inhibitor of NF-κB (IκB). In the noninflammatory state, IκB retains NF-κB in an inhibitory cytoplasmic complex. After inflammatory stimuli, IκB is phosphorylated, dissociates from NF-κB, and undergoes degradation. This permits the translocation of free NF-κB to the nucleus, where it binds to cognate DNA response elements, leading to transactivation of inflammatory genes. Similarly, activation of JNK-AP-1 signaling by inflammatory mediators leads to phosphorylation and activation of JNK, which then phosphorylates the N terminus of c-Jun. This initiates a switch of c-Jun dimers for c-Jun-c-Fos heterodimers, which ultimately stimulate transcription of inflammatory target genes. Both JNK1 and IKK signaling are upregulated in adipose (Weisberg et al., 2003; Xu et al., 2003), skeletal muscle (Bandyopadhyay et al., 2005), and liver (Cai et al., 2005) from insulin-resistant rodents and humans.

Obesity activates JNK and NF-κB signaling by several mechanisms. For example, IL-1 and TNF-α instigate inflammatory signaling through classical activation of their cell surface receptors (Olefsky and Glass, 2010). Alternatively, the inflammatory process can be initiated by activation of pattern recognition receptors (PRRs), which include Toll-like receptors (TLRs) and NOD-like receptors (NLRs). PRRs sense exogenous pathogen-associated molecular patterns (PAMPs), including microbial derived LPS, peptidoglycan, and bacterial DNA, as well as endogenous damage-associated molecular patterns (DAMPs), such as 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; Vandanmagsar 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; Vandanmagsar 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

receptor-bound protein 2 (Grb-2), which leads to the activation of two downstream signaling pathways. 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 mRNAs 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; Quiat 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 myriocin, 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 and support adipose homeostasis (Odegaard 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) (Lumeng 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) (Lumeng 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; Odegaard 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 (Patsouris 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 PPARδ (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., 2006), 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 chemoattractant LTB4 and its specific receptor BLT1 have also been implicated in macrophage recruitment to inflamed adipose tissue. LTB4 is synthesized from arachadonic 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) (Horrillo 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., 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 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 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 causes 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; Obstfeld 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 (Obstfeld 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 IKKβ, 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).

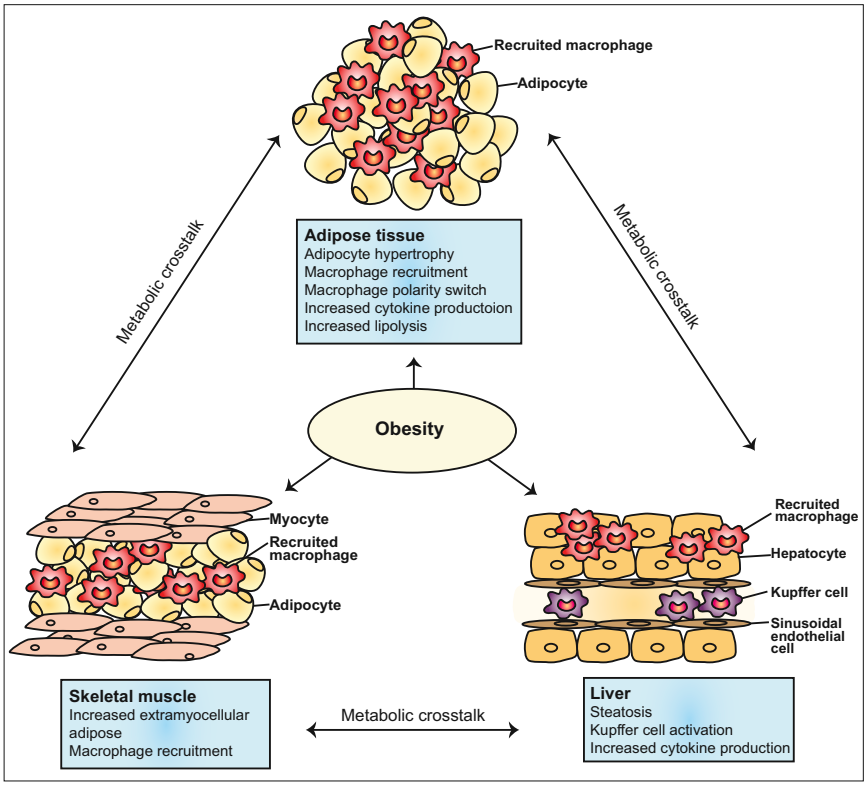

Figure 2. Obesity Induces Inflammation in Adipose Tissue, Liver, and Skeletal Muscle
In the obese state, adipocyte hypertrophy and apoptosis promote the recruitment of monocytes to adipose tissue, where in response to inflammatory stimuli they differentiate into M1 inflammatory macrophages. In muscle, obesity is associated with increased extramyocellular adipose, which is proposed to recruit macrophages to this site. In the liver, obesity causes increased hepatic lipogenesis and inflammatory gene expression that promotes the activation of resident Kupffer cells and the recruitment of monocytes. M1-like macrophages secrete inflammatory cytokines that can induce insulin resistance locally or enter the peripheral circulation and cause systemic insulin resistance and inflammation. Adapted from Olefsky and Glass (2010).

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 chemoattractant LTB4. 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 immunological 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 adipose 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 Rag-1-null mice with $CD4^+$ cells, which predominately 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 MHCII-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 MHCII-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 MHCII expression by these cells (Deng et al., 2013; Morris et al., 2013). In line with these findings, mice deficient in MHCII 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, IgG^+ 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 RAG-1-deficient 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 T cells

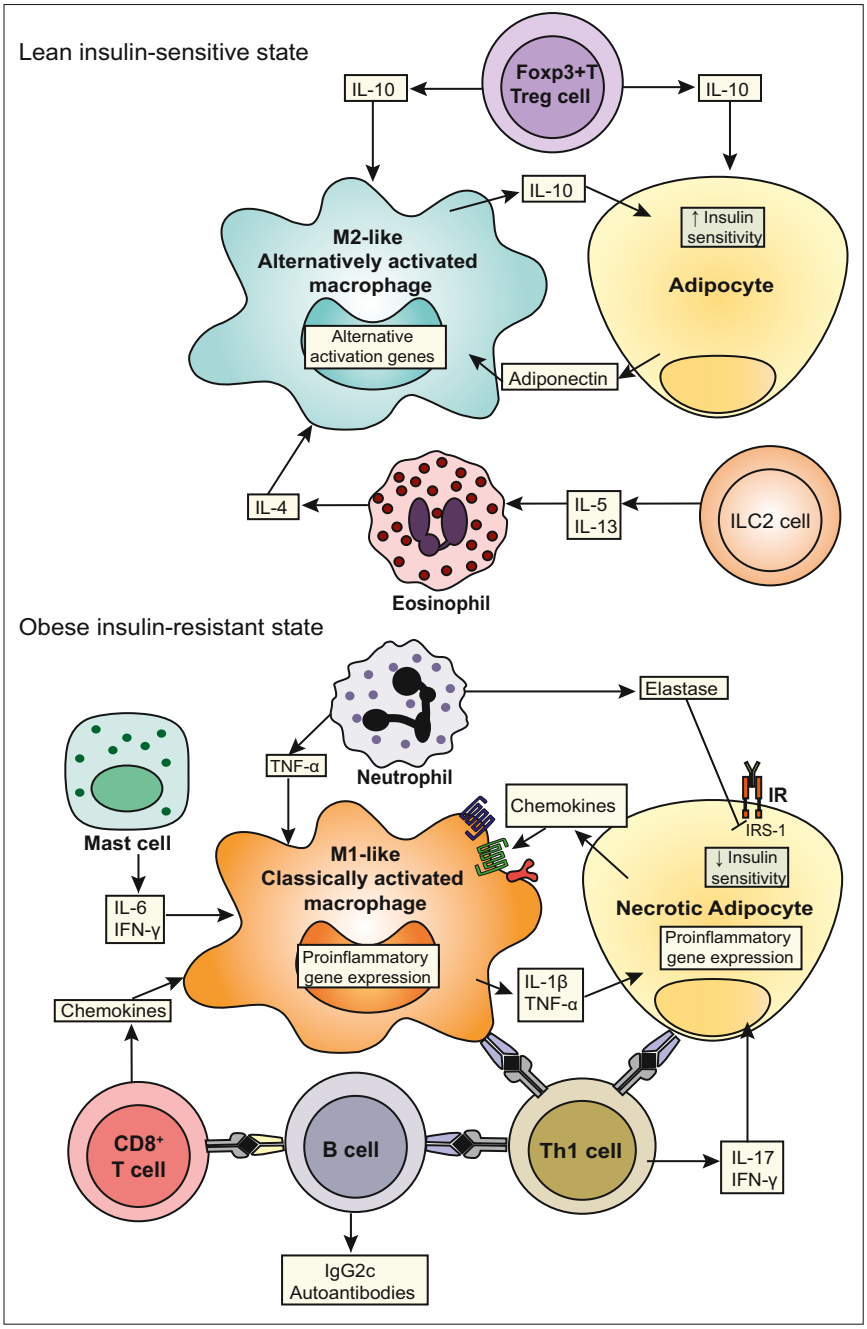

Figure 3. Regulation of Adipose Insulin Sensitivity by Innate and Adaptive Immune Cells

In the lean insulin-sensitive state (top), ILC2 cells produce IL-5 and IL-13, which promote the maturation and recruitment of eosinophils. Eosinophils and FoxP3$^+$ Treg cells maintain the alternative activation of macrophages through the production of IL-4 and IL-10. The production of adiponectin by adipocytes also contributes to the maintenance of AAMs. The alternative activation state of macrophages supports adipocyte insulin sensitivity. In the obese state (bottom), adipocytes undergo cellular stress and secrete proinflammatory cytokines and chemokines, which stimulate the recruitment of monocytes and their differentiation into M1 macrophages. Neutrophils inhibit insulin signaling by the production of elastase. Macrophages and adipocytes are potentially capable of presenting antigens on MHC class II molecules to T cells and B cells, inducing an adaptive immune response. This is hypothesized to induce the clonal expansion of T cells and promote recruitment of B cells. Recruited B cells induce MHCI- and MHCII-dependent proinflammatory cytokine production by CD4$^+$ and CD8$^+$ T cells and produce pathogenic autoantibodies. The production of proinflammatory cytokines and chemokines by Th1 cells, CD8$^+$ cells, and mast cells and neutrophils sets up a feed-forward loop of immune cell recruitment, further contributing to insulin resistance.

and B cells that participate in the maintenance of the inflammatory response.

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 ATM content, increased adipose tissue eosinophil numbers, 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.

ACKNOWLEDGMENTS

This work was supported by NIH grants DK-033651, DK-074868, DK-063491, and P01-DK054441.

REFERENCES

Ahmadian, M., Suh, J.M., Hah, N., Liddle, C., Atkins, A.R., Downes, M., and Evans, R.M. (2013). PPARγ signaling and metabolism: the good, the bad and the future. Nat. Med. *19*, 557–566.

Arkan, M.C., Hevener, A.L., Greten, F.R., Maeda, S., Li, Z.-W., Long, J.M., Wynshaw-Boris, A., Poli, G., Olefsky, J., and Karin, M. (2005). IKK-beta links inflammation to obesity-induced insulin resistance. Nat. Med. *11*, 191–198.

Bandyopadhyay, G.K., Yu, J.G., Ofrecio, J., and Olefsky, J.M. (2005). Increased p85/55/50 expression and decreased phosphotidylinositol 3-kinase activity in insulin-resistant human skeletal muscle. Diabetes *54*, 2351–2359.

Bellingan, G.J., Caldwell, H., Howie, S.E., Dransfield, I., and Haslett, C. (1996). In vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. J. Immunol. *157*, 2577–2585.

Bernstein, L.E., Berry, J., Kim, S., Canavan, B., and Grinspoon, S.K. (2006). Effects of etanercept in patients with the metabolic syndrome. Arch. Intern. Med. *166*, 902–908.

Biswas, S.K., and Mantovani, A. (2012). Orchestration of metabolism by macrophages. Cell Metab. *15*, 432–437.

Bruun, J.M., Helge, J.W., Richelsen, B., and Stallknecht, B. (2006). Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. Am. J. Physiol. Endocrinol. Metab. *290*, E961–E967.

Bu, L., Gao, M., Qu, S., and Liu, D. (2013). Intraperitoneal injection of clodronate liposomes eliminates visceral adipose macrophages and blocks high-fat diet-induced weight gain and development of insulin resistance. AAPS J. *15*, 1001–1011.

Cai, D., Yuan, M., Frantz, D.F., Melendez, P.A., Hansen, L., Lee, J., and Shoelson, S.E. (2005). Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat. Med. *11*, 183–190.

Centers for Disease Control and Prevention (2011). National Diabetes Fact Sheet: National Estimates and General Information on Diabetes and Prediabetes in the United States. (Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention).

Chen, C.Y., Gherzi, R., Andersen, J.S., Gaietta, G., Jürchott, K., Royer, H.D., Mann, M., and Karin, M. (2000). Nucleolin and YB-1 are required for JNK-mediated interleukin-2 mRNA stabilization during T-cell activation. Genes Dev. *14*, 1236–1248.

Chen, A., Mumick, S., Zhang, C., Lamb, J., Dai, H., Weingarth, D., Mudgett, J., Chen, H., MacNeil, D.J., Reitman, M.L., and Qian, S. (2005). Diet induction of monocyte chemoattractant protein-1 and its impact on obesity. Obes. Res. *13*, 1311–1320.

Chen, Q., Wang, H., Liu, Y., Song, Y., Lai, L., Han, Q., Cao, X., and Wang, Q. (2012). Inducible microRNA-223 down-regulation promotes TLR-triggered IL-6 and IL-1β production in macrophages by targeting STAT3. PLoS ONE *7*, e42971.

Christiansen, T., Richelsen, B., and Bruun, J.M. (2005). Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. Int. J. Obes. (Lond.) *29*, 146–150.

Cipolletta, D., Feuerer, M., Li, A., Kamei, N., Lee, J., Shoelson, S.E., Benoist, C., and Mathis, D. (2012). PPAR-γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. Nature *486*, 549–553.

Cramer, T., Yamanishi, Y., Clausen, B.E., Förster, I., Pawlinski, R., Mackman, N., Haase, V.H., Jaenisch, R., Corr, M., Nizet, V., et al. (2003). HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell *112*, 645–657.

DeFuria, J., Belkina, A.C., Jagannathan-Bogdan, M., Snyder-Cappione, J., Carr, J.D., Nersesova, Y.R., Markham, D., Strissel, K.J., Watkins, A.A., Zhu, M., et al. (2013). B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. Proc. Natl. Acad. Sci. USA *110*, 5133–5138.

Deng, T., Lyon, C.J., Minze, L.J., Lin, J., Zou, J., Liu, J.Z., Ren, Y., Yin, Z., Hamilton, D.J., Reardon, P.R., et al. (2013). Class II major histocompatibility complex plays an essential role in obesity-induced adipose inflammation. Cell Metab. *17*, 411–422.

Desvergne, B. (2008). PPARdelta/beta: the lobbyist switching macrophage allegiance in favor of metabolism. Cell Metab. *7*, 467–469.

Dominguez, H., Storgaard, H., Rask-Madsen, C., Steffen Hermann, T., Ihlemann, N., Baunbjerg Nielsen, D., Spohr, C., Kober, L., Vaag, A., and Torp-Pedersen, C. (2005). Metabolic and vascular effects of tumor necrosis factor-alpha blockade with etanercept in obese patients with type 2 diabetes. J. Vasc. Res. *42*, 517–525.

Eller, K., Kirsch, A., Wolf, A.M., Sopper, S., Tagwerker, A., Stanzl, U., Wolf, D., Patsch, W., Rosenkranz, A.R., and Eller, P. (2011). Potential role of regulatory T cells in reversing obesity-linked insulin resistance and diabetic nephropathy. Diabetes *60*, 2954–2962.

Emanuelli, B., Peraldi, P., Filloux, C., Sawka-Verhelle, D., Hilton, D., and Van Obberghen, E. (2000). SOCS-3 is an insulin-induced negative regulator of insulin signaling. J. Biol. Chem. *275*, 15985–15991.

Feig, J.E., Shang, Y., Rotllan, N., Vengrenyuk, Y., Wu, C., Shamir, R., Torra, I.P., Fernandez-Hernando, C., Fisher, E.A., and Garabedian, M.J. (2011). Statins promote the regression of atherosclerosis via activation of the CCR7-dependent emigration pathway in macrophages. PLoS ONE *6*, e28534.

Feingold, K.R., and Grunfeld, C. (1992). Role of cytokines in inducing hyperlipidemia. Diabetes *41* (*Suppl 2*), 97–101.

Feng, B., Jiao, P., Nie, Y., Kim, T., Jun, D., van Rooijen, N., Yang, Z., and Xu, H. (2011). Clodronate liposomes improve metabolic profile and reduce visceral adipose macrophage content in diet-induced obese mice. PLoS ONE *6*, e24358.

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A.B., Benoist, C., Shoelson, S., and Mathis, D. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat. Med. *15*, 930–939.

Fink, L.N., Oberbach, A., Costford, S.R., Chan, K.L., Sams, A., Blüher, M., and Klip, A. (2013). Expression of anti-inflammatory macrophage genes within skeletal muscle correlates with insulin sensitivity in human obesity and type 2 diabetes. Diabetologia *56*, 1623–1628.

Fink, L.N., Costford, S.R., Lee, Y.S., Jensen, T.E., Bilan, P.J., Oberbach, A., Blüher, M., Olefsky, J.M., Sams, A., and Klip, A. (2014). Pro-Inflammatory macrophages increase in skeletal muscle of high fat-Fed mice and correlate with metabolic risk markers in humans. Obesity (Silver Spring) *22*, 747–757.

Funk, S.D., and Orr, A.W. (2013). Ephs and ephrins resurface in inflammation, immunity, and atherosclerosis. Pharmacol. Res. *67*, 42–52.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., and Shimomura, I. (2004). Increased oxidative stress in obesity and its impact on metabolic syndrome. J. Clin. Invest. *114*, 1752–1761.

Gao, Z., Hwang, D., Bataille, F., Lefevre, M., York, D., Quon, M.J., and Ye, J. (2002). Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. J. Biol. Chem. *277*, 48115–48121.

Gao, Z., Zuberi, A., Quon, M.J., Dong, Z., and Ye, J. (2003). Aspirin inhibits serine phosphorylation of insulin receptor substrate 1 in tumor necrosis factor-treated cells through targeting multiple serine kinases. J. Biol. Chem. *278*, 24944–24950.

Goh, Y.P.S., Henderson, N.C., Heredia, J.E., Red Eagle, A., Odegaard, J.I., Lehwald, N., Nguyen, K.D., Sheppard, D., Mukundan, L., Locksley, R.M., and Chawla, A. (2013). Eosinophils secrete IL-4 to facilitate liver regeneration. Proc. Natl. Acad. Sci. USA *110*, 9914–9919.

Goldfine, A.B., Buck, J.S., Desouza, C., Fonseca, V., Chen, Y.-D.I., Shoelson, S.E., Jablonski, K.A., and Creager, M.A.; TINSAL-FMD (Targeting Inflammation Using Salsalate in Type 2 Diabetes–Flow-Mediated Dilation) Ancillary Study Team (2013). Targeting inflammation using salsalate in patients with type 2 diabetes: effects on flow-mediated dilation (TINSAL-FMD). Diabetes Care *36*, 4132–4139.

Gutierrez, D.A., Kennedy, A., Orr, J.S., Anderson, E.K., Webb, C.D., Gerrald, W.K., and Hasty, A.H. (2011). Aberrant accumulation of undifferentiated myeloid cells in the adipose tissue of CCR2-deficient mice delays improvements in insulin sensitivity. Diabetes *60*, 2820–2829.

Hamaguchi, M., and Sakaguchi, S. (2012). Regulatory T cells expressing PPAR-γ control inflammation in obesity. Cell Metab. *16*, 4–6.

Hams, E., Locksley, R.M., McKenzie, A.N.J., and Fallon, P.G. (2013). Cutting edge: IL-25 elicits innate lymphoid type 2 and type II NKT cells that regulate obesity in mice. J. Immunol. *191*, 5349–5353.

Han, M.S., Jung, D.Y., Morel, C., Lakhani, S.A., Kim, J.K., Flavell, R.A., and Davis, R.J. (2013). JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. Science *339*, 218–222.

Haneklaus, M., Gerlic, M., O'Neill, L.A.J., and Masters, S.L. (2013). miR-223: infection, inflammation and cancer. J. Intern. Med. *274*, 215–226.

Hevener, A.L., Olefsky, J.M., Reichart, D., Nguyen, M.T.A., Bandyopadyhay, G., Leung, H.-Y., Watt, M.J., Benner, C., Febbraio, M.A., Nguyen, A.-K., et al. (2007). Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. J. Clin. Invest. *117*, 1658–1669.

Holland, W.L., Bikman, B.T., Wang, L.-P., Yuguang, G., Sargent, K.M., Bulchand, S., Knotts, T.A., Shui, G., Clegg, D.J., Wenk, M.R., et al. (2011). Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J. Clin. Invest. *121*, 1858–1870.

Horrillo, R., González-Périz, A., Martínez-Clemente, M., López-Parra, M., Ferré, N., Titos, E., Morán-Salvador, E., Deulofeu, R., Arroyo, V., and Clària, J. (2010). 5-lipoxygenase activating protein signals adipose tissue inflammation and lipid dysfunction in experimental obesity. J. Immunol. *184*, 3978–3987.

Hotamisligil, G.S. (2010). Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell *140*, 900–917.

Hotamisligil, G.S., Shargill, N.S., and Spiegelman, B.M. (1993). Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science *259*, 87–91.

Huang, J.T., Welch, J.S., Ricote, M., Binder, C.J., Willson, T.M., Kelly, C., Witztum, J.L., Funk, C.D., Conrad, D., and Glass, C.K. (1999). Interleukin-4-dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. Nature *400*, 378–382.

Hudson, B.D., Shimpukade, B., Mackenzie, A.E., Butcher, A.J., Pediani, J.D., Christiansen, E., Heathcote, H., Tobin, A.B., Ulven, T., and Milligan, G. (2013). The pharmacology of TUG-891, a potent and selective agonist of the free fatty acid receptor 4 (FFA4/GPR120), demonstrates both potential opportunity and possible challenges to therapeutic agonism. Mol. Pharmacol. *84*, 710–725.

Hundal, R.S., Petersen, K.F., Mayerson, A.B., Randhawa, P.S., Inzucchi, S., Shoelson, S.E., and Shulman, G.I. (2002). Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J. Clin. Invest. *109*, 1321–1326.

Ilan, Y., Maron, R., Tukpah, A.-M., Maioli, T.U., Murugaiyan, G., Yang, K., Wu, H.Y., and Weiner, H.L. (2010). Induction of regulatory T cells decreases adipose inflammation and alleviates insulin resistance in ob/ob mice. Proc. Natl. Acad. Sci. USA *107*, 9765–9770.

Johnson, A.M.F., and Olefsky, J.M. (2013). The origins and drivers of insulin resistance. Cell *152*, 673–684.

Kamei, N., Tobe, K., Suzuki, R., Ohsugi, M., Watanabe, T., Kubota, N., Ohtsuka-Kowatari, N., Kumagai, K., Sakamoto, K., Kobayashi, M., et al. (2006). Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J. Biol. Chem. *281*, 26602–26614.

Kang, K., Reilly, S.M., Karabacak, V., Gangl, M.R., Fitzgerald, K., Hatano, B., and Lee, C.-H. (2008). Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab. *7*, 485–495.

Kawazoe, Y., Naka, T., Fujimoto, M., Kohzaki, H., Morita, Y., Narazaki, M., Okumura, K., Saitoh, H., Nakagawa, R., Uchiyama, Y., et al. (2001). Signal transducer and activator of transcription (STAT)-induced STAT inhibitor 1 (SSI-1)/suppressor of cytokine signaling 1 (SOCS1) inhibits insulin signal transduction pathway through modulating insulin receptor substrate 1 (IRS-1) phosphorylation. J. Exp. Med. *193*, 263–269.

Kim, C.-S., Park, H.-S., Kawada, T., Kim, J.-H., Lim, D., Hubbard, N.E., Kwon, B.-S., Erickson, K.L., and Yu, R. (2006). Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int. J. Obes. (Lond.) *30*, 1347–1355.

Lanthier, N., Molendi-Coste, O., Cani, P.D., van Rooijen, N., Horsmans, Y., and Leclercq, I.A. (2011). Kupffer cell depletion prevents but has no therapeutic effect on metabolic and inflammatory changes induced by a high-fat diet. FASEB J. *25*, 4301–4311.

Larsen, C.M., Faulenbach, M., Vaag, A., Vølund, A., Ehses, J.A., Seifert, B., Mandrup-Poulsen, T., and Donath, M.Y. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N. Engl. J. Med. *356*, 1517–1526.

Lee, J., and Ozcan, U. (2014). Unfolded protein response signaling and metabolic diseases. J. Biol. Chem. *289*, 1203–1211.

Lee, Y.S., Jung-whan, K., Osborne, O., Oh, D.Y., Sasik, R., Schenk, S., Chen, A., Chung, H., Murphy, A., Watkins, S.M., et al. (2014). Increased adipocyte O_2 consumption triggers HIF-1α, causing inflammation and insulin resistance in obesity. Cell *157*, 1339–1352.

Li, P., Lu, M., Nguyen, M.T.A., Bae, E.J., Chapman, J., Feng, D., Hawkins, M., Pessin, J.E., Sears, D.D., Nguyen, A.-K., et al. (2010). Functional heterogeneity of CD11c-positive adipose tissue macrophages in diet-induced obese mice. J. Biol. Chem. *285*, 15333–15345.

Liu, J., Divoux, A., Sun, J., Zhang, J., Clément, K., Glickman, J.N., Sukhova, G.K., Wolters, P.J., Du, J., Gorgun, C.Z., et al. (2009). Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nat. Med. *15*, 940–945.

Lumeng, C.N., Deyoung, S.M., Bodzin, J.L., and Saltiel, A.R. (2007). Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes *56*, 16–23.

Lumeng, C.N., DelProposto, J.B., Westcott, D.J., and Saltiel, A.R. (2008). Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. Diabetes *57*, 3239–3246.

Mansuy-Aubert, V., Zhou, Q.L., Xie, X., Gong, Z., Huang, J.-Y., Khan, A.R., Aubert, G., Candelaria, K., Thomas, S., Shin, D.-J., et al. (2013). Imbalance between neutrophil elastase and its inhibitor α1-antitrypsin in obesity alters insulin sensitivity, inflammation, and energy expenditure. Cell Metab. *17*, 534–548.

Mathis, D. (2013). Immunological goings-on in visceral adipose tissue. Cell Metab. *17*, 851–859.

Molofsky, A.B., Nussbaum, J.C., Liang, H.-E., Van Dyken, S.J., Cheng, L.E., Mohapatra, A., Chawla, A., and Locksley, R.M. (2013). Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. J. Exp. Med. *210*, 535–549.

Morris, D.L., Cho, K.W., Delproposto, J.L., Oatmen, K.E., Geletka, L.M., Martinez-Santibanez, G., Singer, K., and Lumeng, C.N. (2013). Adipose tissue macrophages function as antigen-presenting cells and regulate adipose tissue CD4+ T cells in mice. Diabetes *62*, 2762–2772.

Mothe-Satney, I., Filloux, C., Amghar, H., Pons, C., Bourlier, V., Galitzky, J., Grimaldi, P.A., Féral, C.C., Bouloumié, A., Van Obberghen, E., and Neels, J.G. (2012). Adipocytes secrete leukotrienes: contribution to obesity-associated inflammation and insulin resistance in mice. Diabetes *61*, 2311–2319.

Murray, P.J., Allen, J.E., Biswas, S., Fisher, E.A., Gilroy, D.W., Goerdt, S., Gordon, S., Hamilton, J.A., Ivashkiv, L.B., Lawrence, T., et al. (2014). Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity *41,* this issue, 14–20.

Neyrinck, A.M., Cani, P.D., Dewulf, E.M., De Backer, F., Bindels, L.B., and Delzenne, N.M. (2009). Critical role of Kupffer cells in the management of diet-induced diabetes and obesity. Biochem. Biophys. Res. Commun. *385*, 351–356.

Nguyen, M.T.A., Favelyukis, S., Nguyen, A.-K., Reichart, D., Scott, P.A., Jenn, A., Liu-Bryan, R., Glass, C.K., Neels, J.G., and Olefsky, J.M. (2007). A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J. Biol. Chem. *282*, 35279–35292.

Nishimura, S., Manabe, I., Nagasaki, M., Eto, K., Yamashita, H., Ohsugi, M., Otsu, M., Hara, K., Ueki, K., Sugiura, S., et al. (2009). CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat. Med. *15*, 914–920.

Nussbaum, J.C., Van Dyken, S.J., von Moltke, J., Cheng, L.E., Mohapatra, A., Molofsky, A.B., Thornton, E.E., Krummel, M.F., Chawla, A., Liang, H.E., and Locksley, R.M. (2013). Type 2 innate lymphoid cells control eosinophil homeostasis. Nature *502*, 245–248.

Obstfeld, A.E., Sugaru, E., Thearle, M., Francisco, A.-M., Gayet, C., Ginsberg, H.N., Ables, E.V., and Ferrante, A.W., Jr. (2010). C-C chemokine receptor 2 (CCR2) regulates the hepatic recruitment of myeloid cells that promote obesity-induced hepatic steatosis. Diabetes *59*, 916–925.

Odegaard, J.I., Ricardo-Gonzalez, R.R., Goforth, M.H., Morel, C.R., Subramanian, V., Mukundan, L., Red Eagle, A., Vats, D., Brombacher, F., Ferrante, A.W., and Chawla, A. (2007). Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. Nature *447*, 1116–1120.

Odegaard, J.I., Ricardo-Gonzalez, R.R., Red Eagle, A., Vats, D., Morel, C.R., Goforth, M.H., Subramanian, V., Mukundan, L., Ferrante, A.W., and Chawla, A. (2008). Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab. *7*, 496–507.

Oh, D.Y., Talukdar, S., Bae, E.J., Imamura, T., Morinaga, H., Fan, W., Li, P., Lu, W.J., Watkins, S.M., and Olefsky, J.M. (2010). GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell *142*, 687–698.

Oh, D.Y., Morinaga, H., Talukdar, S., Bae, E.J., and Olefsky, J.M. (2012). Increased macrophage migration into adipose tissue in obese mice. Diabetes *61*, 346–354.

Olefsky, J.M., and Glass, C.K. (2010). Macrophages, inflammation, and insulin resistance. Annu. Rev. Physiol. *72*, 219–246.

Orr, J.S., Puglisi, M.J., Ellacott, K.L.J., Lumeng, C.N., Wasserman, D.H., and Hasty, A.H. (2012). Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. Diabetes *61*, 2718–2727.

Osborn, O., and Olefsky, J.M. (2012). The cellular and signaling networks linking the immune system and metabolism in disease. Nat. Med. *18*, 363–374.

Ozcan, U., Cao, Q., Yilmaz, E., Lee, A.-H., Iwakoshi, N.N., Ozdelen, E., Tuncman, G., Görgün, C., Glimcher, L.H., and Hotamisligil, G.S. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science *306*, 457–461.

Ozes, O.N., Akca, H., Mayo, L.D., Gustin, J.A., Maehama, T., Dixon, J.E., and Donner, D.B. (2001). A phosphatidylinositol 3-kinase/Akt/mTOR pathway mediates and PTEN antagonizes tumor necrosis factor inhibition of insulin signaling through insulin receptor substrate-1. Proc. Natl. Acad. Sci. USA *98*, 4640–4645.

Patsouris, D., Li, P.-P., Thapar, D., Chapman, J., Olefsky, J.M., and Neels, J.G. (2008). Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab. *8*, 301–309.

Quiat, D., and Olson, E.N. (2013). MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. J. Clin. Invest. *123*, 11–18.

Randolph, G.J. (2008). Emigration of monocyte-derived cells to lymph nodes during resolution of inflammation and its failure in atherosclerosis. Curr. Opin. Lipidol. *19*, 462–468.

Recalcati, S., Locati, M., Gammella, E., Invernizzi, P., and Cairo, G. (2012). Iron levels in polarized macrophages: regulation of immunity and autoimmunity. Autoimmun. Rev. *11*, 883–889.

Ruan, H., Hacohen, N., Golub, T.R., Van Parijs, L., and Lodish, H.F. (2002). Tumor necrosis factor-alpha suppresses adipocyte-specific genes and activates expression of preadipocyte genes in 3T3-L1 adipocytes: nuclear factor-kappaB activation by TNF-alpha is obligatory. Diabetes *51*, 1319–1336.

Saberi, M., Woods, N.-B., de Luca, C., Schenk, S., Lu, J.C., Bandyopadhyay, G., Verma, I.M., and Olefsky, J.M. (2009). Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. Cell Metab. *10*, 419–429.

Sabio, G., Das, M., Mora, A., Zhang, Z., Jun, J.Y., Ko, H.J., Barrett, T., Kim, J.K., and Davis, R.J. (2008). A stress signaling pathway in adipose tissue regulates hepatic insulin resistance. Science *322*, 1539–1543.

Samuel, V.T., and Shulman, G.I. (2012). Mechanisms for insulin resistance: common threads and missing links. Cell *148*, 852–871.

Sartipy, P., and Loskutoff, D.J. (2003). Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc. Natl. Acad. Sci. USA *100*, 7265–7270.

Schmidt, A.M., and Moore, K.J. (2013). The Semaphorin 3E/PlexinD1 axis regulates macrophage inflammation in obesity. Cell Metab. *18*, 461–462.

Schubert, K.M., Scheid, M.P., and Duronio, V. (2000). Ceramide inhibits protein kinase B/Akt by promoting dephosphorylation of serine 473. J. Biol. Chem. *275*, 13330–13335.

Shimizu, I., Yoshida, Y., Moriya, J., Nojima, A., Uemura, A., Kobayashi, Y., and Minamino, T. (2013). Semaphorin3E-induced inflammation contributes to insulin resistance in dietary obesity. Cell Metab. *18*, 491–504.

Solinas, G., and Karin, M. (2010). JNK1 and IKKbeta: molecular links between obesity and metabolic dysfunction. FASEB J. *24*, 2596–2611.

Solinas, G., Vilcu, C., Neels, J.G., Bandyopadhyay, G.K., Luo, J.-L., Naugler, W., Grivennikov, S., Wynshaw-Boris, A., Scadeng, M., Olefsky, J.M., and Karin, M. (2007). JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. Cell Metab. *6*, 386–397.

Solomon, D.H., Massarotti, E., Garg, R., Liu, J., Canning, C., and Schneeweiss, S. (2011). Association between disease-modifying antirheumatic drugs and diabetes risk in patients with rheumatoid arthritis and psoriasis. JAMA *305*, 2525–2531.

Spite, M., Hellmann, J., Tang, Y., Mathis, S.P., Kosuri, M., Bhatnagar, A., Jala, V.R., and Haribabu, B. (2011). Deficiency of the leukotriene B4 receptor, BLT-1, protects against systemic insulin resistance in diet-induced obesity. J. Immunol. *187*, 1942–1949.

Stephens, J.M., and Pekala, P.H. (1991). Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. J. Biol. Chem. *266*, 21839–21845.

Steppan, C.M., Bailey, S.T., Bhat, S., Brown, E.J., Banerjee, R.R., Wright, C.M., Patel, H.R., Ahima, R.S., and Lazar, M.A. (2001). The hormone resistin links obesity to diabetes. Nature *409*, 307–312.

Stienstra, R., van Diepen, J.A., Tack, C.J., Zaki, M.H., van de Veerdonk, F.L., Perera, D., Neale, G.A., Hooiveld, G.J., Hijmans, A., Vroegrijk, I., et al. (2011). Inflammasome is a central player in the induction of obesity and insulin resistance. Proc. Natl. Acad. Sci. USA *108*, 15324–15329.

Stienstra, R., Tack, C.J., Kanneganti, T.-D., Joosten, L.A.B., and Netea, M.G. (2012). The inflammasome puts obesity in the danger zone. Cell Metab. *15*, 10–18.

Sullivan, T.J., Miao, Z., Zhao, B.N., Ertl, L.S., Wang, Y., Krasinski, A., Walters, M.J., Powers, J.P., Dairaghi, D.J., Baumgart, T., et al. (2013). Experimental evidence for the use of CCR2 antagonists in the treatment of type 2 diabetes. Metabolism *62*, 1623–1632.

Talukdar, S., Oh, Y., Bandyopadhyay, G., Li, D., Xu, J., McNelis, J., Lu, M., Li, P., Yan, Q., Zhu, Y., et al. (2012). Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. Nat. Med. *18*, 1407–1412.

Tam, C.S., Sparks, L.M., Johannsen, D.L., Covington, J.D., Church, T.S., and Ravussin, E. (2012). Low macrophage accumulation in skeletal muscle of obese type 2 diabetics and elderly subjects. Obesity (Silver Spring) *20*, 1530–1533.

Tamura, Y., Sugimoto, M., Murayama, T., Minami, M., Nishikaze, Y., Ariyasu, H., Akamizu, T., Kita, T., Yokode, M., and Arai, H. (2010). C-C chemokine receptor 2 inhibitor improves diet-induced development of insulin resistance and hepatic steatosis in mice. J. Atheroscler. Thromb. *17*, 219–228.

Tang, T., Sui, Y., Lian, M., Li, Z., and Hua, J. (2013). Pro-inflammatory activated Kupffer cells by lipids induce hepatic NKT cells deficiency through activation-induced cell death. PLoS ONE *8*, e81949.

Ueki, K., Kondo, T., and Kahn, C.R. (2004). Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol. Cell. Biol. *24*, 5434–5446.

Urano, F., Wang, X., Bertolotti, A., Zhang, Y., Chung, P., Harding, H.P., and Ron, D. (2000). Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. Science *287*, 664–666.

Ussher, J.R., Koves, T.R., Cadete, V.J.J., Zhang, L., Jaswal, J.S., Swyrd, S.J., Lopaschuk, D.G., Proctor, S.D., Keung, W., Muoio, D.M., and Lopaschuk, G.D. (2010). Inhibition of de novo ceramide synthesis reverses diet-induced insulin resistance and enhances whole-body oxygen consumption. Diabetes *59*, 2453–2464.

Vallerie, S.N., Furuhashi, M., Fucho, R., and Hotamisligil, G.S. (2008). A predominant role for parenchymal c-Jun amino terminal kinase (JNK) in the regulation of systemic insulin sensitivity. PLoS ONE *3*, e3151.

van Asseldonk, E.J.P., Stienstra, R., Koenen, T.B., Joosten, L.A.B., Netea, M.G., and Tack, C.J. (2011). Treatment with Anakinra improves disposition index but not insulin sensitivity in nondiabetic subjects with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. J. Clin. Endocrinol. Metab. *96*, 2119–2126.

van Diepen, J.A., Wong, M.C., Guigas, B., Bos, J., Stienstra, R., Hodson, L., Shoelson, S.E., Berbée, J.F.P., Rensen, P.C.N., Romijn, J.A., et al. (2011). Hepatocyte-specific IKK-β activation enhances VLDL-triglyceride production in APOE*3-Leiden mice. J. Lipid Res. *52*, 942–950.

van Gils, J.M., Derby, M.C., Fernandes, L.R., Ramkhelawon, B., Ray, T.D., Rayner, K.J., Parathath, S., Distel, E., Feig, J.L., Alvarez-Leite, J.I., et al. (2012). The neuroimmune guidance cue netrin-1 promotes atherosclerosis by inhibiting the emigration of macrophages from plaques. Nat. Immunol. *13*, 136–143.

Vandanmagsar, B., Youm, Y.-H., Ravussin, A., Galgani, J.E., Stadler, K., Mynatt, R.L., Ravussin, E., Stephens, J.M., and Dixit, V.D. (2011). The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat. Med. *17*, 179–188.

Wan, W., Lionakis, M.S., Liu, Q., Roffê, E., and Murphy, P.M. (2013). Genetic deletion of chemokine receptor Ccr7 exacerbates atherogenesis in ApoE-deficient mice. Cardiovasc. Res. *97*, 580–588.

Wanschel, A., Seibert, T., Hewing, B., Ramkhelawon, B., Ray, T.D., van Gils, J.M., Rayner, K.J., Feig, J.E., O'Brien, E.R., Fisher, E.A., and Moore, K.J. (2013). Neuroimmune guidance cue Semaphorin 3E is expressed in atherosclerotic plaques and regulates macrophage retention. Arterioscler. Thromb. Vasc. Biol. *33*, 886–893.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W., Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. *112*, 1796–1808.

Weisberg, S.P., Hunter, D., Huber, R., Lemieux, J., Slaymaker, S., Vaddi, K., Charo, I., Leibel, R.L., and Ferrante, A.W., Jr. (2006). CCR2 modulates inflammatory and metabolic effects of high-fat feeding. J. Clin. Invest. *116*, 115–124.

Williamson, R.T. (1901). On the treatment of glycosuria and diabetes mellitus with sodium salicylate. BMJ *1*, 760–762.

Winer, S., Chan, Y., Paltser, G., Truong, D., Tsui, H., Bahrami, J., Dorfman, R., Wang, Y., Zielenski, J., Mastronardi, F., et al. (2009). Normalization of obesity-associated insulin resistance through immunotherapy. Nat. Med. *15*, 921–929.

Winer, D.A., Winer, S., Shen, L., Wadia, P.P., Yantha, J., Paltser, G., Tsui, H., Wu, P., Davidson, M.G., Alonso, M.N., et al. (2011). B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nat. Med. *17*, 610–617.

Wu, D., Molofsky, A.B., Liang, H.-E., Ricardo-Gonzalez, R.R., Jouihan, H.A., Bando, J.K., Chawla, A., and Locksley, R.M. (2011). Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science *332*, 243–247.

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., Tartaglia, L.A., and Chen, H. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J. Clin. Invest. *112*, 1821–1830.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., et al. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat. Med. *7*, 941–946.

Yang, H., Youm, Y.-H., Vandanmagsar, B., Ravussin, A., Gimble, J.M., Greenway, F., Stephens, J.M., Mynatt, R.L., and Dixit, V.D. (2010). Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: implications for systemic inflammation and insulin resistance. J. Immunol. *185*, 1836–1845.

Zhang, X., Xu, A., Chung, S.K., Cresser, J.H.B., Sweeney, G., Wong, R.L.C., Lin, A., and Lam, K.S.L. (2011). Selective inactivation of c-Jun NH2-terminal kinase in adipose tissue protects against diet-induced obesity and improves insulin sensitivity in both liver and skeletal muscle in mice. Diabetes *60*, 486–495.

Zhong, J., Rao, X., Braunstein, Z., Taylor, A., Narula, V., Hazey, J., Mikami, D., Needleman, B., Rutsky, J., Sun, Q., et al. (2014). T cell costimulation protects obesity-induced adipose inflammation and insulin resistance. Diabetes *63*, 1289–1302.