Immunometabolic control of homeostasis and inflammation

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Chronic inflammation underlies an array of chronic, non-communicable diseases, including various cardiovascular and metabolic diseases and cancer. Recent studies have also shown that the mechanisms involved in regulating immunity and metabolism are intricately linked. The term immunometabolism refers to that linkage. In this review, we will discuss immune cell-mediated regulation of metabolic homeostasis and pathology in major metabolic tissues. A particular focus is on macrophages, which play diverse roles in the inflammatory processes induced in non-communicable diseases.

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The realization that inflammatory and metabolic signals are closely linked has given rise to the concept of immunometabolism, which refers to the interplay between immunological and metabolic processes. Inflammatory responses are fundamental actions elicited when the body is subjected to a potentially harmful stimulus or an injury. The acute inflammatory response is characterized by the cardinal signs of “dolor, calor, rubor and tumor” (pain, heat, redness and swelling). Such responses are a key part of the body’s defense system, an indispensable protective response mediated by a combination of immune cells, vascular cells and other stromal cells, such as fibroblasts, as well as parenchymal cells. Proper resolution of acute inflammation leads to recovery of normal function and homeostasis. Chronic inflammation is another matter. It is now clear that chronic inflammation underlies a variety of non-communicable diseases (NCDs), including cardiovascular and metabolic diseases such as atherosclerosis, type
2 diabetes (T2D) and cancer. Chronic inflammation is a prolonged condition in which inflammation, tissue injury and attempts at repair coexist\(^1\). Although chronic inflammation may follow acute inflammation, in the most common NCDs of today, it likely begins insidiously as a low-grade, smoldering response with no manifestation of the cardinal signs of inflammation.

The innate immune system serves as the body’s immediate first line of defense. The cells of the innate immune system recognize and respond to pathogens, but they do not confer long-lasting immunity to specific antigens. By contrast, the adaptive immune system creates immunological memory after an initial response to a specific pathogen, which enables an enhanced response to subsequent encounters with that pathogen. Mononuclear phagocytes are essential players in the orchestrated response of the innate and adaptive immune systems. Of particular interest are monocyte-macrophage lineage cells, which have been shown to act as major effector cells in chronic inflammatory processes during the pathological development of NCDs\(^2\). Their functions are diverse and change during the course of chronic inflammatory processes, and it is becoming clear that monocyte-macrophage lineage cells not only play essential roles in host defense, mediating pathogen clearance, but also play pivotal roles in the maintenance of tissue homeostasis. For instance, mononuclear phagocytes are indispensable for tissue remodeling during organ development. In addition, recent studies have shown monocyte-macrophage lineage cells to be involved in the regulation of metabolism in adult animals\(^3\). Monocyte-macrophage lineage cells that are constitutively localized in peripheral tissues are called tissue-resident macrophages. Nearly all tissues in the body contain populations of tissue-resident macrophages exhibiting highly diverse and plastic phenotypes and gene expression profiles, and fulfilling tissue-specific and microenvironment-specific functions under both physiological and pathogenic conditions. In this review, we will summarize what is currently known about the macrophages involved in maintaining tissue homeostasis and inflammatory processes within the major metabolic tissues.

### Macrophage mediated control of liver function

Kupffer cells are resident mononuclear cells within the liver, and account for approximately 10% of the total number of liver cells\(^4\). Kupffer cells act in part as sentinels, capturing antigens and pathogens that enter the liver\(^5\), but they also play major roles in maintaining tissue homeostasis. Kupffer cells are derived from embryonic yolk-sac cells and exhibit a type-2-like (M2-like) “anti-inflammatory” phenotype in the steady state. Nonetheless, Kupffer cells have been implicated in the pathogenesis of several liver diseases, including viral hepatitis, steatohepatitis, alcoholic liver disease, nonalcoholic fatty liver disease (NAFLD), activation of liver rejection during transplantation and liver fibrosis\(^6\).

NAFLD and nonalcoholic steatohepatitis (NASH) are strongly associated with insulin resistance and T2D, and inflammatory activation of Kupffer cells has been implicated in both of those liver pathologies. For example, nuclear factor κB (NF-κB) is activated in Kupffer cells in NASH and is responsible for increased secretion of proinflammatory cytokines\(^6\). Silencing of NF-κB expression in Kupffer cells in obese mice reduces cytokine secretion and improves insulin tolerance. Moreover, genetic ablation of IkB kinase β (IKKβ), an upstream kinase required for NF-κB activation, in myeloid cells reduces macrophage-mediated inflammation and improves systemic and hepatic insulin sensitivity\(^7\). The proinflammatory cytokines secreted from Kupffer cells activate IKKβ and c-Jun N-terminal kinases (JNK) within hepatocytes, thereby inducing insulin resistance\(^8, 9\). Kupffer cells thus appear to promote insulin resistance by activating inflammatory pathways within hepatocytes.

During the progression of T2D, insulin resistance increases
cells ameliorate obesity-induced insulin resistance shown that alternatively activated, anti-inflammatory Kupffer fatty acid oxidation and increased hepatic lipid storage. Activity in hepatocytes, which in turn leads to a reduction in fatty acid oxidation and increased hepatic lipid storage.

In contrast to the insulin resistance and hepatic steatosis-promoting functions of Kupffer cells, studies have also shown that alternatively activated, anti-inflammatory Kupffer cells ameliorate obesity-induced insulin resistance. The Th2 cytokine IL-4 and its downstream transcription factor, PPARδ, mediate the alternatively activated phenotype in Kupffer cells. Transplantation of Ppard−/− bone marrow into irradiated wild-type mice diminishes alternative activation of hepatic macrophages and induces hepatic dysfunction and systemic insulin resistance. In the bone marrow-transplanted mice, expression of the genes involved in oxidative phosphorylation was reduced in the liver, suggesting a deficiency in PPARδ in myeloid cells impairs oxidative metabolism in hepatocytes. Interestingly, medium conditioned by wild-type macrophages has the ability to increase β-oxidation in primary hepatocytes, whereas medium conditioned by PPARδ-deficient macrophages lacks that ability. This suggests factors produced by macrophages regulate hepatocyte metabolism. Another recent study also showed that Kupffer cell activation may not always lead to insulin resistance. Ldr−/− mice fed a high-fat, high-cholesterol diet developed severe liver inflammation with Kupffer cell activation, but they did not develop hepatic or systemic insulin resistance. Whether inflammatory activated Kupffer cells induce insulin resistance, may depend on how they are activated and on the state of their microenvironment. In addition, the effects of Kupffer cells on insulin resistance may also vary depending on their activation states.

Macrofages in skeletal muscle development and function
In addition to being a motor organ, skeletal muscle is a primary site of glucose uptake and accounts for approximately 80% of insulin-stimulated glucose disposal in humans. Skeletal muscle contains a small resident macrophage population under physiological conditions (~200 cells/mg of muscle), but obesity in both mice and humans is reportedly associated with increases in muscle inflammatory gene expression, which is accompanied by macrophage infiltration. These macrophages are largely localized in small intermuscular adipose depots that arise within skeletal muscle in obese individuals. On the other hand, other reports show no obesity-related increase in skeletal muscle macrophage number, and the origin of the increased mononuclear cells seen in some obese subjects has not yet been determined. The role of macrophages in the mechanism underlying insulin resistance also remains unclear, though it is possible that inflammatory factors released from the intramuscular macrophages exert paracrine effects that cause local insulin resistance, as is seen in the liver.

Skeletal muscle has a remarkable ability to repair itself after injury. This regeneration is a highly orchestrated process involving the concerted activation of a variety of cellular and molecular responses. Macrophages are essential for skeletal muscle regeneration and remodeling. In a freeze-injury model, for instance, depletion of circulating monocytes using clodronate liposomes not only attenuates the inflammatory response, it also impedes repair processes, prolonging the clearance of necrotic myofibers and increasing muscle fat accumulation. Previous studies also showed that macrophages stimulate myogenic progenitor cell growth through both secretion of soluble mitogenic factors and the establishment of direct anti-apoptotic cell-cell contacts.

Following a muscle injury, a phenotype transition among macrophages is reportedly essential for the muscle repair process. For example, notexin-induced muscle injury leads to rapid recruitment from blood of inflammatory CX3CR1+Ly-6C+ monocytes that exhibit a nondividing, F4/80+ proinflammatory M1-type phenotype. The accumulation of CX3CR1+Ly-6C+ monocytes/macrophages reaches a maximum within 24 h of an injury, after which the recruited CX3CR1+Ly-6C+ cells acquire a CX3CR1+Ly-6C− anti-inflammatory M2-type phenotype and become proliferative. The population then increases until reaching a plateau 7 days after injury, at which time the cells further differentiate into F4/80−CD11c+ mature macrophages. Given that LPS/IFN-γ-treated proinflammatory human muscle macrophages promote myogenic cell proliferation, while IL-4 or dexamethasone/IL-10-treated anti-inflammatory macrophages promote myogenic differentiation, it appears switching from a proinflammatory to an anti-inflammatory phenotype is important for supporting muscle regeneration.
In addition, phagocytosis of muscle cell debris appears to switch the macrophage phenotype via activation of AMPK signaling.

**Macrophages in type 2 diabetes**

Insulin resistance and β cell dysfunction are two key pathological mechanisms contributing to T2D. The observation that proinflammatory signaling pathways can inhibit insulin signaling\(^26\) provides a link between inflammation and insulin resistance. Recent studies have shown that inflammation is also involved in the development of β cell dysfunction. In T2D subjects, for example, pancreatic islets exhibit histological changes characteristic of inflammation, including amyloid deposition\(^27\), immune cell infiltration\(^28\), cell death and fibrosis\(^29\). In addition, sections of pancreas from patients with T2D, C57BL/6 mice fed a high-fat diet, db/db mice and GK rats all show elevated numbers of macrophages within islets\(^28\). High intake of glucose or palmitate reportedly induces secretion of chemokines from islets, which promotes monocyte migration, suggesting that the T2D islet milieu promotes macrophage infiltration.

We recently found that there are two subpopulations of macrophages within islets: CD11b\(^+\)Ly-6C\(^+\) monocytes/macrophages and CD11b\(^+\)Ly-6C\(^-\) macrophages\(^30\). Under basal conditions, islet-resident macrophages are primarily CD11b\(^+\)Ly-6C\(^-\) cells, which exhibit an M2-type phenotype. The numbers of these M2-type cells are not altered in T2D mice, but the numbers of CD11b\(^+\)Ly-6C\(^+\) monocytes/macrophages exhibiting the M1-type phenotype is selectively increased such that the macrophage polarity is shifted toward M1 in T2D islets.

Palmitate is the most abundant saturated FFA in blood, and the deleterious effects of palmitate on β cells, collectively termed “lipotoxicity,” are well documented\(^31\). *In vitro* studies have shown that β cell lipotoxicity is directly induced by palmitate, at least in part via pathways primarily involving endoplasmic reticulum stress and reactive oxygen species. After developing a method to increase serum free palmitate levels through infusion of emulsified ethyl palmitate, we tested the effects of palmitate on β cells *in vivo*. We found that palmitate rapidly induces β cell dysfunction, in part by inducing expression of chemokines CCL2 and CXCL11 via TLR4 activation. These chemokines in turn recruit M1-type monocytes/macrophages to the islets. Blocking the accumulation of M1-type monocytes/macrophages suppresses palmitate-induced β cell dysfunction, indicating the causal involvement of M1-type macrophages. In this model the proinflammatory cytokines IL-1β and TNF-α produced by M1 macrophages promote β cell dysfunction. Thus the chemokines from β cells and cytokines from M1 macrophages form a vicious cycle that exacerbates islet inflammation (Fig. 2). M1 macrophage accumulation within islets appears to similarly contribute to β cell dysfunction in db/db and KKAy mice, two models of T2D. These results clearly demonstrate that activation of inflammatory processes within islets leads to β cell dysfunction.

**Appetite control by macrophages**

In addition to peripheral metabolic tissues such as adipose tissue and liver, obesity also reportedly activates inflammatory signaling within the central nervous system (CNS), including in the hypothalamus\(^32\). The hypothalamus is the control center for regulating whole-body energy homeostasis, and inhibiting hypothalamic inflammatory mediators such as c-Jun N-terminal kinase (JNK) and NF-κB diminishes insulin resistance in obese animals\(^33\). Moreover, inhibition of inflammatory signaling specifically within Agouti-related peptide (AgRP) neurons, which stimulate feeding behavior, protects against obesity and glucose intolerance.

Microglia are the resident macrophages in the CNS. They arise from progenitors in the yolk sac and are maintained by self-renewal\(^34\). During development, they colocalize with dying neurons, suggesting they are involved in regulating neuronal number and development. Microglia share many functions with peripheral tissue macrophages, including an ability to carry out phagocytosis and release various...
cytokines, including TNF-α, IL-1β and IL-10. Microglia can be activated by proinflammatory signals in response to brain injury, resulting in the production of cytokines that act locally on other CNS cell types.

High-fat diet-induced obesity leads to chronic low-grade hypothalamic inflammation that involves activation of both microglia and astrocytes. In rats, hypothalamic inflammation that includes elevated expression of IL-6 and TNF-α mRNA is evident within 1 to 3 days after starting a high-fat diet, prior to substantial weight gain. This inflammatory process eventually leads to neuronal injury. Indeed, markers suggestive of neuronal injury (e.g., Hsp72) are detected in the hypothalamic arcuate nucleus and adjacent median eminence within the first week after initiating a high-fat diet, and are associated with the development of reactive gliosis reflected by recruitment of microglia and astrocytes. Interestingly, pro-opiomelanocortin (POMC) neurons are much more vulnerable to a high-fat diet than other types of neurons in the arcuate nucleus. As POMC neurons stimulate energy expenditure and reduce appetite, long-term microgliosis could potentially perpetuate weight gain through inflammation-induced damage to these anorexigenic neurons. It has also been proposed that hypothalamic inflammation plays a role in central leptin resistance through cytokine-mediated inhibition of signal transducer and activator of transcription-3 (STAT3), which is a key component of the leptin signaling pathway. In addition, NF-κB, a major regulator of inflammatory responses, mediates leptin resistance in the hypothalamus. It thus appears that a high-fat diet and obesity lead to activation of inflammatory signaling within the hypothalamus, which can modulate hypothalamic function, altering the regulation of feeding behavior and systemic metabolism.

Long-chain saturated fatty acids, but not unsaturated fatty acids, trigger hypothalamic inflammatory responses through activation of TLR4 on microglia, leading to leptin resistance. Inhibition of TLR4 signaling not only suppresses inflammatory cytokine expression within the hypothalamus, it suppresses weight gain. Leptin also reportedly activates microglia and increases IL-6 production through the leptin receptor, PI3K and NF-κB pathways, which suggests microglia are able to sense systemic adiposity and in turn mediate inflammatory responses (Fig. 3).

Microglia are also sensitive to microenvironmental changes caused by exercise. Regular exercise reduces the induction of microglia-specific calcium binding protein Iba1, a marker of hypothalamic microglial activation in Ldr−/− mice. This effect is independent of changes in body weight, and suggests regular exercise may prevent diet-induced inflammation mediated by microglia in the hypothalamic arcuate nucleus. Accordingly, microglia appear to respond to systemic metabolic alterations and control inflammatory signaling in the hypothalamus.

Conclusions and future perspectives

In this review we provide an overview of the physiological and pathological activities of macrophages located within the major metabolic tissues and the hypothalamus. In each tissue, macrophages respond to microenvironmental as well as systemic cues, including nutrient and hormonal signals, and make crucial contributions to the tissue responses to local and systemic stress. The inflammatory processes executed by macrophages may impair the normal function of each tissue. However, as exemplified by the activities of skeletal muscle macrophages, their inflammatory activation may also be essential for proper adaptive responses to stress and injury. In addition, it appears Kupffer cells control hepatocyte metabolism, while macrophages within pancreatic islets are thought to support β cell proliferation during development. This suggests macrophages also control processes unrelated to inflammation and may play diverse roles in development, maintenance of homeostasis and other metabolic functions.
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and pathology. Future studies are clearly needed to further elucidate the many roles played by macrophages.

Recent studies have also revealed the multiple origins of macrophages and the proliferative capacity of subsets of tissue macrophages. This means the macrophages found in both healthy and diseased tissues may include macrophages of fetal origin, circulating monocyte-derived newly differentiated macrophages, and macrophages generated through self-replication of monocyte-derived macrophages. In addition, recent studies have also shown that the epigenomes and transcriptomes of macrophages are highly plastic, and the tissue microenvironment can transform an enhancer landscape of exogenous macrophages into one mimicking that of resident macrophages within a given tissue. It will be important to further define the different roles of macrophages of different origin (e.g., fetal tissue-derived vs. monocyte-derived) in physiology and pathology, and determine how macrophages respond to stimuli and acquire their diversity.

As we have seen, inflammation within metabolic tissues can affect systemic metabolism. However, the linkage between immunity and metabolism is not limited to the involvement of immune cells in the regulation of homeostasis and the dysregulation of metabolic tissues. Recent studies have shown that the inflammatory functions of immune cells are crucially affected by their intrinsic metabolic processes. For instance, activation of effector T cells associates with a metabolic switch from fatty acid oxidation to aerobic glycolysis. The ability of the systemic metabolic status to affect cellular metabolism adds further complexity to the immunometabolic interplay at the cellular level. It is therefore anticipated that a better understanding of the immunometabolic mechanisms underlying NCDs will provide a variety of novel therapeutic targets.

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Conflict of interests
We have no conflict of interest to declare.

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