Non-traditional cytokines: How catecholamines and adipokines influence macrophages in immunity, metabolism and the central nervous system

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Abstract

Catecholamines and adipokines function as hormones; catecholamines as neurotransmitters in the sympathetic nervous system, and adipokines as mediators of metabolic processes. It has become increasingly clear, however, that both also function as immunomodulators of innate and adaptive immune cells, including macrophages. Macrophages can respond to, as well as produce their own catecholamines. Dopamine, noradrenaline, and adrenaline are the most abundant catecholamines in the body, and can induce both pro-inflammatory and anti-inflammatory immune responses in macrophages, as well as non-immune processes such as thermogenesis. Though they are responsive to adipokines, particularly lipoproteins, leptin, and adiponectin, macrophages generally do synthesize their own adipokines, with the exception being resistin-like molecules. Adipokines contribute to adverse metabolic and immune response by stimulating lipid accumulation, foam cell formation and pro-inflammatory cytokine production in macrophages. Adipokines can also promote balance or resolution during metabolic and immune processes by promoting reverse lipid transport and expression of Th2 cytokines. This review will explore the mechanisms by which catecholamines and adipokines influence macrophage function in neural pathways, immunity and metabolism.

Keywords

Adipokine; dopamine; macrophage; resistin-like molecules; atherosclerosis; sepsis

1. Introduction

Macrophages are essential components of the innate immune system. First identified by Metchnikoff for their potent phagocytic capabilities, which explains their name "big eater" in Greek, their function in engulfing and eliminating microbial pathogens is well-recognized. The importance of macrophages in other immune contexts, such as influencing

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adaptive immunity, mediating wound healing and downregulating inflammation is also appreciated. New studies, however, have revealed that the macrophage function extends beyond the immunological realm, affecting both the central nervous system and metabolism. First, macrophages respond to and can produce catecholamines, which are neurotransmitters that signal through the sympathetic nervous pathway. Second, macrophages make and respond to adipokines that influence the outcome of several metabolic diseases such as atherosclerosis. This suggests the requirement for multidisciplinary research spanning immunology, neuroscience and metabolism for the improved understanding of these critical cell-types. Here we review the main mediators of these neural-immune or metabolic-immune circuits, which are either synthesized by macrophages or that influence their function, and discuss their function in neural pathways, immunity and metabolism.

2. Catecholamines

Catecholamines are hormones produced in both the adrenal medulla and the central nervous system. As neurotransmitters, catecholamines are an integral part of the sympathetic nervous pathway, also known as the "fight-or-flight response", which mediates essential physiologic responses including increased heart rate and blood pressure, mobilization of energy stores and control of core body temperature [1]. In addition to their hormonal and neurotransmitter roles, catecholamines also influence immune responses, and the importance of this neural-immune cross-talk via neurotransmitters and cytokines has been increasingly recognized [2]. For instance, stimulation of the vagus nerve can regulate inflammatory cytokine production, and conversely, macrophages and lymphocytes are able to synthesize catecholamines that influence the central nervous system (CNS) [3–5]. Additionally, immune cells express adrenergic receptors and are therefore responsive to catecholamines [6]. Catecholamine signaling in immune cells exerts a number of effects including cell activation, proliferation and apoptosis [7, 8]. Furthermore, catecholamines can be locally produced by immune cells and act in both autocrine and paracrine ways [6]. Here, we focus on the macrophage-specific modulatory effects of catecholamines.

The most abundant catecholamines in the human body are dopamine, adrenaline and noradrenaline. Catecholamines are synthesized from the non-essential amino acid tyrosine by a series of enzymatic pathways [9]. First, tyrosine hydroxylase removes a hydroxyl group from tyrosine to produce the dopamine precursor L-DOPA. L-DOPA is decarboxylated to form dopamine, which is then catabolized to noradrenaline and adrenaline by hydroxylases. Dopamine binds dopamine receptors, while noradrenaline and adrenaline bind α and β-adrenergic receptors, all of which belong to a family of G protein-coupled receptors that signal through phospholipase C and cAMP/protein kinase A pathways [10, 11]. In the immune system, myeloid cells express α and β-adrenergic receptors, while lymphocytes primarily express β-adrenergic receptors [1].

Functionally, catecholamine receptor signaling in macrophages has significant effects on the inflammatory response. Inhibition of the β-adrenergic receptor with the β-blocker propranolol, or depletion of adrenal catecholamines by adrenalectomy, led to increased LPS-induced tumor necrosis factor (TNF) α production in peritoneal macrophages [12]. Alveolar macrophages recovered from mice chronically treated with β-blockers produced more

noradrenaline, interleukin (IL) 6 and TNFα following LPS treatment *ex vivo* [13]. Conversely, adrenaline, noradrenaline and dopamine treatment of RAW 264.7 macrophages inhibited LPS-induced production of nitric oxide [14]. Finally, treatment of RAW cells with dopamine or noradrenaline decreased proliferation and increased apoptosis [8]. Taken together, these studies suggest that macrophage responsiveness to catecholamines via the β-adrenergic receptor exerts an important immunoregulatory mechanism to reduce inflammation. Supportive of this, treatment of mice with β2-adrenergic agonists ameliorated LPS-induced endotoxemia and acute lung inflammation [15]. This was associated with alternatively activated macrophage (AAM) polarization, characterized by increased IL-4, IL-10 and Arginase-1 expression, and decreased expression of iNOS and IL-12 [16].

Recent data suggest that catecholamines can auto-regulate their levels and function by controlling expression of both tyrosine hydroxylase as well as catecholamine receptors [12]. For instance, adrenal catecholamines contribute to the paracrine regulation of macrophage synthesis of catecholamines and expression of the β-adrenergic receptor. Adrenalectomy resulted in decreased expression of β2-adrenergic receptor and increased expression of tyrosine hydroxylase by peritoneal macrophages presumably as a compensatory mechanism to increase catecholamine levels. Consistent with this, treatment with the β-blocker propanonol increased macrophage expression of tyrosine hydroxylase.

In contrast to the anti-inflammatory effect of β-adrenergic receptor signaling, stimulation of the α-adrenergic receptor of murine peritoneal macrophages in combination with LPS treatment led to increased TNFα and IL-1β expression compared with LPS alone [17]. Additionally, treatment of human monocytes with the α1-adrenergic receptor agonist phenylephrine hydrochloride promoted LPS-induced IL-1β [18]. Use of protein kinase C and MAP kinase inhibitors demonstrated that these signaling pathways were downstream of the α-adrenergic receptor-induced inflammatory response. Together, these observations suggest that the differential roles of catecholamines on macrophages may depend on the adrenergic receptor. Specifically in the context of LPS-induced inflammation, β-adrenergic receptors agonists inhibit inflammation, while α-adrenergic receptor signaling or β-adrenergic receptor blockers promote pro-inflammatory responses. The differential responses between α-adrenergic and β-adrenergic receptors is likely due to variance in G protein pairings with the receptors [11]. Briefly, α1 preferentially binds noradrenaline and signals via the PKC-activating Gq subunit, while α2 preferentially binds adrenaline and stimulates Gi, thereby decreasing cAMP. β1-adrenergic receptor equivalently binds noradrenaline and adrenaline, which leads to Gs subunit-mediated increase of cAMP. Though the β2 receptor also couples with the Gs subunit, its preferential binding partner is adrenaline. Influencing differential adrenergic receptor expression and G protein pairing on macrophages could therefore have therapeutic potential in dictating the inflammatory outcome of several disease conditions such as endotoxemia or acute respiratory disease.

In addition to regulation of inflammation by the sympathetic nervous system via catecholamine-adrenergic receptor signaling, macrophages are also influenced by the parasympathetic/cholinergic nervous system, through recognition of acetylcholine by nicotinic receptors. In this neural immune circuit, termed the inflammatory reflex, stimulation of the vagus nerve leads to the release of acetylcholine that acts on macrophages

to downregulate expression of inflammatory cytokines such as TNFα. In a mouse model of sepsis, this pathway was critical in limiting inflammation, and was dependent on acetylcholine production by a small subset of memory T cells [19]. In more recent studies, Ulloa and colleagues utilized electroacupuncture at the sciatic nerve to protect mice from fatal sepsis induced by LPS treatment [4]. This protective mechanism was associated with decreased levels of TNFα, CCL2, IL-6, and IFN-γ in the serum, and dependent on vagal nerve stimulation and adrenal-derived catecholamines. Specifically, vagotomy or adrenalectomy abolished the production of catecholamines, and treatment with dopamine receptor agonists could rescue the adrenalectomized mice from fatal sepsis. Together, these studies demonstrate the importance of both dopaminergic and cholinergic nervous pathways in the regulation of the inflammatory immune response during sepsis.

In contrast to its role in preventing sepsis, macrophage exposure to dopamine may increase susceptibility to HIV [20, 21]. Macrophages are the main cell type in the CNS that are infected with HIV, and recent studies showed that dopamine treatment of human peripheral blood monocyte-derived macrophages led to a two-fold increase in CCR5-mediated HIV entry and increased HIV replication. Supportive of these studies, another group reported a positive correlation between dopamine levels and CNS viral loads in SIV-infected macaques [22]. These studies implicate catecholamines as immunomodulatory molecules and elucidate a potential role for these neurotransmitters in HIV-associated neurocognitive disorders. Since therapeutic drugs, such as ritalin and some antidepressants, and illicit drugs, such as cocaine, can lead to increased CNS dopamine, these drugs may contribute to increased HIV virulence.

Catecholamine signaling also negatively impacts the rate of wound repair. The stress induced by injury can lead to a surge in catecholamines, with 10-fold increases in circulating adrenaline in severe burn injuries [23]. Macrophages and neutrophils that are recruited to the injury respond to and produce catecholamines. Wounding studies in mice and in skin biopsies have allowed evaluation of the effects of systemic and local elevation in catecholamines in wound healing. Burn wounds generated in excised human skin exhibited delayed re-epithelialization when treated with high levels of adrenaline [24]. This was due to the effects of adrenaline on inhibiting the migration of keratinocytes, which express the β2-adrenergic receptor. Treatment with β2-adrenergic receptor antagonists rescued the wound healing process. α2-adrenergic receptor$^{-/-}$ mice had accelerated wound closure [25], supporting the negative effect of both α and β adrenergic receptor signaling in wound healing. In another study, mice that were chronically delivered adrenaline via an osmotic pump, exhibited impaired wound healing associated with persistent neutrophil trafficking. Interestingly, the chronic inflammation was mediated by β2-adrenergic receptor signaling in macrophages that promoted IL-6 production. Therefore, while β2-adrenergic receptor signaling is protective in downregulating excessive inflammation during endotoxemia, in response to persistent exposure to adrenaline, it can have detrimental effects by promoting inflammation and impairing keratinocyte responses that are necessary for wound healing [26].

In addition to the effects of catecholamines in modulating macrophage immune responses, recent studies have shown that macrophages can potently affect the central nervous system

(CNS), demonstrating that bi-directional communication exists in these neural immune circuits [27, 28]. Like all tissues in the body, the CNS has a resident population of macrophages. Referred to as microglia, these cells play essential roles promoting optimal brain function by editing neuronal synapses and providing growth factors promoting neuroprotection during health, injury or infection. Chronic activation of microglia as can lead to dysregulated and/or neurotoxic functions contributing to neurodegeneration, neuropathic pain and/or decreased cognitive ability. Most studies have focused on the role of pathogen associated molecular patterns (PAMP) or danger associated molecular pattern (DAMP) molecules as the primary signals triggering maladaptive microglial activation in CNS injury and disease function [27, 28]. However, in vitro and in vivo studies now reveal that norephinephrine (NE) plays a non-redundant and complementary role to DAMP and PAMP signals. For example, ATP acting via P2 purinergic receptors potently promotes microglial process extension, phagocytosis and inflammasome activation [29, 30]. Using both in vitro and in vivo approaches, Gyoneva and Traynelis [31] demonstrated that activation of microglial β2 adrenergic decreased their base line rate as well as the higher ATP induced rate of process extension and migration. These data suggest that the decreasing levels of NE observed in progressive degenerative disorders such as Alzheimer's disease directly contributes to decreased ability to inhibit microglial activation by classic DAMPs, Conversely, drugs of abuse associated with activation of microglial adrenergic receptors will lead to altered microglial surveillance, decreased responses to CNS DAMP signals with likely alterations in microglial regulations of neuronal synapses.

Macrophages can also serve as a source of catecholamines and serve essential roles in maintaining physiologic homeostasis. As previously mentioned, macrophages express tyrosine hydroxylase in response to numerous stimuli including LPS but also as a compensatory mechanism when local catecholamine levels are low [8]. More recently, it was shown that IL-4/IL-13-induced AAM were a critical extraneuronal source of catecholamines in thermogenesis. Thermogenesis is an essential physiologic response in mammals that maintains constant body temperature in response to temperature changes [32, 33]. In a mouse model of adaptive thermogenesis, where mice were exposed to cold temperatures, maintenance of body temperature in wild-type mice was associated with catecholamine production by AAM in the brown adipose tissue. In contrast, macrophage-specific STAT6$^{-/-}$ mice, which lack alternatively activated macrophages, had decreased catecholamine levels and were unable to maintain body temperature homeostasis following thermogenic stress. Conversely, wild-type mice treated with IL-4 exhibited increased AAM-derived tyrosine hydroxylase and noradrenaline. Mechanistically, the catecholamine-producing AAM that infiltrated white adipose tissue spurred the development of thermogenic beige adipose tissue. AAM polarization, and subsequent development of beige adipose tissue, led to increased energy expenditure, mediated by uncoupling protein 1 and fatty acid metabolism, and the generation of non-shivering thermogenesis. This previously unrecognized function of macrophages in instructing beige adipose tissue development and subsequent energy expenditure has significant implications for the role of these cells in metabolic disorders.

These studies dramatically demonstrate the potent effects of catecholamines in regulating and mediating macrophage contributions to immunity and physiology. However,

conclusions from these studies are often cited and applied to contexts quite different from those tested in the original experiments as if macrophage populations are homogenous and essentially identical throughout development and between tissues. Macrophages are highly plastic cells and multiple different types of activation states can be observed within a tissue dependent on local environmental cues [27].

Three studies of macrophages acutely isolated from the spleen and brain reveal the effects of development, activation state and local tissue environment [30]. For example, careful characterization of signal transduction revealed that only a subset of microglia are responsive to catecholamines, with only 7% of adult cortical microglia demonstrating responsiveness to dopamine [30]. Cytokine activation of microglia regulated dopamine responsiveness but the regulation was developmentally regulated. While IFNg tripled the number of neonatal microglia responsive to dopamine, IFNg had no effect on adult microglial responsiveness. Conversely, IL-4 had no effect on dopamine responsiveness of neonatal microglia, but did decrease dopamine responsiveness of adult microglia. While most microglia appear to express adrenergic receptors, the ratio of B2 to alpha adrenergic receptor expression dramatically increases upon activation by B-amyloid or PAMP signals. Thus activated microglia are preferentially inhibited by NE as compared to homeostatic microglia. Even within the spleen, there is substantial heterogeneity in macrophage responsiveness to catecholamines. Clenbuterol is a β2 agonist with some structural and pharmacological similarities to epinephrine and salbutamol. Within the spleen, Shirato and colleagues compared three macrophage populations and found that only the bacterial phagocytosis by the small but MARCOhi expressing macrophages was inhibited by Clenbuterol.

Taken together, these observations illustrate the diverse, context-dependent, complex roles of catecholamines and acetylcholine in macrophage responses, and reveal that non-traditional therapeutic strategies, such as acupuncture, that target neural-immune circuits could provide new effective treatments for infection, inflammation and metabolism (Figure 1).

3. Adipokines

Adipose-derived hormones, also known as adipokines, are molecules that exhibit hormone characteristics during metabolic processes as well as cytokine functions in modulating the immune response [34, 35]. Their metabolic functions range from stopping hunger signals to promoting lipid and glucose uptake and metabolism [34, 36]. Adipokines act on both innate and adaptive immune cells to increase cell activation, survival and chemotaxis, Also, depending on the particular adipokine, they can increase pro-inflammatory or anti-inflammatory cytokine production [37]. Here, we discuss the lipoproteins, leptin, and adiponectin due to their profound influence on macrophages during metabolic and immune processes.

3.1 Lipoproteins

An amalgamation of lipids and proteins, lipoproteins provide hydrophilic properties to lipids, allowing them to be transported within aqueous environments inside and outside of

cells. Some well-studied lipoproteins include Apolipoprotein (Apo) A and E that bind lipids reversibly to form high density lipoprotein (HDL) and Apo B that binds lipids irreversibly to form low density lipoprotein (LDL) [38, 39]. One of the main functions of HDL is to promote cholesterol efflux from cells, such as foam cells that contribute to arterial plaques. As such, decreased HDL levels are indicative of increased atherosclerosis and cardiovascular events. In addition to being a fat molecule transporter, HDL also has a number of anti-inflammatory properties including decreasing expression of adhesion molecules, TNF and CCL2 in endothelial cells. LDL is also a fat molecule transporter; it differs from HDL in that it contains higher proportions of fat molecules. In conditions of oxidative stress, LDL is susceptible to oxidation, and can form aggregates. These oxLDL aggregates form fat droplets that are recognized by scavenger receptors on macrophages and lead to macrophage development into foam cells. Together, the accumulation of oxLDL aggregates and foam cell activation contribute to plaque formation in artery walls that precipitate atherosclerotic events.

One mechanism by which oxidized LDL (oxLDL), as well as cholesterol, may promote atherosclerosis is by causing dysfunction in macrophage lysosomal activity that contributes to processing of lipids [40]. Peritoneal macrophages treated *in vitro* with oxLDL or cholesterol exhibited altered lysosomal function and morphology. Furthermore, macrophages from cardiovascular plaques displayed similar lysosomal dysfunction. Lysosomal biogenesis is controlled by transcription factor EB; in the presence of pro-atherosclerotic lipids, TFEB was less able to translocate to the nucleus to turn on protective autophagy genes. Overexpressing TFEB rescued lysosomal function, enhanced cholesterol efflux and decreased lipid-mediated inflammation by reducing inflammasome activation and IL-1β production.

In addition to directly modulating macrophage activity, oxLDLs can indirectly influence macrophages during atherogenesis by promoting expression of adhesion molecules on endothelial cells [41]. OxLDLs increased expression of vascular cell adhesion molecule (VCAM) 1 and intercellular adhesion molecule (ICAM) 1, subsequently promoting macrophage adhesion to endothelial cells. OxLDLs, the glycoprotein fibronectin, and its receptor, integrin α5, form a pro-atherogenic network that contributes to the formation of aortic plaques. Treatment of atherosclerosis-prone mice with integrin α5 inhibitor led to decreased lipid accumulation, VCAM-1 expression, and macrophage infiltration, which ultimately led to reduced plaque formation. Another important therapeutic strategy to reduce the pathogenic effects of oxLDL is treatment with lipoprotein mimetic molecules. These are synthetic peptides that mimic the ApoA and ApoE, which are components of HDL, the protective cholesterol. Treatment with mimetic peptides can counteract the pro-atherogenic and pro-inflammatory functions of LDLs, and human clinical trials testing these peptides are underway [42]. RAW 264.7 macrophages treated with mimetic peptides neutralized negatively charged LDLs and, prevented LDL uptake and foam cell formation [43]. Furthermore, production of pro-inflammatory cytokines IL-1α, IL-6, and chemokine CCL2, were decreased in macrophages after treatment. *In vivo* challenge with oxLDL led to increased IL-6 secretion into plasma, while pre-treatment of the oxLDL molecules with mimetic peptides decreased inflammation.

Other indirect mechanisms that impact macrophage biology include lipoprotein enzymes that catalyze the formation of immune-modulating metabolites. Lipoprotein lipase (LPL), a lipoprotein hydrolyzing enzyme, contributes to atherogenesis by liberating free fatty acids from lipoproteins [44]. Exposing THP-1 macrophages to LPL-hydrolyzed lipoproteins products led to decreased expression of cholesterol transporter genes including ATP-binding cassette transporters, peroxisome proliferator-activated receptors (PPARs), HDL scavenger receptor and liver x receptor. Treatment of macrophages with free fatty acids isolated via LPL hydrolysis caused decreased expression of transporter genes and impaired reverse transport of cholesterol from cells.

Finally, lipoproteins modulate the functions of macrophages by influencing their polarization into classically activated macrophages, which are associated with exacerbated disease progression in atherosclerosis or AAM, which are considered atheroprotective. Phosphatidylcholine is a major component of oxLDL that forms pro-inflammatory lysophosphotydalcholine (lysoPC) when metabolized. In human macrophage differentiation cultures, lysoPC promoted production of conventional classically activated macrophage cytokines IL-1β, IL-12, IL-6 and TNFα [45]. This stimulatory effect was dependent on the G protein-coupled receptor G2A. In contrast, the HDL-associated lipid, sphingosine-1-phosphate (S1P) was atheroprotective and promoted AAM polarization [46]. S1P exposure in macrophages reduced expression of pro-inflammatory cytokines, but stimulated production and secretion of prototypical AAM cytokine IL-4. In conjunction with increased macrophage-derived IL-4, macrophages exhibited augmented production of other AAM proteins including IL-13, arginse-1, and IL-4 receptor. S1P-mediated macrophage polarization resulted in attenuated expression of CD36, a scavenger receptor that recognizes oxLDL, and increased expression of ATP-binding cassette transporter, suggesting that S1P prevents lipid accumulation in macrophages. Indeed, macrophages treated with S1P exhibited decreased lipid storage in an IL-4 dependent manner. These data provide insights into opposing roles for LDL and HDL in macrophage polarization and the subsequent effects in exacerbating or inhibiting atherosclerosis.

3.2 Leptin

Leptin is a hormone produced in the adipose tissue that was discovered by studies of ob/ob mice that have a spontaneous mutation in the leptin gene, leading to obese and developed diabetes [47]. Functionally, leptin affects the hypothalamus region of the brain, where it triggers satiety signals and helps regulate food intake by counter-acting ghrelin, the hunger hormone, but also functions to promote energy expenditure in peripheral tissues [48]. Leptin expression is directly related to the amount of adipose tissue a person has, with increased adipose tissue leading to greater expression of leptin. Chronically high leptin levels can lead to leptin resistance and changes in the dynamics of fat storage, glucose metabolism and insulin signaling.

In contrast to its metabolic function in reducing obesity, leptin also acts as an immune mediator where it promotes activation, chemotaxis and survival of both innate and adaptive immune cells [49]. Leptin shares structural similarity with IL-6 and acts on immune cells via the leptin receptor, which belongs to the cytokine receptor family. Stimulation of the leptin

receptor activates JAK-STAT signal transduction, utilizing JAK2 and STAT3 to relay its signals [50]. Because it shares a similar signal transduction mechanism as cytokines, leptin signaling can promote obesity-associated induction of pro-inflammatory mediators [51]. Leptin receptor deficient bone marrow cells were transferred into irradiated wild-type mice. Deficiency of leptin receptor led to decreased adipose tissue infiltration of inflammatory macrophages and reduced formation of crown-like structures, foci of macrophages that contribute to disease pathogenesis. In agreement with decreased inflammatory macrophages, expression of pro-inflammatory cytokines including TNFα, IL-6 and CCL2 were decreased in adipose tissue. Furthermore, leptin also stimulated IL-18 secretion from THP-1 macrophages. Increased IL-18 release from leptin-stimulated cells was not dependent upon increasing IL-18 transcription, suggesting leptin promotes IL-18 release via activation the inflammasome/caspase-1 to cleave pro-IL-18. Indeed, inhibiting caspase-1 activity abolished leptin-stimulated IL-18 secretion. Since both leptin and IL-18 are increased during obesity, these data provide further insight into potential pathogenic mechanism of obesity-associated inflammation [52].

In addition to inflammation, zinc deficiency is another potential consequence of obesity observed in humans. Mice that were fed a zinc deficient high fat diet exhibited enhanced alterations in adipose tissue expression of zinc transporters compared to mice that were fed zinc sufficient high fat diet [53]. Zinc deficiency also augments leptin production, increases leptin receptor expression, and increased infiltration of macrophages and formation of crown-like structures in adipose tissue. The mechanism by which zinc deficiency contributes to leptin-mediated inflammation during obesity remains elusive. However, the authors speculate that because zinc can exhibit antioxidant properties and leptin production can be augmented by pro-inflammatory cytokines, altered zinc metabolism and oxidative stress resultant of zinc deficiency contributes to leptin production and inflammation.

Leptin may also contribute to Systemic Lupus Erythematosus, an autoimmune disorder. Leptin promotes uptake of apoptotic self-antigen in peritoneal macrophages [54]. Macrophages then transfer antigen to self-reactive T cells. These data indicate leptin in promoting crosstalk between innate and adaptive immune cells, and suggest the inhibiting leptin signaling could alleviate SLE.

Contrary to its pro-inflammatory effects, leptin can also reduce adipose tissue inflammation by enabling a leptin-catecholamine signaling axis [55]. Mice challenged with LPS exhibited induction of pro-inflammatory cytokines, which was attenuated with prostaglandin E2, a hormone that spurs production of cAMP. PGE2-mediated suppression of inflammation occurred via HDAC4, a histone deacetylase that can inhibit NF-κB-mediated inflammation, dephosphorylation, nuclear translocation, and association with genes that transcribe pro-inflammatory cytokines, namely TNFα and IL-12. Administration of exogenous leptin increased expression of noradrenaline in adipose tissue, which increased cAMP production, ultimately leading to dephosphorylation and nuclear translocation of HDAC4 in bone marrow-derived macrophages during short-term high fat diet feeding to mice. Loss of HDAC4 promoted increased expression of pro-inflammatory cytokines in macrophages, as well as increased crown-like structure formation in adipose tissue. These effects were more modest during long-term feeding. As mice become leptin resistant, HDAC4 function

decreased and contributed to metabolic dysfunction. These data support an earlier study that showed decreased HDAC4 expression in obese individuals [56].

3.3 Adiponectin

Initially discovered as hormone produced exclusively in adipose, adiponectin was first described as a modulator of glucose levels; adiponectin stimulates a decrease in gluconeogenesis, while increasing glucose uptake [57]. Adiponectin also regulates fat metabolism by promoting β-oxidation of lipids. Though adiponectin is primarily expressed in adipose tissue, it is also produced in endothelial cells, as well as skeletal and cardiac myocytes [37]. Expression of adiponectin can be enhanced by PPARs, contrary to catecholamines, which inhibit its expression. Pro-inflammatory cytokines, including TNFα and IL-6, also suppress expression of adiponectin. Given the inflammatory nature of obesity-related diseases, this offers one potential explanation for decreased adiponectin expression during insulin resistance, metabolic syndrome, etc. Outside of its metabolic functions, adiponectin also exerts anti-inflammatory effects on macrophages. Adiponectin stimulates production of IL-10 and IL-1R antagonist, decreases phagocytic activity, and suppresses pro-inflammatory cytokine production by inhibiting NF-κB [58–60]. Below, we discuss some of the mechanisms by which adiponectin protects against cardiovascular and metabolic dysfunction.

Adiponectin has been proposed as a protective mediator against obesity-related atherogenesis. Rosiglitazone, a PPARγ agonist, stimulated adiponectin production in adipose tissue and was associated with decreased inflammatory cytokine production, as well as decreased macrophage infiltration [61]. In addition, rosiglitazone decreased aortic inflammation and plaque formation. Increased adiponectin led to an induction of Irak3, a negative regulator of NF-κB-mediated inflammation. Increased Irak3 expression in bone marrow-derived macrophages, and led to a reduction in CCL2. The protective role of adiponectin/Irak3 in obesity-related atherogenesis was supported in high fat diet mouse studies. HFD-fed mice exhibited decreased PPARγ, adiponectin and Irak3 expression, but augmented plaque formation and inflammation.

Furthermore, foam cell formation can be reduced by exposure to adiponectin [62]. Adiponectin treatment of primary macrophages from diabetic patients lead in increased cholesterol efflux in an adiponectin-receptor dependent manner. Signaling via adiponectin receptor increased expression of ATP-binding cassette transporter and liver x receptor α, both of which are important in mediating cholesterol efflux.

In a model of alcoholic liver disease, which can lead to inflammation and metabolic dysfunction, adiponectin suppresses both MyD88 dependent and independent TLR4 signaling [63, 64]. LPS stimulation of rat liver macrophages, or Kupffer cells, leads to MyD88-dependent production of TNFα. Adiponectin treatment reduced LPS-induced TNFα in rat Kupffer cells by decreasing ERK signaling and increasing IκB stability. In contrast, LPS-induced MyD88-independent TLR4 signaling leads to production of immune mediators CXCL10 and IFNβ. Adiponectin reduced CXCL10 and IFNβ production in LPS treated, ethanol exposed macrophages. Inflammation was reduced by adiponectin-mediated expression of heme oxygenase 1, a potent anti-inflammatory molecule. In accordance with

its ability to decrease inflammation, adiponectin also promotes polarization of RAW 264.7 macrophages from the classically activated phenotype to anti-inflammatory alternatively activated phenotype [65]. Macrophages treated with adiponectin exhibited increased IL-4 production, together with increased expression of alternatively activated macrophage markers including Arginase 1, mannose receptors and IL-1 receptor antagonist. Adiponectin-mediated alternatively activated macrophage polarization was partially dependent upon the adiponectin-heme oxygenase 1 axis. Finally, macrophage polarization differentially affects adiponectin receptor expression [66]. Classically activated macrophages, stimulated by IFN-γ-LPS, decreased expression of adiponectin receptors, while anti-inflammatory cytokines IL-4 and IL-10 stimulated adiponectin receptor expression in peritoneal and bone marrow-derived macrophages.

Collectively, these data point to important roles for lipoproteins, leptin, and adiponectin in promoting or ameliorating diseases associated with metabolic dysfunction, including atherosclerosis and alcoholic liver disease, by altering lipid storage and metabolism, and shifting the balance of immune responses to pro-inflammaory or anti-inflammatory (Figure 2).

4. Resistin and Resistin-like molecules

The Resistin-Like Molecules (RELM) are a family of secreted mammalian proteins that have both hormonal and immune functions. In mice, resistin was first described as a gene expressed by adipocytes that caused resistance to insulin thereby leading to the protein family's name [67]. In a screen for adipocyte genes that were sensitive thiazolidinedione (TZD), a PPARγ ligand that improves insulin sensitivity in diabetic patients, resistin was identified as gene that was profoundly inhibited by TZD. Subsequent studies in both mouse models and clinical studies of obese or diabetic individuals have implicated resistin in mediating obesity-induced diabetes [68]. Interestingly, in humans, resistin is expressed by immune cells, where it promotes inflammatory cytokine production. Additionally, the related proteins RELMα, RELMβ and RELMγ are highly expressed in infection, inflammatory diseases and metabolic disorders [69]. Here, we will discuss the recent studies demonstrating the complexity in function of these proteins in modulating macrophage function.

In metabolic studies, resistin contributes to insulin resistance by increasing production of hepatic glucose, while impairing insulin-mediated glucose metabolism [70]. Research studies using mice support the involvement of resistin in promoting obesity-related pathologies, however, resistin studies with human subjects are controversial. Though increased resistin levels are correlated with obesity, and is predictive of adverse cardiovascular events by promoting vascular inflammation and lipid uptake [71], other studies have not seen a significant correlation between resistin and adiposity or insulin resistance [72]. Another difference in physiology of resistin between mice and humans relates to the cellular source; mouse resistin is expressed primarily in adipose, while human resistin is produced by macrophages, and to a lesser extent, adipocytes. Increased resistin expression observed during obesity-related pathologies could be related to increased infiltration of macrophages into the adipose tissue. In a model of atherosclerosis using

rabbits, resistin-expressing macrophages infiltrated aortic plaques after cholesterol feeding or surgical injury [73]. Adenoviral expression of human resistin induced macrophage migration to the plaque. This process was mediated by integrins; resistin induced macrophage expression of integrins and expression of VCAM-1 and ICAM-1 by vascular endothelial cells, which led to increased macrophage-endothelial cell adhesion. In addition, resistin promoted macrophage survival, and chemotaxis both directly and indirectly. Macrophages migrated toward resistin in the absence of other chemokines, while migration was enhanced in the presence of resistin and CCL2. Macrophage infiltration was associated with increase lipid accumulation and decreased plaque stability. Resistin also promotes chemotaxis of primary human macrophages by inducing expression of fractalkine (FKN) [74]. Using an endothelial cell-smooth muscle cell co-culture system to mimic cell interactions within vessel walls, the presence of resistin in conjunction with smooth muscle cells in the sub-endothelial space promoted macrophage transmigration. Resistin augmented production of FKN and CCL2 in endothelium, and this response was enhanced in the presence of smooth muscle cells. Resistin-mediated increases in CCL2 was also shown to be partially dependent upon FKN up-regulation, however, macrophage transmigration could be reduced by inhibiting FKN or CCL2. Additionally, inhibiting both abolished macrophage transmigration, pointing to a compensatory role for FKN and CCL2 in promoting macrophage transmigration. Finally, resistin-mediated macrophage transmigration was dependent upon expression of FKN receptor, CX3CR1, and CCL2 receptor, CCR2. These data suggest that resistin contributes to promotion and sustainment of adverse cardiovascular events by stimulating macrophage chemotaxis directly, or indirectly via modulation of other chemokines.

Resistin is also a key immune mediator. Resistin directly stimulates NF-κB-mediated inflammation, including the promoting expression and secretion of TNFα, IL-1β, IL-6 and IL-12 [71]. Recent data from our lab indicate that the immune stimulatory effect of human resistin is detrimental in helminth infection and impairs worm expulsion [75]. Transgenic mice expressing human resistin exhibited increased expression of resistin and infiltration of pro-inflammatory monocytes following infection with the helminth *Nippostrongylus*. Mechanistically, human resistin promoted a pro-inflammatory environment, including increased expression of Toll-like receptor 4, IL-1β, and CCL2, without influencing the type 2 T helper cytokine immune response. These observations were confirmed in helminth-infected humans, who exhibited increased serum levels of resistin that was associated with increased parasite burden and circulating levels of CCL2 and TNFα.

The related murine protein RELMα is also expressed by immune cells and is immunomodulatory [69]. RELMα is a prototypical marker for AAMs, and its expression is spurred by stimulants that induce Th2 immune responses such as allergens and helminths. Although RELMα is a marker for AAMs, it acts as a negative regulator of Th2 immune responses during helminth infection [76]. RELMα$^{-/-}$ mice challenged with *Schistosoma* eggs exhibited increased lung granuloma formation and exacerbated production of IL-4, IL-13 and IL-5, and circulating IgE. RELMα$^{-/-}$ AAMs co-cultured with CD4$^+$ T cells promoted increased proliferation and Th2 cytokine production. These data illustrate a role for AAM-derived RELMα in regulating Th2 responses during helminth infection.

RELMα$^{-/-}$ mice also showed enhanced immunity to *Nippostrongylus* infection, associated with increased Th2 immune responses [77]. RELMα is also expressed by dendritic cells [78], and in contrast to AAM-derived RELMα, dendritic cell-derived RELMα was important in T cell priming and production of IL-13 and IL-10 [79]. In non-infection Th2 inflammatory settings such as murine asthma models, the function of RELMα is controversial. Delivery of RELMα into the lungs promoted Th2 cytokine-mediated fibrosis by the DNA damaging agent bleomycin [80]. Conversely, RELMα$^{-/-}$ mice exhibited reduced bleomycin-induced fibrosis. In contrast, transgenic mice that overexpressed RELMα were protected from ova-induced allergic inflammation and exhibited reduced Th2 cytokines [81]. These studies suggest that the immune function of RELMα is complex and may depend on which cell-type expresses RELMα, the RELMα levels and the type of inflammatory environment.

In a model of bacterial-induced colitis with gram negative bacterium *Citrobacter*, we showed that RELMα exhibited a pro-inflammatory role [82]. *Citrobacter* infection led to colitis and increased RELMα expression by intestinal epithelial cells and infiltrating macrophages and eosinophils. RELMα$^{-/-}$ mice were protected from *Citrobacter*-induced colitis; however, treatment with exogenous RELMα restored *Citrobacter*-related pathologies in RELMα$^{-/-}$ mice in an IL-17A dependent manner. These results suggest that RELMα contributes to intestinal inflammation following bacterial infection by promoting a Th17 inflammatory environment. RELMα is also involved in pathogenesis of non-bacterial colitis [83]. RELMα stimulated intestinal production of IL-6 in response to DSS-induced colitis. Additionally, LPS and RELMα acted synergistically to induce IL-6 and TNF-α expression following *ex vivo* stimulation of bone marrow-derived macrophages.

New studies have identified a critical metabolic function for RELMα in protection against atherosclerosis in both high fat diet fed mice and LDL receptor deficient mice [84]. Mice lacking the LDL receptor (*ldlr*$^{-/-}$) cannot efficiently remove circulating LDL, leading to increased formation of atherosclerotic plaques in the context of high fat diet. However, *ldlr*$^{-/-}$ mice that were deficient in RELMα suffered from exacerbated atheroscleoric disease compared to RELMα sufficient *ldlr*$^{-/-}$ mice, evidenced by increased circulating cholesterol, and increased number and size of aortic plaques. Additionally, overexpression of RELMα in high fat diet fed mice was protective and reduced circulating cholesterol levels. This atheroprotective function for RELMα is conflicting with the pathogenic role for human resistin in related metabolic disease, suggesting that although related in protein structure, these proteins may have opposing functions. Interestingly, in an inflammatory environment mediated by DSS, a compound that is toxic to intestinal epithelial cells, RELMα$^{-/-}$ mice showed ameliorated metabolic function compared to wild-type mice and were protected from hyperglycemia induced by glucose challenge [83]. This suggests that RELMα promotes metabolic dysfunction in the context of ongoing inflammation. Similar to resistin, the effects of RELMα may depend on the inflammatory and metabolic environment.

Similar to RELMα, RELMβ is induced following helminth-induced Th2 immune responses. Their expression pattern, however, varies. RELMβ is primarily produced by mucus-producing goblet cells, as opposed to hematopoietic cells that are a main cellular source for RELMα [69]. Following helminth infection with *Nippostronglus* and *Heligmosomoides,*

RELMβ$^{-/-}$ mice exhibited impaired worm expulsion [85]. *In vitro* studies showed that RELMβ could bind to the helminths and decrease their fecundity and viability. In contrast to this host protective role by directly acting on the worm, RELMβ also had an immunostimulatory function following *Trichuris* infection where it promoted activation of splenic and bone marrow-derived macrophages, and production of inflammatory cytokines, analogous to the function of human resistin [86]. While RELMβ has been shown to be almost exclusively expressed in goblet cells in helminth infection, foam cells also express RELMβ in atherosclerotic plaques [87]. RELMβ was expressed in human aortic lesions, and expression was co-localized with macrophage marker CD68. ApoE$^{-/-}$ mice, which are susceptible atherosclerosis, were bred with RELMβ$^{-/-}$ mice to determine its role in aortic lesions. Presence of RELMβ augmented aortic lipid accumulation and macrophage infiltration in ApoE$^{-/-}$ mice. Additionally, RELMβ supported lipid uptake and the formation of foam cells by down-regulating cholesterol efflux mediators. Similar to the *Trichuris* infection studies, RELMβ promoted expression of pro-inflammatory molecules TNFα, IL-1β, and IL-6 in macrophages, which likely contributes to RELMβ-mediated atherosclerotic pathogenesis.

The function of RELMγ, which is expressed by haematopoietic cells, is less clear. In high fat fed diet mice and obese leptin receptor deficient mice, both RELMγ and RELMβ serum levels were significantly upregulated [88], suggesting that analogous to the other RELM proteins, RELMγ is also induced in metabolic dysfunction. In conclusion, these multiple studies on RELM proteins highlight the complexity in function of this protein family as important adipokines that regulate metabolism, immunity and inflammation (Figure 3).

5. Conclusion

Macrophage phenotypes are as diverse as the stimuli that activate them [89]. In both *in vitro* and *ex vivo* experiments, culture conditions such as media, growth factors and the type of culture dish may affect the physiological readouts. Additionally, the tissue source of macrophages can account for differences in macrophage responses. Investigators should therefore consider how using immortal or primary cells, bone marrow derived or tissue resident macrophages, and mouse or human macrophages, could influence the experimental outcome. In this review, we have summarized recent studies that encompass many of these different sources of macrophages to highlight the significance of catecholamines, adipokines and RELM proteins in macrophage function.

Catecholamines and adipokines have long been recognized as hormone signaling molecules, but recent studies have elucidated previously unrecognized functions for these proteins in modulating the immune system specifically through effects on macrophages. These advances in knowledge of neuro-immune and metabolism-immune interactions offer valuable perspective when considering human health and physiology. For example, while the health benefits of exercise are well known from the metabolic perspective, these studies provide insight into new immune mechanisms that influence positive health outcomes via the CNS and metabolic processes. With the discovery that thermostress promotes an immune response that mediates adipogenesis of energy-burning beige fat, while electroacupuncture triggers CNS-mediated anti-inflammatory pathways, exploring these

non-traditional macrophage-mediated pathways may identify innovative treatments for metabolic and inflammatory diseases.

Abbreviations

AAM	alternatively activated macrophage
Apo	apolipoprotein
CNS	central nervous system
FKN	fractalkine
HDL	high density lipoprotien
ICAM	intercellular adhesion molecule
IL	interleukin
LDL	low density lipoprotein
LPL	Lipoprotein lipase
lysoPC	lysophosphatidylcholine
MCP	monocyte chemoattractant protein
oxLDL	oxidized LDL
PPAR	peroxisome proliferator-activated receptor
RELM	resistin-like molecule
S1P	sphingosine-1-phosphate
TNF	tumor necrosis factor
TZD	thiazolidinedione
VCAM	vascular cell adhesion molecule

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Highlights

- Macrophages modulate immunity in response to, and by producing, catecholamines.

- Adipokines influence metabolic outcomes by modulating macrophage phenotype.

- RELM proteins promote adverse metabolic outcomes such as lipid accumulation.

- RELM proteins can both promote and decrease susceptibility to infection.
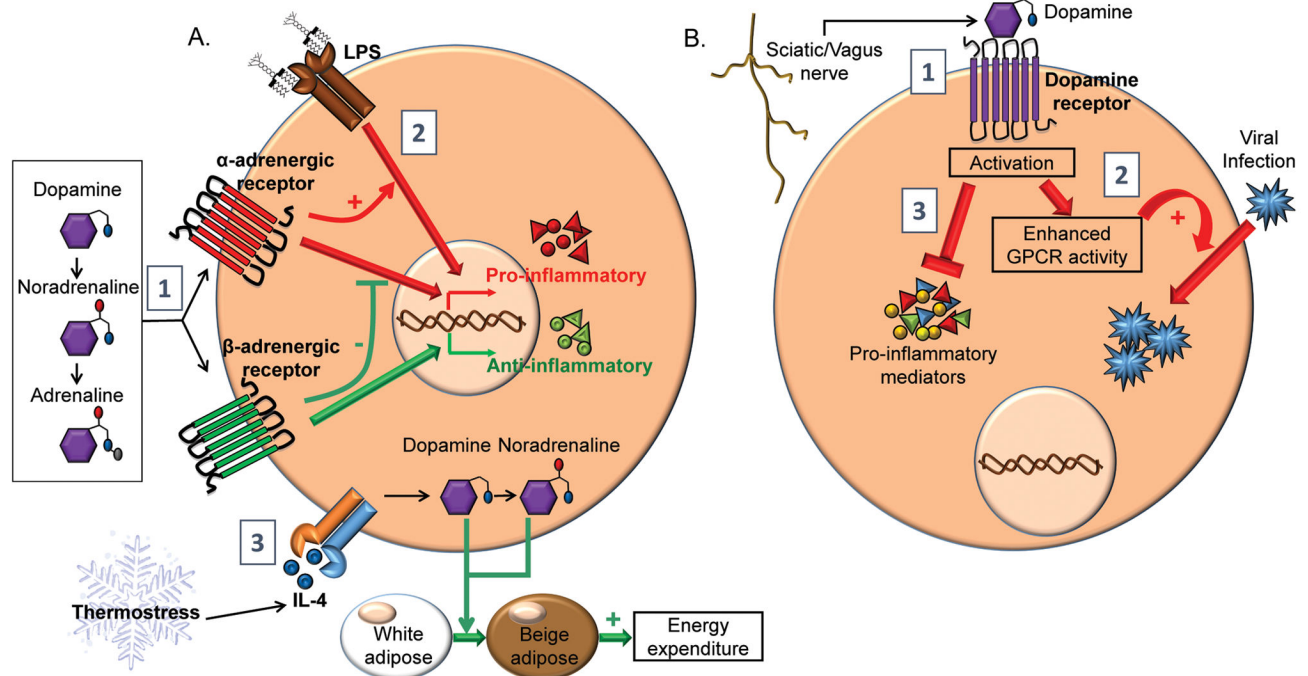
Figure 1. Catecholamine signaling in macrophage function

A. Catecholamines are recognized by α and β-adrenergic receptors (1). Signaling through α-adrenergic receptors is pro-inflammatory and promotes LPS-induced gene expression whereas β-adrenergic receptor signaling inhibits this and induces expression of anti-inflammatory cytokines (2). Cold temperature induces macrophage synthesis of catecholamines which act to increase white to beige adipose tissue conversion and energy expenditure (3). B. Sciatic and vagus nerve stimulation promotes dopamine synthesis (1). Dopamine receptor signaling enhances GPCR activity leading to increased viral entry and replication (2). Dopamine signaling also inhibits pro-inflammatory cytokine production (3).
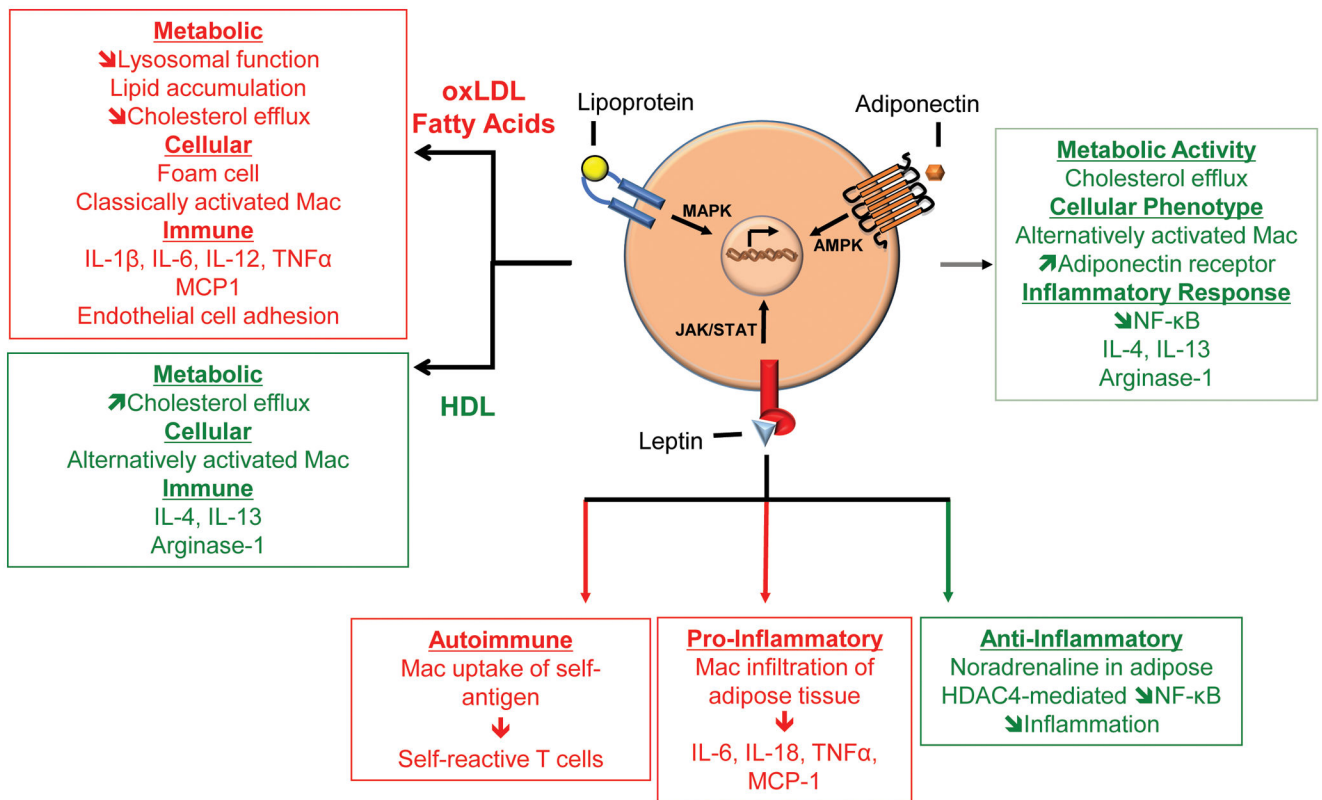
Figure 2. Adipokine influence on macrophage function
In addition to influencing the metabolic status of macrophages, adipokines and lipoproteins also exert both pro-inflammatory and anti-inflammatory effects on macrophages.
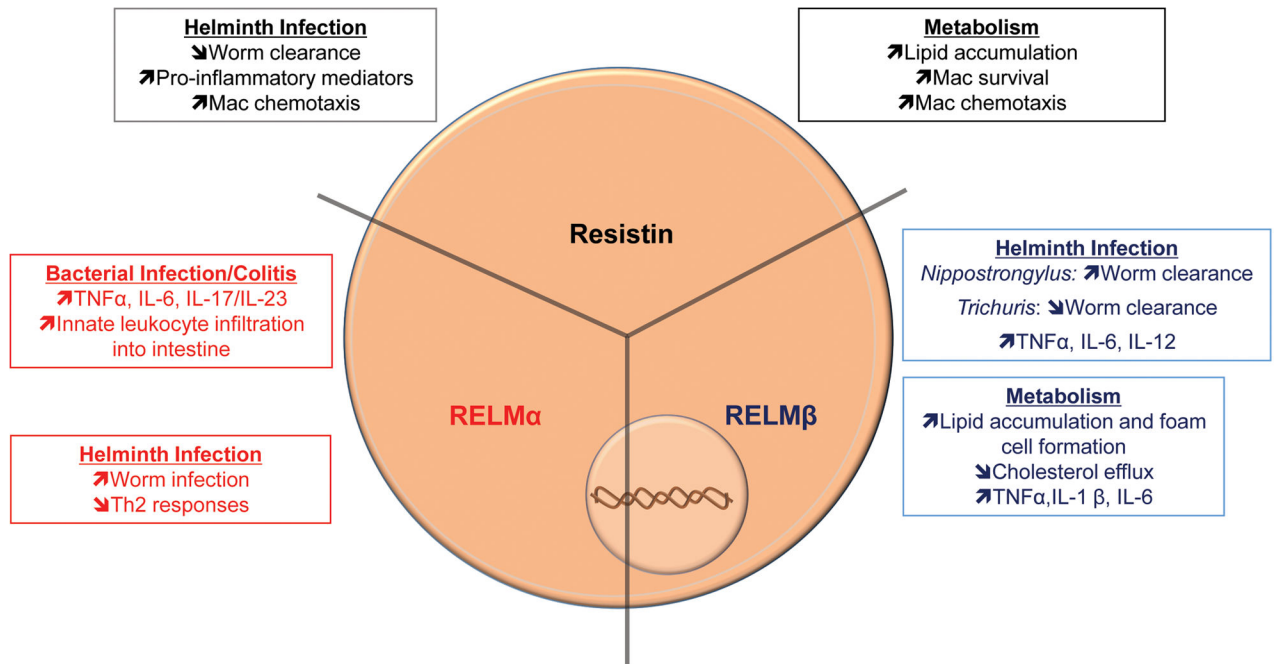
Figure 3. Resistin-like molecules influence macrophage physiology during in infection and metabolic disease

In general, RELM proteins promote pro-inflammatory responses during infection and metabolic dysfunction, leading to detrimental effects on the host. In some cases, however, the presence of RELM proteins can be beneficial, such as RELMβ promoting resolution of *Nippostrongylus* infection.