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Macrophage polarization and Metainflammation

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Abstract

Chronic over-nutrition and obesity induces low-grade inflammation throughout the body. Termed "metainflammation," this chronic state of inflammation is mediated by macrophages located within the colon, liver, muscle and adipose tissue. A sentinel orchestrator of immune activity and homeostasis, macrophages adopt variable states of activation as a function of time and environmental cues. Metainflammation phenotypically skews these polarization states and has been linked to numerous metabolic disorders. The past decade has revealed several key regulators of macrophage polarization, including the Signal Transducer and Activator of Transcription (STAT) family, the peroxisome proliferator-activated receptor gamma (PPARγ), the CCAAT-enhancer-binding proteins (C/EBP) family and the Interferon regulatory factors (IRFs). Recent studies have also suggested that microRNAs (miRNAs) and long noncoding RNA (lncRNA) influence macrophage polarization. The pathogenic alteration of macrophage polarization in metainflammation is regulated by both extracellular and intracellular cues, resulting in distinct secretome profiles. Metainflammation-altered macrophage polarization has been linked to insulin insensitivity, atherosclerosis, inflammatory bowel disease, cancer and autoimmunity. Thus, further mechanistic exploration into the skewing of macrophage polarization promises to have profound impacts on improving global health.

Key terms

obesity; metainflammation; macrophage polarization

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Introduction

Chronic over nutrition disturbs the body's homeostasis and associates with physiologic disturbances such as high blood pressure, hyperglycemia, hyperlipidemia, and obesity. The constellation of these pathologies is termed metabolic syndrome, and it is affecting a growing percentage of the world's population. Metabolic syndrome is associated with comorbidities such as diabetes, cardiovascular disease, osteoarthritis and cancer, greatly impacting mortality and morbidity (1). The inciting trigger of metabolic dysfunction has yet to be mechanistically elucidated, however chronic over-nutrition and resultant obesity are recognized as a cause of chronic, low-grade inflammation, historically described as "Syndrome X" (2), "The Deadly Quartet" (3), and "Insulin-Resistance Syndrome" (4). The unresolvable immune activation occurs in the absence of overt infection or frank autoimmune disease, but is recognized to not only affect local tissues, but systemic physiology through chronic, low-grade inflammation induced by obesity(5) (6) that is termed metainflammation.

The link between adipose tissue and inflammation was first substantiated in the 1990s, when tumor necrosis factor α (TNFα) mRNA was isolated from adipose tissue of obese rodents and discovered to attenuate phosphorylation events during insulin signaling (7) (8). Transcriptome analysis in obese mice then revealed a positive correlation between macrophage-related genes and increased body mass in 2003 (9). When compared to other tissues, the upregulation of these macrophage genes was observed earliest and in the greatest magnitude within white adipose tissue (10).

Macrophages represent an integral compartment of the innate immune system and are critical regulators of both normal homeostasis as well as pathology. Derived from hematopoietic progenitors, macrophages are crucial for proper tissue remodeling during fetal development (11). Furthermore, macrophages are equipped with the capacity to sense and respond to homeostatic alterations in adults (12). Macrophages are seeded throughout the body where they play important homeostatic roles. Key examples include within osteoclasts in bone, lung alveolar macrophages, and Kupffer cells in the liver (13). Within these organs, macrophages protectively remove dead cells, debris and lipoproteins from their environment (12). Beyond maintenance of homeostasis, macrophages also play critical roles in the repair of injured tissue (14) (15). Critical to this reparative function is the capacity for macrophages to sense tissue damage, then orchestrate a localized induction, then resolution of inflammation. Metainflammation conversely, is characterized by the unrelenting, chronic activation of macrophages which significantly, if not indefinitely, alters the body's balance of inflammatory cues.

Since obesity and inflammation were first associated, much has been uncovered with respect to how macrophages take residence within specific tissues and assume differential states of activation under the influence of metainflammation. This review will cover these topics, with special emphasis on how macrophages maintain a chronic, meta-inflamed environment and how this environment instigates an imbalance of physiology, ultimately resulting in development of metabolic syndrome.

Macrophage Distribution and Tissue-residence in Metainflammation

This review will focus on the effects of obesity-associated meta-inflammation on macrophages at the sentinel site of nutrient absorption, gastrointestinal tract, as well as within metabolically active organs such as adipose tissue, the liver, and muscle.

a. Kinetics of Macrophage Localization

Macrophage residency is established through two primary mechanisms: (i) prenatally, or (ii) during adulthood from circulating monocytes (11) (13). (i) Seminal fate-mapping work using state-of-the-art genetic mouse models has established key characteristics of prenatally derived macrophage populations, which include liver Kupffer cells, skin Langerhans cells, and lung alveolar macrophages. These macrophages are generated from HSC without an intermediary monocyte phenotype and if absent, result in significant growth retardation, or mortality (16) (17). Once established prenatally, these macrophage populations are further self-sustaining in the physiologic steady state, expanding to fill their tissue resident niche with minimal input from circulating monocytes.(18) (19) (20).

During adulthood, bone marrow HSCs yield both myeloid and lymphoid progenitors, but it is from the myeloid progenitor pool that macrophages can be derived. HSC differentiate into monocytes, which then egress from the bone marrow via C-C Motif Chemokine Receptor 2 (CCR2). Within circulation, monocytes can be broadly categorized into $Ly6C^{high}$ or $Ly6C^{low}$ populations. It is thought that $Ly6C^{low}$ populations patrol and survey for intravascular damage, while $Ly6C^{high}$ populations take residence within peripheral tissues (21). While there is some debate as to the plasticity of these initial cell surface phenotypes in relation to their terminal fate, the dogma of “classical” monocyte-inflammatory, tissue resident macrophage is based on $Ly6C^{high}$ monocytes (22) (23) (13).

b. Adipose Tissue Macrophages

Originally characterized as an inert tissue depot of triglycerides, adipose tissue has since been recognized dynamic organ that orchestrates metabolic, endocrine and immune responses (24). Adipose tissue serves as a reservoir of fatty energy during times of fasting, but in settings of obesity, it releases serum factors such as adipokines, cytokines and chemokines to elicit recruitment of immune cells. In adipose tissue, infiltrating immune cells, such as macrophages, B cells, T cells, are the second largest population after adipocytes and play critical roles in regulating tissue function and homeostasis (25). Being a major portion of adipose tissue immune cells, the activity of adipose tissue macrophages (ATM) are closely correlated with inflammatory responses under obesity stress.

Adipose tissue can be classified into three categories based on function and appearance: White, Brown, and Beige.

1) White Adipose—White adipose tissue (WAT) comprises the majority of “fatty tissue” and is capable of releasing free fatty acids into the circulation when circulating glucose levels are low. WAT also secretes factors that modulate insulin sensitivity, and it was within WAT that an association between obesity and immune cell activation was first conceived (26). In comparing lean to obese mice, macrophage infiltration of adipose tissue was

demonstrated to increase 10-fold – from 5% to up to 50% after reaching the state of obesity (9). Studies using obese animal models suggested a positive correlation of increased C-C Motif Chemokine Ligand 2 (CCL2, or monocyte chemoattractant protein-1, MCP-1) with increased adipose tissue mass. This link was substantiated by dominant-negative CCL2 expression mitigating insulin resistance and macrophage infiltration associated with high-fat diet and genetically-induced diabetes (27). More recently, the mRNA upregulation of the alarmin S100 calcium-binding protein A8 (S100A8) has been associated with early stages of high fat diet feeding, precipitating macrophage chemotaxis in both in vitro assays using RAW264.7 and 3T3-L1 adipocytes, and in vivo tracking models using $LysM^{EGFP}$ and intravital adipose tissue imaging (28). Though there has long been a clear association between inflammation and insulin resistance, the mechanistic steps linking these two events was largely unknown until 2011. Through time course analyses of macrophage content and insulin sensitivity of wildtype versus immunocompromised mice, it was elucidated that macrophage-induced inflammation was necessary for the sequelae of long-term insulin resistance upon high fat feeding (29).

Within expanding WAT, macrophages, both resident and infiltrating, localize to dying adipocytes and form crown-like structures (CLS) fusing into pro-inflammatory, multinucleated giant cells(30) (31). These CLS have been reported to account for over ninety percent of infiltrating macrophages and are a histologic hallmark of inflammation within adipose tissue (31) (32). In addition to causing adipose hypertrophy, obesity has also been associated with excessive free fatty acids (FFA). These FFA have been shown to activate Toll-like receptor 4 (TLR4) signaling from the 3T3-L1 adipocyte cell line and adipose tissue harvested from diabetic mouse models, further substantiating a link between inflammatory macrophage activation and insulin resistance (33).

2) Brown and Beige Adipose—In contrast to white adipose tissue, brown adipose tissue (BAT) possess high levels of a mitochondrial uncoupling protein and specializes in thermogenesis (34). Specifically, uncoupling protein-1 (UCP1) allows for the energy of ATP to be expended as heat. In contrast to white adipose, brown fat is also richly innervated, vascularized and shares a progenitor with muscle tissue (34). Similar to BAT, “beige” adipocytes can express high levels of UCP-1. Its alternative names including “brite” (“Br”own and wh”ite”) tissue, or inducible “brown-like” adipocytes highlight its phylogenetic link to WAT, but nevertheless functional similarity to BAT. While there is debate regarding what beige tissue is induced, it is largely believed that it basally expresses low levels of UCP1 that can be upregulated upon appropriate environmental cues (35) (36) (37) (38)

Promoting the beiging of WAT has served as a therapeutic target since genetic upregulation of “beige” genes have been linked to obesity-resistance in mouse models (39). Recent studies have suggested that macrophages play an active role in the beiging process, or development of BAT. In 2011, it was reported that mice exposed to low temperatures would upregulate their non-shivering thermogenesis genes in a macrophage-dependent manner. Through the genetic, *in vivo* manipulation of cytokines required for alternative M2 macrophage polarization, it was concluded that alternatively activated macrophages were required for the orchestration of thermogenic responses to cold through upregulation of

catecholamine synthesis pathway (40) (41) (42). This notion of macrophage-mediated release of catecholamines to promote the browning of adipose tissue, however, has since been directly contradicted. In a recent study spanning six institutions, *Fischer et al* demonstrated that administration of IL-4 had no effect on either energy expenditure, or the browning of adipose tissues. Furthermore, the catecholamine synthesis enzyme implicated in the previous studies (tyrosine hydroxylase; *Th*) was absent in their rdTomato reporter mice, with no detectable levels of *Th* mRNA within macrophages isolated from BAT or inducible WAT (43). While the influence of alternatively activated macrophages being able to promote the browning of tissue is under debate, several reports have suggested that macrophage-induced inflammation may actively suppress adipose browning (38) (44) (45) (46). Thus, the true physiologic significance of macrophages in the beiging process or development of BAT has yet to be completely elucidated (35), but remains a critical gap in the literature.

c. Colon

The GI tract houses the largest total number of macrophages, and is the first organ to encounter nutrient excess (47). Besides playing a critical role in pathogen clearance, colonic macrophages also regulate inflammatory responses, local homeostasis and insulin sensitivity (48) (49). Intestinal macrophages play critical roles in chronic inflammation in the gut following obesity induced dysbiosis and intestinal barrier deficiency. Obesity in mice is associated with altered microbial composition in the gut (50, 51). Similarly, alterations in intestinal bacterial gene landscape correlates with obesity and its associated metabolic syndrome in humans (52) (53) (54). A consequence of altered microbiota composition in the gut is the increased intestinal permeability, which allows infiltration of bacteria and their degradative products, such as LPS, into lamina propria (55) (56) (57). Bacterial products trigger the activation of lamina propria macrophages in the intestine, resulting in chronic inflammation that triggers the development of metabolic diseases (55) (56) (57). In addition, in Inflammatory Bowel Disease (IBD), macrophages have been observed to take on a foamy appearance within lamina propria and to further potentiate inflammation, paralleling their well-known role in the pathophysiology of atherosclerosis.

d. Liver

Macrophages comprise a large proportion of the liver (20–40%) of the liver, a critical site of metabolic conversion (58). For instance, the liver is responsible for maintaining and regulating the body’s supply and excretion of cholesterol and has been demonstrated to be responsive to, and perpetuating of systemic energy excess. Notably, the gene Mgl2 which encodes the protein CD301A, a prototypical “M2” marker” has been recently implicated in guiding the maintenance of systemic energy surplus (59). Through inducible diptheria toxin depletion of Mgl2 in mononuclear phagocytes (MNP), Kumamoto et al showed that Mgl2 depletion surprisingly tracked with weight loss, improved insulin sensitivity, and decreased serum cholesterol levels. Central to this conclusion was that CD301+ MNPs secreted Relmα, which altered metabolic liver enzymes, including ones that regulate cholesterol elimination from the body via bile acids (60). Beyond responses to macrophage signals, the liver is also a directly affected by chronic fat overload. Nonalcoholic fatty liver disease (NAFLD) is a disease that affects almost a third of the Western population, and is associated with hepatocyte death, release of DAMPS, and resultant macrophage activation (58).

Though both the liver and adipose tissue are capable of lipogenesis, the large majority of organismal fatty acids are conferred through dietary consumption(61) However, in obese states, human metabolic studies have shown that hepatic lipogenesis is increased, perhaps contributing to excessive fat mass(62). Within mice, proteomic and lipidomic analysis of obese versus lean hepatic endoplasmic reticulum demonstrated that obese mice had a higher phosphatidylcholine (PC) to phosphatidylethoanolamine (PE) ratio that disrupted the sarco/endoplasmic reticulum calcium-ATPase (SERCA), leading to ER stress that further stimulates lipid secretion from the liver, creating a vicious cycle linking obesity with insulin resistance and type 2 diabetes(63). During the progression of NAFLD, liver macrophages were found to enhance hepatic lipid accumulation (64, 65) and release of TNF-α, IL-1b and CCL2 (66) (67, 68). These cytokines not only triggered tissue damage, but they also lead to further activation of tissue residing macrophages, which promotes downstream liver fibrosis through production of TGF-β and PDGF (13, 69).

e. Muscle

As muscle is a major site of energy expenditure, it has a crucial role in overall energy homeostasis and whole-body metabolism. Under normal physiologic conditions, it helps normalize the body's stores of glucose upon insulin stimulation (70). Similar to adipose tissue, muscle experiences increased macrophage infiltration in the setting of obesity and Type 2 Diabetes (70) (71). Histological studies have revealed that macrophages infiltrate intermuscular and perimuscular fat depots, albeit in markedly reduced total percentage in comparison to liver and adipose tissue. Nevertheless, it is hypothesized that skeletal muscle macrophages do indeed affect whole-body insulin resistance. Differentially activated macrophages conduct varying functions in muscle healing after injury (72). Additionally, macrophages also regulate cardiac tissue regeneration, maintain cardiac homeostasis and modulate the electric conduction of cardiomyocytes (73) (74).

Regulatory Mechanisms of Macrophage Polarization

In the 1980s, interferon gamma (IFNγ) was identified as an activator of macrophage phagocytic function (75) (76) (77). Since then, much has been discovered with regard to the molecular pathways involved in the macrophage priming process as well as the diversity of phenotypes that macrophages can adopt. In the 2000's, substantial work seemed to elucidate a clear paradigm of macrophage polarization mirroring the opposing phenotypes of, for example, Th1 and Th2 cells. Once tissue resident, macrophages adopted either a proinflammatory M1 phenotype, or an anti-inflammatory M2 phenotype. They are generally accepted as transient, reversible, and occurring along a spectrum (5). **M1** represents the "classically-activated" proinflammatory phenotype of macrophages capable of eliminating pathogens and cells infected by viruses or that have become transformed (5). They produce cytokines and inducible nitric-oxide synthase (iNOS), which provides effector molecules for microbicidal activities that oxidative stress that can inhibit proliferation of nearby cells (6, 78, 79). **M2** represents an "alternatively" activated phenotype with anti-inflammatory activities that can promote wound healing (7).

Macrophage polarization state was first implicated in obesity when *Lumeng et al.* uncovered a propensity for M1 inflammatory gene expression over that of M2 after high-fat feeding(80). This skewing depended on expanding adipose tissue in obese mice expressing the macrophage chemoattractant CCL2, whereas lean mice, in the absence of CCL2 expression, had a predominate M2 gene profile (8) (81). The extent of how these differential macrophage phenotypes influence the browning of fat, or how fat might conversely influence macrophage polarization status, is of current debate. Groups had previously proposed that thermogenic BAT is sustained by M2 macrophages in response to cold or exercise (41) (82). However, more recent studies suggest that while there are intrinsic properties to white and brown adipose tissue that might sustain macrophage polarization (83), the contribution of M2 macrophages to brown adipose maintenance is minimal (43). However, the link between inflammation and suppression of beige fat, or suppression of UCP1 expression in brown fat has been consistently shown (80) (84).

a. Regulatory pathways of macrophage polarization

Within the past decade, several key regulators of macrophage polarization have been elucidated. Interferons have longed been recognized to prime inflammatory macrophage and members of the Signal Transducer and Activator of Transcription (STAT) family have been identified as key mediators of these responses. In addition, regulators of lipid metabolism, transcription factor families, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) have been shown in both in vitro and or in vivo models to be key regulators of macrophage polarization.

1) STAT family—The Janus Kinase (JAK)-STAT pathway is known to promote M1-like macrophages. In particular, STAT1 dimerizes in response to interferon-gamma (IFNγ) and induces M1-associated genes (85). In mice, STAT1 deficiency abolishes responsiveness to IFNγ and IFNα (86). Lipopolysaccharide (LPS), another well-known M1 inducer, functions through induction of IFNβ, which promotes the formation of STAT1-STAT2 heterodimers that mediates the induction of M1-associated genes by forming the IFN-stimulated gene factor 3 (ISGF3) complex (87).

In contrast to STAT1/2, STAT6 is associated with M2 macrophage polarization. IL-4 and IL-13 have both been shown in vitro and in vivo studies to induce M2 polarization (88) (89) (90). STAT6 mediates IL-4a signaling and regulates many M2 signature genes (88) (91). STAT6 signaling is further mediated by MCP-1-induced protein (MCPIP) by inducing reactive oxygen species, endoplasmic reticulum stress, and autophagy (92).

2) PPARγ and LXR signaling mediated polarization—The lipid metabolism regulator Peroxisome Proliferator-Activated Receptor gamma (PPARγ) is a negative regulator of pro-inflammatory genes (93) (94). PPARγ knockout in murine myeloid cells reduces M2-like activation and induces susceptibility to obesity, insulin resistance, and glucose intolerance (95). Evidence suggests that IL-4 and IL-13 acts upstream of PPARγ and regulates its expression in murine thioglycollate-elicited macrophages and human peripheral blood monocytes (96).

PPARγ has also been shown to bind the promoter region of miR223, a micro RNA implicated in myeloid differentiation (97) (98) (see section on miRNA for more detail on this mechanism). Interestingly, interactions between PPARγ and STAT6 have been shown in cultured mouse primary macrophages, in which STAT6 acts as a cofactor, facilitating the induction of PPARγ-regulated genes (99).

Additionally, the liver X receptors (LXRs), which, similar to PPARγ, are nuclear transcription factors that heterodimerize with the retinoid X receptor (RXRα), have been association with suppression of inflammatory pathways in macrophages (100) (101, 102). LXRs are largely associated with retrograde cholesterol transport and their activation has recently been implicated with the M2-promoting transcription factor MafB, which is downregulated by miR155 and miR33(103). While LXR activation has historically been tied to amelioration of autoimmune diseases and regression of atherosclerotic plaques, their role in metainflammation is an area that will surely be explored in the future.

3) The CREB-C/EBP pathways—Several members of the **CCAAT-enhancer-binding proteins** (C/EBP) family play important roles in macrophage activation. C/EBPβ mediates toll-like receptor-induced expression of arginase1 (ARG1), a signature gene of the M2 phenotype. C/EBPβ is also reported to promote expression of several M2 specific genes (such as Arg1 and mannose receptor c-type 1: Mrc1)), which itself is induced by the transcription factor cAMP-responsive element-binding protein (CREB). Deletion of CREB binding sites in the promoter region of C/EBPβ impaired muscle tissue repair in mice and inhibited expression of M2 related genes: Macrophage Scavenger Receptor 1 (MSR1), IL-10, IL-13 Receptor subunit receptor α1 (IL-13RA1), and ARG1 within macrophages, with no effect on the transcription of inflammatory, M1-associated genes (104). Upon LPS stimulation, CREB inhibited expression of M1-associated genes through p38 mediated induction of IL-10 and dual specificity protein phosphatase 1 (DUSP1) (105) (106). Another C/EBP family member, C/EBPσ was shown to induce M1-like pro-inflammatory responses in mouse bone marrow-derived macrophages, and its activity was inhibited by microRNA Let-7c (107). Another member of this family, C/EBPα, was required for both M1 and M2 activation of mouse macrophages (108). Altogether, CREB-C/EBP signaling plays a central role in the regulation of both M1- and M2-like macrophage polarization.

4) The interferon regulatory factors—Interferon regulatory factors (IRFs) have long been classified as regulators of type I interferons (IFN), and increasing evidence suggests that they also play important roles in regulating macrophage functions (109).

Knockout of IRF1 or IRF2 abolished pro-inflammatory responses in murine macrophages in response to LPS or IFNγ stimulation (110). Another member of the IRF family, IRF5 was suggested to promote M1 polarization while inhibiting M2-associated markers in human peripheral blood macrophages (111), and IRF6 was recently implicated in the negative regulation of M2 polarization of murine bone-marrow-derived macrophage (BMDM) through PPARγ inhibition (112). In contrast, some other IRFs mediated the anti-inflammatory type I Interferon responses. For example, IRF3 mediated anti-inflammatory signaling and contributed to M2 activation of human microglia (113) and IRF4 mediated IL4 induced M2 activation of murine BMDM (114). Further, studies on murine primary

macrophages demonstrated a role for Lysine Demethylase 6B (KDM6B, or Jmjd3)-mediated histone demethylation of the Irf4 gene during the induction of M2 macrophage activation in response to parasites or fungi (115). In addition, IRF9 was suggested to be involved in the anti-inflammatory and M2-promoting effects of interferon tau in murine BMDM (116).

b. microRNA and lncRNA regulated macrophage polarization

In recent years there has been increasing evidence suggesting that microRNAs (miRNAs) and long noncoding RNA (lncRNA) play critical roles in regulating macrophage polarization, often through binding interactions with several key transcription factors.

1) miR223—As mentioned above, PPARγ is a crucial regulator of macrophage activation. Upon stimulation with Th2 cytokines (IL-4 or IL13), PPARγ activation in murine macrophages induced miR223 expression by binding upstream of miR223 which in turn inhibited expression of Nuclear Factor of Activated T-Cells 5 (NFATt5) and RAS p21 Protein Activator 1 (RASA1), promoting the development of an anti-inflammatory M2-like phenotype. This is consistent with the observation in mice with ablation of miR223 that desensitized the PPARγ-regulated anti-inflammatory responses in macrophages (117). In addition to promoting M2-like phenotypes, miR223 has also been shown to suppress M1-like pro-inflammatory responses through suppressing NFκB/JNK via inhibiting PBX/Knotted 1 Homeobox 1 (Pknox1) expression (118). This M1-suppressive activity of miR223 has been illustrated within murine intestinal macrophages, where miR223 suppresses C/EBPβ expression, thereby blunting the differentiation of THP-1 cells and peripheral human blood monocytes into inflammatory macrophages (119) (120).

2) miR155—In contrast to miR223, miR155 has been shown to facilitate the development of the pro-inflammatory M1-like phenotype. First identified as gene commonly induced in B-cell lymphomas, miR155 expression has since been shown to increase upon TLR activation (LPS, hypomethylated DNA or Pam3CSK4) or pro-inflammatory cytokine (TNF-α, IFNβ or IFNγ) stimulation within cultured murine macrophages (121). Expression was dependent on JNK signaling and increased levels of miR155 positively correlated with pro-inflammatory responses (O'Connell et al, 2007). Similarly, in a study by Tili el. (122) miR155 promoted TNF-α production and targeted several members of TLR4 signaling. During alcoholic liver disease, NF-κB activation induced miR155 expression in liver macrophages, which enhanced TNF-α synthesis by stabilizing its mRNA (123). In addition to promoting pro-inflammatory factors, miR155 facilitated inflammatory responses through direct suppression of anti-inflammatory regulators such as suppressor of cytokine signaling 1 (SOCS1) and phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1 (SHIP1) (124) (125). In addition, miR155 can promote M1 polarization through inhibition of IL13RA1, thus blocking STAT6 activation (126). Interestingly, despite numerous studies supporting its pro-inflammatory role, there is also evidence suggesting that in mouse bone marrow-derived macrophages miR155 down-regulates pro-inflammatory cytokine production in response to LPS stimulation, possibly through targeting TGF-β Activated Kinase 1/MAP3K7 Binding Protein 2 (TAB2) (127).

3) miR125b and Let7c—Another mechanism that microRNAs utilize to regulate macrophage polarization is by repressing the expression of critical transcription factors. miR125b is one such miRNA that promotes M1-like pro-inflammatory responses of macrophages. For example, when murine macrophages were induced by IFNγ, increased expression of miR125b suppressed its target gene IRF4, which is an important transcription factor that promotes M2 activation. The resulting response was an M1-like phenotype (128); however, in mouse Raw 264.7 macrophages, miR125b negatively regulated TNF-α production and was down-regulated upon LPS stimulation (122). In contrast, microRNA Let-7c has been shown to play a crucial role in maintaining M2 activation through suppression of the pro-inflammatory transcription factor C/EBPσ. In M1 polarized macrophages, ectopic expression of Let-7c reduced IL-12 levels and major histocompatibility complex class II surface expression, which collectively suggests suppressed inflammatory activity. Knockdown of Let-7c, on the other hand, caused reduced M2-associated activity of macrophages, despite stimulation with Th2 cytokines (107).

4) Other miRNAs—In addition to the above mentioned species, several other miRNAs are also reported to be involved in macrophage polarization. Among them, miR9 and miR127 enhanced M1 polarization by suppressing expression of peroxisome proliferator-activated receptor δ (PPAR δ) and B-cell lymphoma 6 protein (Bcl6), respectively (129) (130). On the other hand, miR124 has been shown to promote M2 polarization through STAT3 and TNF-α converting enzyme targeting (131). Other miRNAs that have been implicated in inducing M2-like phenotypes are miR132 and miR146a which inhibit NF-κB signaling pathways (132) (133). Alternatively, miR21, has an unclear role in macrophage polarization, with evidence suggesting a role in promoting both M1 (134) and M2 (135) activation.

5) lncRNAs—Long noncoding RNAs (lncRNA) are non-coding transcripts over 200 nucleotides. Recently, lncRNAs have been proposed to have a regulatory role across a broad spectrum of biological processes, including macrophage polarization. A recent study found that M1 and M2 polarized macrophages presented distinct lncRNA profiles (136). One example is lncRNA E330013P06, which was induced in macrophages isolated from insulin-resistant type 2 diabetic (T2D) mice and monocytes of T2D humans. Overexpression of lncRNA E330013P06 in macrophages enhanced expression of pro-inflammatory genes and inflammatory responses (137). Similarly, lncRNA-Cox2 was induced by inflammatory stimuli in murine BMDM and its overexpression enhanced or suppressed expression of a multitude of immune response genes, including several key regulators of macrophage polarization (138). While this is a burgeoning area of research, lncRNAs have been found to target NFκB and TNF-α signaling pathways, both of which are critical regulators of inflammatory responses (139) (140) (141).

Macrophage Polarization and Metainflammation

a. Pathogenic alteration of macrophage polarization during metainflammation

Alterations in macrophage polarization could contribute to obesity-induced insulin resistance. The residing macrophages in adipose tissues of lean individuals generally have anti-inflammatory, M2-like phenotypes (112) (142). Under the stress of obesity, a population

of pro-inflammatory M1-like macrophages are recruited into adipose tissues. Specifically, Patsouris *et al.* demonstrated a clear contribution of pro-inflammatory macrophages to obesity-associated inflammation and insulin resistance, which could then be resolved through depletion of these macrophages (143). Additionally, Shi et al. suggested that during obesity, M1-like ATM undermined insulin responses of adipocytes and enhanced inflammatory responses through production of pro-inflammatory cytokines (33). In addition, obesity stress not only promoted infiltration of pro-inflammatory macrophages into adipose tissue, but also caused the M2-like residing macrophages to undergo a phenotypic switch to M1 (80).

b. Mechanisms of altered macrophage polarization during obesity

Several mechanisms of altered macrophage polarization during obesity have been recently suggested. These hypotheses are centered on several signaling pathways and regulation by other adipose tissue-resident cells.

T cells have long been recognized as critical regulators of macrophage development and function. During diet-induced obesity in mice, recruitment of $CD8^+$ T cells into adipose tissue precedes the increase of ATM, and blocking T cell recruitment reduces the number of M1-like macrophages in the tissue with no effect on M2-like macrophage recruitment (144). Conversely, T regulator cells (Tregs) may contribute to M2 polarization and improve insulin response (145). In addition to T cells, adipocytes, the largest cell population within adipose tissue, have also been demonstrated as an orchestrator of ATM polarization. During the progression of obesity, the adipocyte secretome shifts to a pro-inflammatory profile that induces M1-like polarization (146). Furthermore, *in vitro* co-culture of BMDM with adipocytes enhanced surface expression of CD11c, a pro-inflammatory macrophage marker (147).

In recent years, several studies have implicated key signaling pathways as major players in the process of altered macrophage polarization during obesity. Arkan *et al.* described a role for IKKβ regulation of M1 polarization, since depletion of IKKβ in myeloid cells reduced tissue inflammation and improved insulin sensitivity in mice fed a high fat diet (148). Similarly, depletion of Mitogen-Activated Protein Kinase 8 (MAPK8) in hematopoietic cell lineages decreased adipose tissue inflammation and reduced insulin resistance (149). TLR4 signaling is another potent regulator of macrophage polarization. TLR4 is activated during obesity by several types of molecules, including saturated fatty acids and oxidized low-density lipoprotein (Ox-LDL) (150) (142) (151). Obesity induced an increase in TLR4 expression in ATM (33), and activation of TLR4 signaling in macrophages increased pro-inflammatory cytokines production, which blocked insulin signaling in adipocytes in co-culture studies (152).

Interleukin 6 (IL-6) has both pro-inflammatory and anti-inflammatory roles in regulating immune responses. In models of acute inflammation and sepsis, IL-6 promotes inflammation and mediates monocyte recruitment (153) (154). In contrast, the anti-inflammatory function of IL-6 was shown in a study by Mauer *et al.*, in which disruption of IL-6 signaling in myeloid cells caused exaggerated systemic inflammation in high-fat diet fed obese mice (155). IL-6 was described to augment IL-4 signaling in mouse BMDMs through STAT3-

mediated IL-4Rα expression, hence driving M2 polarization of mouse BMDMs and ATM. Braune *et al.* recently corroborated this duality of IL-6 mediated macrophage responses, showing that IL-6 promoted local proliferation of M2-like macrophages in adipose tissue during obesity, likely through sensitization to IL-4 through IL-4Rα upregulation (156).

In addition, epigenetic mechanisms are also involved in regulating macrophage polarization during obesity. DNA methyltransferase 1 (DNMT1)-mediated DNA methylation suppressed M2-like activation of ATM in obese mice, while inhibition of DNA methylation promoted ATM M2-like phenotype and down-regulated inflammatory markers in ATM. Interestingly, PPARγ1 expression, the previously described regulator of macrophage polarization, was controlled by DNA methylation in those cells and DNA-demethylation induced PPARγ1 expression and M2 macrophage polarization (157). Besides the above mentioned mechanisms, dietary factors associated with obesity are found to contribute to the altered macrophage functions and phenotypes. Links between systemic hyperlipidemia and altered macrophage phenotype have long ago been made, primarily in the context of impaired macrophage directed wound healing in diabetes (158, 159). While lipid accumulation within macrophages plays a critical role in the transition of macrophage to foam cell in the context of atherosclerosis, arthritis, and neurodegeneration, it is still unclear what role it might have in the context of obesity induced meta-inflammation(160). In addition, saturated fatty acids induced proinflammatory responses in mouse peritoneal macrophages (161) and macrophage cell line RAW 264.7 (162). In contrast, polyunsaturated fatty acids triggered anti-inflammatory effects in RAW 264.7 and in mouse intraperitoneal macrophages (163).

c. Secretome of polarized macrophages

Release of secretory products is a major way that macrophages interact with and regulate other cells to coordinate inflammatory or homeostatic responses. As one might predict, the secretome of M1 and M2 polarized macrophages are distinct (164). M1 macrophages generally produce pro-inflammatory cytokines such as TNF-α, IL-1β, IL-12, and IL-23 (165) (166), while M2 macrophages secrete anti-inflammatory cytokines and growth factors, such as IL-10 and TGFβ (167). For example, LPS, a classic inflammatory stimulus of M1 macrophages, induces M2 polarized human PBMC derived macrophages to produce significantly higher IL-10 and lower TNFα, IL-6 and IL-12p40 than their M1 counterparts (168). A recent study implicated inflammasome assembly as a mediator of these distinct secretory patterns (164). Among the macrophage-derived cytokines, IL-6 is a particularly interesting one as it is secreted by both M1 macrophages and subtypes of M2 macrophages (169). As mentioned above, IL-6 have both proinflammatory and anti-inflammatory functions, depending on the specific scenario. Obesity or exercise induced IL-6 elevation enhanced insulin secretion by inducing Glucagon-like peptide-1 (GLP-1) secretion from pancreas and intestine (170). In addition, IL-6 was needed for macrophage recruitment and myoblast proliferation, which promoted tissue repair of skeletal muscle (171).

In addition to cytokines, M1 macrophages are also characterized by production of nitric oxide (NO) and reactive oxygen species (ROS), which are mediated by SOCS3 (172) and Cytochrome B-245 β chain (CYBB alternatively named NADPH Oxidase 2 or NOX2)

(173), respectively. NO and ROS have crucial regulatory roles in obesity-associated chronic inflammation (174) (175), (176) (177).

Recently, several studies have suggested that miRNAs are important components of the immune system's secretome (178) (179). Studies on human cell lines revealed a critical role for Argonaute (AGO) proteins in mediating microRNA exportation out of cell membranes (180) (181). AGO proteins bind to miRNAs and protect them from nuclease degradation. AGO-associated miRNAs are released from cells either within vesicles or in vesicle-free forms and enter acceptor cells through membrane fusion, gap junction channels, or other unknown mechanisms (182).

Extracellular RNAs have now been accepted as a new communication venue between cells and tissues. However, our understanding of how they are produced and are packaged for secretion is still in its infancy, which much less known about their secretion by macrophages. Cultured human macrophages were able to transfer miR142 and miR223 to co-cultured hepato-carcinoma cells through gap junctions (183), and LPS stimulation could alter the level of miRNAs detected in the supernatant of human monocyte cell line THP-1-derived macrophages (178). (178). Recent data from our lab shows that the extracellular miRNA (exRNA) profile of BMDMs is different from their intracellular miRNAs (inRNA) profile, and varied with polarization status (Fig 1.). While the function of these secreted miRNAs are not yet fully understood, evidence from recent years' studies suggest that extracellular RNAs could play critical roles in cell-cell communication. For example, miRNAs released by macrophages and other cell types have been shown to regulate cancer metastasis (184) (185), tissue remodeling (186) and immune response (187) (188). The role of extracellular miRNAs in mediating polarized macrophage function, especially in the scenario of obesity-induced metainflammation, however, is still unknown. Thus, this understudied area represents a new area of opportunity for developing novel strategies for therapeutic intervention.

d. Meta-Inflammation moderates further disease pathogenesis

The skewing of macrophage polarization affects human physiology and unsurprisingly, pathology as well. Abnormally altered macrophage polarization greatly contributes to local tissue pathology. Atherosclerosis is well known as a chronic inflammatory disease (189), and is characterized by conglomeration of atheromatous immune cells and debris within the artery wall. Both M1-like and M2-like macrophages have been found in human atherosclerotic plaques (190) (94). Interestingly, shoulder regions of plaques, which are most prone to rupture and initiating sequelae of thrombosis and vessel occlusion, contained more M1-like pro-inflammatory macrophages (191), while regions that contained M2-like macrophages that were more stable and resistant to foam cell formation (192), which is critical for atherosclerosis progression.

Polarization of colonic macrophages is also involved in pathogenesis of inflammatory bowel disease (IBD). M1-like macrophages accumulated in the colon during IBD and became the predominant phenotype (193), which were likely to promote disease (194). On the other hand, IL-10-producing, M2-like macrophages also presented at sites of inflammation and were suggested to reduce inflammatory reactions and contribute to IBD resistance (195).

In addition to local effects, the skewing of macrophage activation induced by meta-inflammation has profound systemic impacts. For example, insulin resistance is closely associated with chronic inflammation (196), where pro-inflammatory cytokines released by macrophages are major contributing factors to the development of insulin resistance(26) (197). In adipose tissues, excess nutrients trigger inositol-requiring enzyme 1α (IRE1α) signaling, which impairs adaptive thermogenesis, disturbing the energetic homeostasis in favor of a M1 ATM phenotype (198). However, beyond the well-documented history of meta-inflammation and diabetes, there is emerging evidence implicating chronic macrophage activation in graft-vs-host diseases (GVHD) and the inflammatory conditions of osteoarthritis (199), systemic lupus erythematous, and gastrointestinal cancers (200). GVHD occurs when effector immune cells from a donor attack host tissue, and it has been suggested that host macrophages normally play a protective role through the engulfment of autoreactive immune cells (201). As acute GVHD has been epidemiologically linked to obese populations with insulin insensitivity, it is possible that a skewing of macrophage polarization plays a role in this epidemiologic finding (201) (202). Osteoarthritis has long been associated with metabolic syndrome, and it has since been suggested that a proinflammatory secretome of the predominant M1-skewed macrophage population inhibits the synthesis of protective extracellular matrix, accelerating cartilage degradation and bone resorption (203). Obesity has also been epidemiologically linked to increased incidence of gastrointestinal cancer, perhaps because M1-like macrophages possess a secretome that might promote carcinogenesis (204). In mouse models of lymphocyte-derived DNA induced systemic lupus erythematosus, transplantation of M2, but not M1 macrophages, relieved macrophage-depleted mice from lupus like pathology (205). While the precise mechanisms underlying these associations have yet to be uncovered, they suggest that they are significant contributors to the pathologic burden on human health.

Summary

In summary, the significance of macrophage infiltration and activation is beginning to be recognized as a pivotal instigator of meta-inflammation. In response to over-nutrition, peripheral tissues alter their metabolic phenotypes, release distinct secretome profiles, and shift the body’s homeostasis into one that promotes macrophage invasion, and supports various downstream inflammatory cascades.

Several important regulators are involved in regulation of macrophage polarization, including STAT, PPARα, CREB-C/EBP and IRFS. Several microRNAs modulate macrophage activation by suppressing expression of critical regulators that promote either M1 or M2 polarization. In addition, regulatory roles of lncRNAs have also been identified. Certainly the polarization of macrophages is affected under obesity stress, and T cell and adipocyte activity, signaling of regulatory proteins and changes in DNA methylation status contribute to those alternations. Polarized macrophages orchestrate tissue functions through secreting cytokines and/or reactive chemical species, which present distinct patterns between M1- and M2-like macrophages. Abnormally altered macrophage polarization not only contributes to local tissue pathology, but also has an extensive impact in promoting insulin resistance and its consequent symptoms. Despite the great achievements made in the past decades, a lot of questions on mechanisms of macrophage polarization and meta-

inflammation are yet to be answered. The heterogeneous and plastic nature of macrophages make the interaction between macrophages and their microenvironment a complex and dynamic process. Our current knowledge of such interactions *in vivo* is limited. The signaling pathways underlying the impacts of polarized macrophages on physiological functions and pathological alterations are yet to be deciphered. New technologies including single cell analysis and computational biology approaches are being incorporated in this field and will hopefully help address those challenges. And transitions from scientific research to clinical application, such as discoveries of new molecular targets for therapies, will be one of the future directions. The metainflammation-induced alternations of macrophage polarization, and their impacts on tissue/organ functions are summarized in Figure 2.

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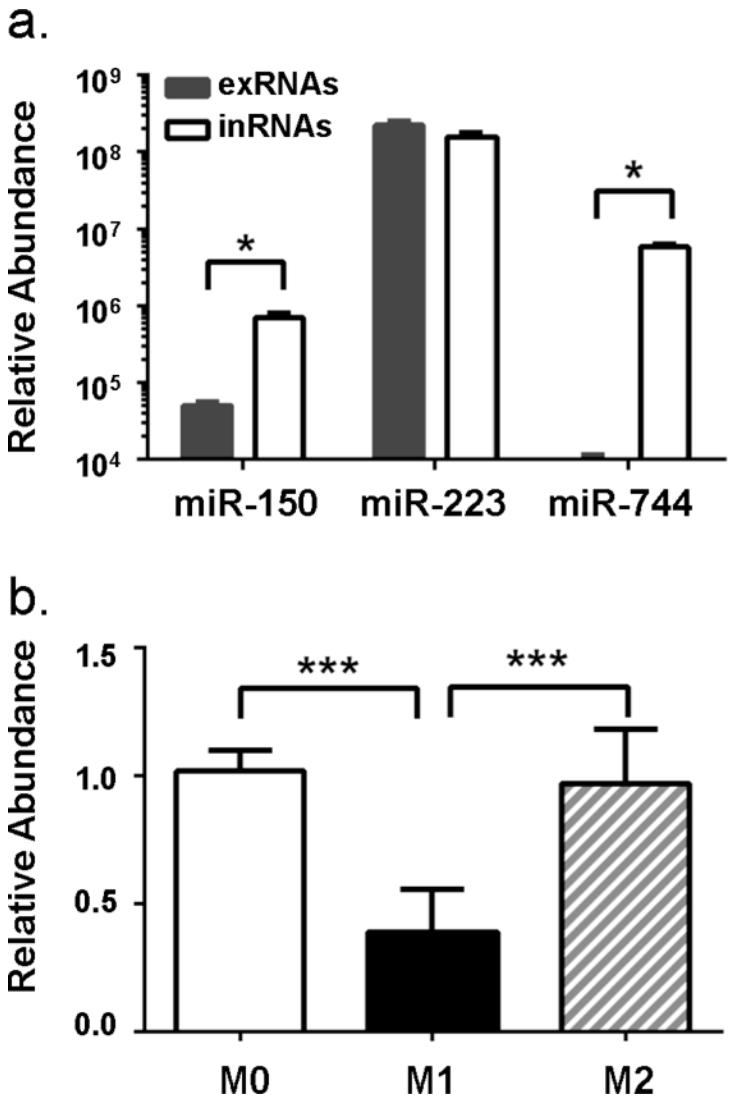

Figure 1. Intracellular and Extracellular miRNA profile of BMDMs

(a) Total extracellular RNAs (exRNAs) were extracted from conditioned medium of BMDMs 48 hours post-stimulation and intracellular RNAs (inRNAs) were extracted from the same batch of BMDMs. qRT-PCR analysis confirmed that miR-150 and miR-744 were highly expressed in the BMDMs, but secreted at low levels. Interestingly, miR-223, a potent regulator of macrophage polarization, was abundant in both the intra- and extra-cellular compartment. (b) BMDMs were either not activated (M0) or stimulated with 100 ng/mL LPS (M1) or 20 ng/mL IL-4 (M2). Total extracellular RNAs were extracted from cell culture supernatants 24 hours post-stimulation and miR-223 expression was quantified by qRT-PCR. exRNAs were normalized using Cel-miR-39 as spike-in controls. Data are presented as mean ± SEM; n=4, *, $p<0.05$ (student's t test).

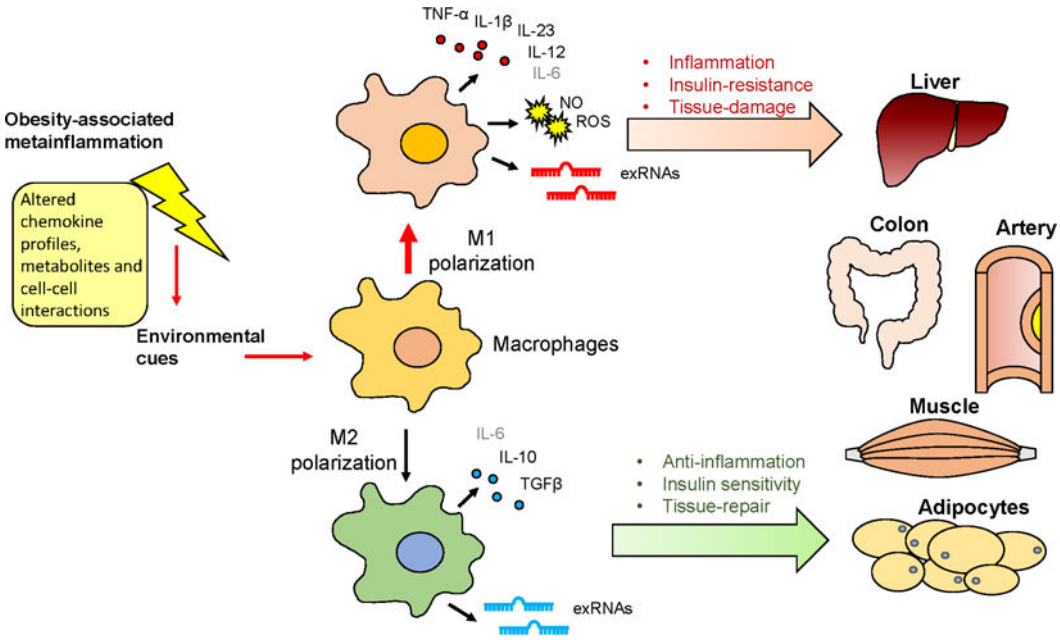

Figure 2. Macrophage polarization modulated tissue/organ functions during obesity-induced metainflammation

Obesity-associated metainflammation orchestrates macrophage polarization patterns through altering environmental cues, which generally favors an M1 activation state. M1- and M2-like macrophages differently modulate tissue/organ functions by secreting specific cytokines and/or reactive chemical species. Metainflammation-altered macrophage polarization profiles contribute to local tissue pathology at sites such as liver, colon and arterial walls, and further impose systemic effects by regulating insulin sensitivity in the liver, muscle and adipose tissues.