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Macrophage-targeted therapeutics for metabolic disease

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Abstract

Macrophages are cells of the innate immune system that are resident in all tissues, including metabolic organs such as the liver and adipose tissue. Because of their phenotypic flexibility, they play beneficial roles in tissue homeostasis, but they also contribute to the progression of metabolic disease. Thus, they are ideal therapeutic targets for diseases such as insulin resistance, non-alcoholic fatty liver disease, and atherosclerosis. Recently, discoveries in the area of drug delivery have facilitated phenotype-specific targeting of macrophages. In this review, we discuss advances in potential therapeutics for metabolic diseases via macrophage-specific delivery. We highlight micro- and nano-particles, liposomes, and oligopeptide complexes, and how they can be used to alter macrophage phenotype for a more metabolically favorable tissue environment.

Keywords

macrophage; nanoparticle; liposome; therapeutics; metabolic disease; obesity

Rationale for macrophages as therapeutic targets in metabolic disease

Cells of the immune system have important functions in health and disease. Their roles in autoimmune and inflammatory diseases are obvious; however, more recently, their contributions to cancer [1], cardiovascular disease [2, 3], and diabetes [4–6], have also been elucidated. Although all cells of the immune system can theoretically impact tissue homeostasis and contribute to disease, macrophages are particularly important to consider as therapeutic targets because they are resident in all tissues and show tremendous plasticity. Furthermore, they are phagocytic [7] and thus capable of endocytosing particles that are nanometers to micrometers in size. This review will focus on recent literature that shows the therapeutic potential of targeting macrophages in metabolic diseases as well as novel delivery systems being developed to capitalize on their phenotypic variability.

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Contribution of macrophages to metabolic disease

Macrophages are historically understood for their role as recruited and differentiated monocytes that respond to acute infection as phagocytes and secretors of inflammatory cytokines [7]. Resident macrophages are present in every tissue and are involved in development, as well as maintenance of tissue homeostasis, and have been given special names based upon their tissue-of-residence, *e.g.* adipose tissue macrophages [**ATMs;** See Glossary)] and **Kupffer** cells of the liver [Reviewed in [4, 8]; Box 1]. Macrophages can act as accessory cells and positively contribute to tissue homeostasis [9]. Briefly, macrophages secrete chemokines, cytokines, growth factors, and extracellular vesicle-encapsulated material that can influence the function of neighboring parenchymal cells. Ongoing studies are focused on macrophage-parenchymal cells in tissue homeostasis; however, to date, this has most often been studied in the context of disease, *e.g.* inflammatory cytokines can cause insulin resistance (**IR**),rather than how these interactions promote tissue health. Thus, many times, the pathogenicity of macrophages derives from their ability to modulate the function of neighboring cells – a characteristic that can be exploited when considering their potential as therapeutic targets.

BOX 1

Tissue Associated Macrophages

Resident macrophages are known to play critical roles in tissue homeostasis and in disease. These macrophages are given special names based upon their tissue-intrinsic function. Most tissue resident macrophages are yolk sac-derived and self-renew; however, circulating monocytes can also infiltrate tissues. Macrophages from each tissue also have distinct cell surface markers that could ultimately be used for tissue specific macrophage targeting in the future [8, 90].

Arterial Macrophages

Lipid laden foamy macrophages in atherosclerotic plaques are thought to contribute to lesion formation due to their accumulation of cholesterol and secretion of inflammatory cytokines. In addition, they may contribute to plaque instability, intraplaque hemorrhage, and rupture. In this setting, targeting them to reduce their inflammatory nature and increase their pro-fibrotic tendencies are strategies to improve CVD [41].

Kupffer Cells

Kupffer cells play roles in liver iron handling, bilirubin processing, scavenging of gut-derived pathogens, cholesterol metabolism, and immune surveillance [31]. When over-activated, they can also be pathogenic by accumulating lipid, secreting inflammatory cytokines, and activating stellate cells to produce α-smooth muscle actin, leading to fibrosis and ultimately non-alcoholic fatty liver disease (NAFLD). Elimination of macrophages is known to reduce progression of NAFLD. It should be noted that monocytes can be recruited from the circulation to the liver and subsequently differentiate into macrophages; however, these have distinct cell surface markers compared to Kupffer cells.

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Red pulp macrophages (RPMs)

RPMs are responsible for recycling iron retrieved from senescent red blood cells following erythrophagocytosis [91]. In fact, 10 times more iron is recycled in RPMs than is absorbed by enterocytes. Because elevated tissue iron stores are correlated with systemic IR, RPMs could be a potential target for metabolic disease in certain settings.

Tumor Associate Macrophages (TAMs)

Myeloid cells are recruited to the tumor microenvironment where they can be differentiated into TAMs. They retain an M2-like phenotype and are thereby immunosuppressive–preventing immune detection and destruction of malignant cells [92]. Thus, their presence is associated with increased tumor burden and worsened prognosis. Use of mannosylated, siRNA delivery nanoparticles has been recently utilized to activate NF-κB in macrophages and is a promising anti-cancer therapy [93].

Microglia

Macrophages in the brain are called microglia and are essential to central nervous system homeostasis [94]. Developmental programming, synapse remodeling, phagocytosis of dead cells are among their many functions. Microglial activation is a feature of neurodegeneration and also diabetic retinopathy, and thus, reducing their inflammatory potential is a potential target for these diseases.

Macrophages are polarized across a broad spectrum, from an M1-like state that is considered to be pro-inflammatory, to an M2-like state in which they are anti-inflammatory and pro-fibrogenic. This diversity of phenotype is relevant to acute infections where macrophages initially fight off pathogens and phagocytose dead cells, but then convert their phenotype and subsequently assist in the wound healing process. The various properties of macrophages have been reviewed [10], but it is important to note that traditional M1 and M2 classification has been challenged, and more recent studies have suggested a spectrum of inflammatory phenotypes for macrophages [11]. Nonetheless, these same M1-like and M2-like properties can be detrimental when exaggerated or unregulated in chronic inflammation. For example, excess inflammatory cytokine secretion can lead to IR in neighboring cells – a finding particularly relevant in adipose tissue (AT). Conversely, excess fibrogenic stimulation can lead to fibrosis in diseases such as nonalcoholic fatty liver disease (**NAFLD**). Thus, when considering macrophages as therapeutic targets, one needs to be discerning about the phenotype of the macrophages targeted and pathogenesis of the disease treated. Although it is now appreciated that macrophages can contribute to the development of metabolic diseases, their contributions are tissue-specific and context-dependent. Understanding the complexity and diversity of macrophages is also critical.

Macrophages in obesity-related adipose tissue inflammation and insulin resistance

AT was one of the first organs in which macrophage contribution to metabolic disease was discovered, and is arguably the best studied. Less than 15 years ago it was found that the number of macrophages increases in obese compared to lean AT and that this imparts an overall inflammatory milieu [12, 13]. The increase in macrophages is mostly due to recruitment, although proliferation is also thought to occur [14, 15]; however, regardless of

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how they arise, the new macrophages are more M1-like and pro-inflammatory. Because inflammatory cytokines can interfere with insulin signaling, this results in insulin resistant AT, unchecked basal lipolysis, and ectopic lipid storage in other metabolic tissues such as muscle and liver.

Due to the contribution of these M1-like inflammatory macrophages to obesity-related IR, the selection of macrophage-specific therapeutics should focus on their involvement. Potential areas of modification are to reduce M1-like polarization and inflammatory cytokine production via targeting of specific cytokines such as tumor necrosis factor alpha (**TNF-α**), Interleukin 6 (**IL-6**), and Interleukin 1 beta (**IL-1β**), or chemokines such as Chemokine (**C-C motif**) ligands 2, 3, and 5 (**CCL2**, **CCL3**, and **CCL5**, respectively). Other inflammatory signal transduction intermediates such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and signaling molecules in the inflammasome pathway may prove reasonable targets as well. Conversely, increasing the M2-like anti-inflammatory ATM phenotype via adiponectin, peroxisome proliferator-activated receptor gamma (PPARγ), or type 2 cytokines such as Interleukins 4, 13, and 33 (**IL-4**, **IL-13**, and **IL-33**, respectively) is an alternative [reviewed in [16]]. There are at least 3 caveats with this strategy that should be noted: 1) M1 macrophage content is increased in obese human AT, but not to the same degree as mouse adipose [17–20]. 2) Systemic reduction of inflammation has not been effective at improving metabolic parameters [16], but it is possible that specifically modifying ATMs may be a more viable approach due to the paracrine rather than endocrine nature of M1 macrophage interactions with nearby parenchymal cells. 3) In early stages of AT expansion, mild inflammation is beneficial [21], so the timing of this approach needs to be carefully considered. Despite these caveats, reducing ATM inflammatory potential remains a promising avenue for treatment of obesity-accelerated IR and diabetes.

Other macrophage subsets in adipose tissue

Recently, a new population of metabolically active macrophages (**MMe**) in the AT has been described [22, 23]. Although MMe macrophages secrete pro-inflammatory cytokines, they have distinct cell surface markers that distinguish them from both M1 and M2 macrophages. Specifically, MMes do not express CD38, CD319, or CD273, which are traditional M1 markers, but instead express ABCA1, CD36, and PLIN2, which are associated with M2 macrophages.

In addition to MMes, macrophages with iron-related phenotypes have also been identified in AT. Iron-handling macrophages were first identified in atherosclerotic plaques [24–27] and have been shown to be M2-like and to express high levels of mannose receptor and the haptoglobin/hemoglobin receptor, **CD163**. They have been given names such as **M(Hb)** and **Mhem** and are reviewed in [28]. In plaques, these iron-handling macrophages are considered to play a role in clearing intra-plaque hemorrhage. Our laboratory has found a similar population of iron handling macrophages in AT that we call **MFe^{hi}** [29]. These MFe^{hi} macrophages have a 2-fold increase in iron content and in iron handling genes compared with non – iron-handling AT macrophages. Interesting, they have a strong M2-like phenotype with high expression of mannose receptor and CD163–just like the M(Hb) and

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Mhem macrophages, even in the presence of excess iron. Their relevance to AT homeostasis and protection from IR is under investigation, but they highlight the point that both M1-like and M2-like macrophages could be targets of therapy for obesity-related metabolic disorders.

Hepatic macrophages in nonalcoholic fatty liver disease

Similar to the AT transition, hepatic macrophages undergo changes in polarization, recruitment, and proliferation during the pathogenesis of NAFLD [30]. Hepatic macrophages are dynamic players in maintaining liver homeostasis, via protection against bacteria and microbes from the intestine and restoring tissue integrity following liver injury [reviewed in [31]]. The liver contains a number of distinct macrophage subsets important for liver disease, with embryonic-derived Kupffer cells and monocyte-derived macrophages being the primary subsets driving the progression of NAFLD. Kupffer cells are the resident macrophages of the liver and make up ~20–25% of the non-parenchymal cell content [32]. NAFLD develops due to long-term high carbohydrate or fat consumption, resulting in hepatocyte lipid accumulation and cell death. The inflamed hepatocytes secrete chemokines, such as CCL2 and TNF-α, leading to the activation and proliferation of Kupffer cells and conversion to what could be considered an M1-like phenotype, with expression and secretion of cytokines such as IL-6, IL-1β, TNF-α, and inducible nitric oxide synthase 2 (**iNOS2**) [33]. In addition inflamed hepatocytes and Kupffer cells signal for the infiltration of CCR2+Ly6C+ monocytes that differentiate into monocyte-derived macrophages, also expressing an M1 phenotype [34, 35].

Recently, macrophage inflammation has been targeted for treatment of NAFLD. Impairment of recruitment and/or depletion of hepatic macrophage subsets have been shown to successfully suppress hepatic steatosis and inflammation in mouse models of NAFLD [36, 37]. It has been shown that pediatric NAFLD prognosis could be improved through treatment with docosahexaenoic acid due to its effects on macrophage polarization, pushing them toward an M2-like phenotype [38]. Dietary carotenoids have also been proposed for NAFLD treatment due to their effects on macrophage polarization [reviewed in [39]]. More recently, altering the polarization state of hepatic macrophages to an M2 phenotype by increasing PPARγ activity was found to reduce NAFLD in mice [33].

Macrophages in atherosclerosis

Like AT and the liver, the healthy artery wall contains resident macrophages [40], and in hyperlipidemic conditions, the recruitment of additional macrophages leads to atherosclerotic lesion formation [41]. High fat feeding and hyperlipidemia can both induce myelopoiesis in the bone marrow and recruitment of monocytes into the intimal space [42, 43]. Once there, they differentiate into macrophages and express scavenger receptors allowing them to endocytose oxidized lipids. This lipid accumulation results in foamy macrophages that are highly inflammatory and that also send chemotactic signals to recruit additional monocytes, exacerbating the lipid-enriched inflammatory milieu [44]. Recently, a subset of arterial macrophages [Mhem, M(Hb)] have been shown to express receptors involved in heme scavenging and can clear debris during intraplaque hemorrhage as discussed earlier [45]. In addition, a novel macrophage population that uniquely responds to

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oxidatively modified lipids in the atherosclerotic plaque, called “Mox”, has been identified [46]. Understanding the biology of these unique subsets of arterial macrophages and developing strategies similar to those suggested for AT, *i.e.* reduction of their inflammatory potential, may provide therapeutic opportunities for atherosclerosis. Additionally, decreasing their ability to take up lipid or increasing their ability to efflux lipid are other possibilities. In fact, mannose functionalized nanoparticles have recently been utilized to deliver therapeutic molecules to plaque macrophages in a mouse model of atherosclerosis [47]. Although, this is a very new area of research, the high level of mannose receptor expression on M(Hb) and Mhem cells, could provide a method to target them with mannosylated particles in order to dampen inflammation and improve their iron handling phenotype.

Macrophages as a therapeutic target in metabolic disease

Targeting strategies: Intracellular access

Currently, most macrophage-targeted therapeutic strategies provide specificity by capitalizing on receptor-mediated phagocytosis. Compounds designed to encapsulate therapeutics can have surface modifications that are recognized by receptors on macrophages. There are defining receptors such as F4/80, CD11b, and CD68 that are expressed on all macrophages [48–50], but subpopulations can additionally express others– such as those for mannose, lectin, adenosine [51], and folate [52]. Although not exclusively macrophage-specific, they allow targeting of cells of particular phenotypes and activation states. This receptor-focused approach offers a direct route of import into macrophages while minimizing off-target effects.

There are at least three methods of internalization that can be exploited for delivery purposes [reviewed in [53]]. 1) *Activation of therapeutic agents in the acidic lysosomal compartment*: Once recognized and engulfed, entrapped particles are packaged into a phagosome prior to fusion with destructive lysosomes. Utilizing the microenvironment of the phagosome or lysosome to initiate therapeutic activity is promising, but has been primarily limited to nanoparticle encapsulation approaches. In these strategies, the acidic lysosomal interior initiates breakdown of the nanoparticle capsule and release of its contents [54] Alternatively, lysosomal enzymes degrade the nanoparticle shell to release core contents [55]. 2) *Cytoplasmic delivery via endocytosis and lysosomal escape:* Another strategy to avoid phagocytic destruction is to design particles that escape phagosomes after endocytosis but prior to fusion with the lysosome [56]. 3) *Utilization of endocytic receptors:* A third strategy takes advantage of active transport into the macrophage mediated by coat proteins, such as clathrin and caveolin [57]. Once a particle has entered the intracellular space, it can deliver its phenotype/function altering cargo. Methods to accomplish this in macrophages are discussed below.

Therapeutic strategies: Changing the role of the macrophage

Once inside the macrophage, therapeutic strategies are diverse - depletion, proliferation, inflammation, and gene silencing are commonly used. Approaches that modulate macrophage number often aim to deplete macrophages by inducing apoptosis following accumulation of toxic particles that are released into the macrophage [58]. Targeting

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proliferation may also reduce macrophage number [59], and could be relevant for atherosclerosis; however, macrophages are often recruited in the context of obesity, so this strategy may not be as useful for IR. Modification of inflammatory signal transduction pathways inside the macrophage is another approach. By introducing anti-inflammatory agents to the macrophage cytoplasm, inflammatory cytokine production and release can be modulated [60]. Perhaps the most attractive approach to modifying macrophage polarization is to reduce inflammatory gene expression through RNA interference, as multiple genes can be downregulated simultaneously. Potential targets include inflammatory mediators such as cytokines, *e.g* TNF-α, IL-6, IL-1β; chemokines, *e.g.* CCL2, CCL3, CCL5; and transduction targets involved in promoting inflammation such as members of the NF-κB signaling cascade [61, 62]. These therapeutic strategies, coupled with techniques to target specific macrophage subsets, offer a variety of ways to modulate macrophage number and activity at the site of metabolic disease.

Methods of Delivery

Nanoparticles

Nanoparticle design and delivery is a logical strategy for macrophage therapeutics. By definition, nanoparticles are small agents ranging from 1–1000 nm. Their surface coating dictates whether they target a particular cell type–due to receptor-mediated specificity – or an entire tissue due to chemical properties that encourage attraction to that depot [63]. This approach provides target specificity that aims to minimize toxicity and off-target effects. One area of particular interest is development of nanoparticles that target antigen presenting cells, such as macrophages and dendritic cells, via receptors of the C-type lectin family, [reviewed in [64]].

In one recent example, polysaccharide-based delivery systems were utilized to target inflammatory ATMs in obese mice [65]. The 4–30 nm particles were made with dextran and were readily taken up by macrophages following recognition by dextran-binding C-type lectins and scavenger receptors. In lean mice, the nanoparticles preferentially accumulated in liver after intraperitoneal (IP) injection, but in obese mice they accumulated in the adipose depots. The authors showed that ~90% of the cells taking up the particles in gonadal, perirenal, and subcutaneous AT were of myeloid origin. The nanocarriers were used to deliver the anti-inflammatory drug, dexamethasone, specifically to the obesity-associated inflammatory macrophages. Upon delivery, there was a marked decrease in pro-inflammatory genes, *Tnfa*, *Il6*, and *Ccl2*. Although, these preliminary studies show promise in the nontoxic treatment of chronic inflammation in obese AT, the IP delivery method is not realistic for clinical use. The investigators suggest that extended release will be necessary in order to minimize the invasiveness of delivery, but other modes of delivery will need to be explored, and AT specificity will need to be confirmed regardless of the administration route.

There are nanoparticle technologies that have targeted macrophage receptors in other diseases, but not yet in metabolic disease. For example, superparamagnetic iron oxide nanoparticles (**SPIONs**) have traditionally been used as imaging tools, but now have also been shown to impact M2 macrophages by altering their activation state and iron handling

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capabilities [66]. The M2-like phenotype of iron-handling macrophages means that their use as a therapeutic target must be considered differently than M1-like macrophages. With regard to MFe^{hi}, Mhem, and M(Hb) macrophages, a potential use for SPIONs could be to induce an iron recycling phenotype and/or increase their anti-inflammatory phenotype. Along these lines, “click” chemistry has been used to create mannosylated nanoparticles that deliver **siRNA** specifically to M2-polarized TAMs that express mannose receptor on their surface [67]. While these The ability of these nanoparticles to specifically target the M2 macrophages that are present in lean AT makes them a potentially promising technology for further exploration. More research is needed regarding the roles of MFe^{hi}, Mhem, and M(Hb) macrophages in AT and the artery wall so that siRNA therapy can be targeted to pathways that are relevant to these iron-handling macrophages.

Liposomes

Liposomes are a third drug delivery platform that has been investigated. Liposomes are phospholipid vesicles, about 15–1000 nm in size, that can carry either hydrophilic or lipophilic drugs in their bilayer membrane or core, respectively. The encapsulation design allows for drug protection and stabilization; one method of which is PEGylation, which increases circulation time [68]. As with nanoparticles, liposome shells can be engineered with ligands or antibodies to target specific macrophage phenotypes based on receptor specificity. For example, F4/80-targeted liposomes would go to all macrophages, while IL-6 receptor or CD163-targeted liposomes would be taken up by M1 or M2 polarized macrophages, respectively. Additionally, the contents of the liposomes can be altered. These design features allow for more effective and efficient targeting because the outer shell of the compound can be changed depending on where it needs to go, while the inner contents can be altered according to therapeutic needs. While advances have been made in the design of liposomes [reviewed in [69–71]], clinical usage has proven difficult. Due to the complexity of liposome compounds, the associated trials are typically longer and more complex, which leads to cost burden. Off-target effects of treatment are also common because of leakiness of the liposome and degradation of the particles through circulation. Nonetheless, liposomes remain attractive therapeutics as there are many ways that they can be engineered to target macrophages in metabolic disease.

Liposomes can enter macrophages purely because of the cells’ phagocytic properties. Clodronate-loaded liposomes are used to deplete macrophages by inducing apoptosis when internalized. This technology has been historically used in many tissues, but in 2011 depletion of visceral ATMs with consequent effects on tissue health was shown. IP injection of clodronate liposomes, resulted in an improved glucose and insulin tolerance that was associated with increased circulating adiponectin, an insulin sensitizing adipokine [70]. In 2013, another study showed similar data: under the same conditions–animals fed a high fat diet gained less weight, showed improved glucose tolerance and decreased fasting glucose, insulin, and free fatty acid levels, compared to control groups [72]. Notably, these studies potentially represent an additional disease preventative approach. Current efforts focus on lifestyle changes in diet and exercise, but adjuvant therapy to reduce the inflammatory environment of AT by targeting ATMs should be considered. Nonetheless, this research tool provides mechanistic insight into obesity-related disease progression and is a method that

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can be further optimized to more specifically target macrophage subsets involved in metabolic disease.

As with the nanoparticle technologies, discoveries in liposome delivery outside of the metabolism field may prove interesting for the treatment of metabolic disease. For example, CD163 antibody coated liposomes were shown to be taken up by CD163+ monocytes *in vitro* and to kill cells when loaded with doxorubicin [73]. CD163+ macrophages are resident in lean healthy AT, but their numbers are overshadowed during obesity related inflammation. If the healthy cells can be targeted and encouraged to proliferate, the obese tissue microenvironment can conceivably be therapeutically altered to resemble its lean, and healthier, counterpart.

Glucan Shell Microparticles

β-glucans are sugars most commonly found in the cell walls of bacteria. Dectin-1, a macrophage receptor for β-glucans has been proposed as a potential therapeutic with antitumor and antimicrobial activity [74, 75]. Yeast-derived β-glucans (**Y-BGs**) have been reviewed recently and are known to have beneficial effects in models of obesity, allergy, and cancer [76]. For example, orally administrated Y-BGs induced expression of the anti-inflammatory cytokine, IL-10, in the AT of obese humans and increased serum IL-10 levels [77]. Once taken up by macrophages, Y-BGs appear to cause increased reactive oxygen species (**ROS**) formation and phagosomal maturation, ultimately resulting in the induction of autophagy [78]. A recent study suggests IL-10 is an important mediator of macrophage autophagy necessary for removing dysfunctional mitochondria [79]. Taken together, these findings suggest Y-BGs promote anti-inflammatory activity in macrophages through an IL-10 mediated mechanism.

Interestingly, Y-BGs have been used as unique encapsulation tools that can target macrophages in an activation-independent manner. These 2–4 μm hollow particles are able to encapsulate potential therapeutics, including siRNA and small molecules [80–82]. For example, glucan shells have been utilized to encapsulate gene-silencing molecules for macrophage targeted therapies. Orally delivered β1,3-d-glucan-encapsulated siRNA particles (**GeRPs**) containing siRNA directed toward mitogen activated protein kinase kinase kinase kinase 4 (*Map4k4*) reduced systemic inflammation by reducing *Tnfα* mRNA in macrophages [80]. In this design, the siRNA is anchored to Endo-Porter peptide, which simplifies compound preparation and also facilitates release of internalized GeRPs into the macrophage cytoplasm. This peptide-facilitated phagosomal escape is critical for siRNA to reach the cytoplasm where it can function to silence inflammatory genes. Macrophages exposed to orally delivered GeRPs migrated to other tissues and resulted in a 40–80% knockdown of *Map4k4* expression depending on tissue site. These data also suggest that delivery of GeRPs to specific tissue sites can be carried out by the macrophages themselves upon migration to peripheral tissues. IP injection of GeRPs resulted in specific delivery to epididymal ATMs in obese mice, resulting in silencing of *Tnfα* and *Opn* [83]. While these studies show promise, IP delivery of GeRPs would not be favorable for clinical use. Upon further analysis of their efficacy, the investigators should determine whether other modes of delivery are possible.

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Oligopeptide Complexes

Oligopeptides have been known for decades to display tissue specificity [84–86]. Many tissues are difficult to target for non-viral mediated gene delivery, and oligopeptides complexed with gene modulating molecules are an attractive solution. These oligopeptide complexes exhibit specificity by utilizing peptide-bound oligonucleotide sequences designed to target specific cell populations and are complexed with modulatory molecules, such as siRNA or shRNA, that function by silencing genes. One such oligopeptide gene carrier has been designed and is effectively taken up by mature adipocytes through binding a cell surface protein called prohibitin [85]. However, ATMs have also been shown to express prohibitin, and these oligopeptide gene carriers were found in the the stromal vascular fraction of AT that contains immune cells. This unexpected finding may prove to be beneficial in attempts to specifically deliver non-viral gene altering technology to adipose depots and ATMs.

Another potential way to therapeutically target ATMs is through tumor necrosis factor-α converting enzyme (**TACE**) oligopeptides. TACE is an anti-inflammatory metalloproteinase, which is critical for generating soluble TNF-α, and therefore presents a remarkable opportunity for disrupting TNF-α mediated inflammation [87]. Complexes of TACE shRNA (shTACE) and adipocyte-targeting sequence (ATS-9R) oligopeptides have been produced and are specifically taken up by visceral ATMs following IP injection. These complexes display specificity for AT, and function by silencing TACE, resulting in the cell's inability to generate soluble TNF-α. Accumulation of injected oligopeptides in visceral ATMs without accumulation in the spleen or liver after 4 hours has been observed [86]. Although shTACE oligopeptide complexes are enzymatically degraded, 8 repeated injections over a month long period resulted in improved insulin sensitivity and glucose tolerance, which was attributed to reductions in adipose TACE activity.

Concluding Remarks and Future Perspectives

Macrophages have become a unique target for treating metabolic disease since the discovery of their importance in metabolic tissues and their change in phenotype upon disease progression. This review presents discoveries that couple the ability to alter macrophage function with the technologies that ensure their specific targeting. There is ongoing research to define specific macrophage subsets, identify surface markers unique to these subsets, and capitalize on their unique properties to target specific functions. Many of the delivery systems described in this review can be engineered to transport gene-silencing agents such as siRNA; however; challenges remain in successfully eliminating the targeted gene expression. While CRISPR-Cas9 technology can not yet be used *in vivo* [88], it would be interesting to determine whether this technology could be delivered specifically to tissue macrophage subsets in order to alter their polarization and functional phenotype. Additionally, it has recently been elucidated that epigenetic regulation is involved in macrophage polarization, [89]. Targeting epigenetic modifications via genetic or pharmacological perturbation is another promising area of research.

While strides have been made in elucidating mechanisms involved in maintaining healthy macrophages in metabolic tissues, questions still remain regarding the therapeutic potential

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and feasibility of specifically treating macrophages in metabolic disease (see Outstanding Questions). Ultimately, the future of macrophage-specific treatment relies on continued efforts to identify and produce therapeutics that can be specifically targeted to cells of the appropriate phenotype and tissue location.

Outstanding Questions

- Can we identify and utilize unique macrophage markers to provide tissue-specific therapy?
- Will antisense oligonucleotide therapies targeting macrophages be useful for treatment of metabolic disease?
- Are there less expensive and more streamlined methods to design liposomes that target specific phenotypes of macrophages in metabolic tissues?
- Can the delivery modalities presented here, *e.g.* liposomes and nanoparticles, be engineered to maintain potency when administered orally?
- Modulation of epigenetic programming has grown in cancer research and drug development, but has more recently been related to inflammation in metabolic disease. What is the future of applying compounds that alter epigenetic regulation in obesity associated inflammation?
- Permanent genetic alteration via CRISPR is a cutting edge technique, but has not been optimized for *in vivo* use. Can it be utilized to more robustly affect macrophage polarization genetically and replace siRNA in the presented therapeutics?
- Many novel therapeutics rely on genetic technologies. How responsive will the public be to genome or epigenome altering treatments?
- What are the long-term effects of all of these novel strategies?

Glossary

Adipose tissue macrophage (ATM)
Macrophages in the adipose tissue.

CD163
A high affinity scavenger receptor for the hemoglobin-haptoglobin complex used to identify an M2-polarized macrophage marker.

Chemokine (C-C motif) ligands 2, 3, and 5 (CCL2, CCL3, and CCL5, respectively)
Cytokine ligands involved in the recruitment of leukocytes.

CRISPR-Cas9
Bacterial-based immune defense that leads to destruction of specific genetic sequences. This can be delivered and utilized in other systems to silence target genes of interest.

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β1,3-d-glucan-encapsulated siRNA particles (GeRPs)
Yeast-derived glucan shells that encapsulate macrophage-targeted siRNA.

Inducible nitric oxide synthase 2 (iNOS2)
An enzyme that produces reactive free radicals of nitric oxide when induced by a combination of cytokines and lipopolysaccharide.

Insulin resistance (IR)
A condition in which cellular response to the hormone insulin is reduced, leading to dysfunctional glucose homeostasis.

Interleukin 1 beta (IL-1β)
Pro-inflammatory cytokine produced by activated macrophages.

Interleukin 4 (IL-4)
Anti-inflammatory cytokine produced by T cells that promotes alternative activation and inhibits classical activation of macrophages.

Interleukin 6 (IL-6)
Pro-inflammatory cytokine produced by T cells and macrophages that stimulates an immune response.

Interleukin 13 (IL-13)
Anti-inflammatory cytokine produced by T cells, basophils, and eosinophils that promotes alternative activation of macrophages.

Interleukin 33 (IL-33)
Anti-inflammatory cytokine produced by macrophages and dendritic cells that drives production of other anti-inflammatory cytokines, including IL-4 and IL-13 by T cells.

Kupffer cells
Macrophages that are resident in the liver.

M1-like macrophage
Classically activated macrophage with a pro-inflammatory phenotype most often associated with host defense.

M2-like macrophage
Alternatively activated macrophage with an anti-inflammatory and pro-fibrogenic phenotype most often associated with tissue repair.

Metabolically active macrophages (MMe)
Macrophages that are activated by metabolic growth factors and are resident to adipose tissue. They produce pro-inflammatory cytokines, but express markers that are more similar to M2-like macrophages, including ABCA1, CD36, and PLIN2.

MFe^{hi}
ATMs with high iron content that can recycle iron and display a M2-like phenotype.

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M(Hb)
Macrophages with unique phenotype resulting from uptake of haptoglobin-hemoglobin complexes.

Mhem
Macrophages with unique phenotype resulting from uptake of heme.

Non-alcoholic fatty liver disease (NAFLD)
A class of liver disease characterized by fat deposition in the liver that is not associated with alcohol consumption.

Reactive oxygen species (ROS)
Oxygen containing chemical species that are produced by cells at high levels in response to pathogens or inflammatory stimuli.

Small interfering RNA (siRNA)
Gene silencing technology that interferes with transcription of genes.

Superparamagnetic iron oxide nanoparticles (SPIONs)
Engineered tool that targets macrophages and was originally used for imaging techniques, but can also be targeted for delivery of compounds to M2-polarized macrophages.

Tumor necrosis factor alpha (TNF-α)
Pro-inflammatory cytokine produced by macrophages and adipocytes that activates NF-κB and induces insulin resistance.

Tumor necrosis factor-α converting enzyme (TACE)
Metalloproteinase that generates soluble TNF-α.

Yeast-derived β-glucans (Y-BGs)
β-D-glucose polysaccharides extracted from the cell walls of *Saccharomyces cerevisiae* that have shown promise in altering obesity via IL-10 mediated effects.

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Trends Box

- Macrophages are complex cells with phenotypic plasticity to meet ever-changing tissue environment demands, and their phagocytic properties make them ideal therapeutic targets.
- Depletion of all macrophages via clodronate-loaded liposomes was the historical way to therapeutically alter the inflammation associated with macrophage activation in metabolic tissues such as liver and adipose. Recent advances allow for more directed targeting, with regards to both specific macrophage subpopulations (M1-like vs M2-like) and with modulation of certain function (gene targeted knockdown).
- Receptor mediated endocytosis can be utilized to facilitate transport of therapeutic vectors with high specificity.
- Therapeutics that modulate inflammatory gene expression can be transported in nanoparticle and oligopeptide vectors.