Chapter

The Pivotal Role of Macrophages in Metabolic Distress

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Abstract

Obesity is a prevalent condition with several associated co-morbidities including the development of metabolic diseases. In obesity there is immune cell infiltration into the white adipose tissue and this is associated with the generation of inflammation and insulin resistance (IR). A large majority of the infiltrating leukocytes in obese adipose tissue are pro-inflammatory macrophages, which upon activation induce a switch in metabolism from oxidative phosphorylation, as is utilised by macrophages in lean adipose tissue, towards aerobic glycolysis. The signalling pathways evoked in the recruited macrophages induce the release of pro-inflammatory cytokines, in signalling pathways which directly interfere with insulin signalling and thus induce a state of IR. As macrophages appear to play such a pivotal role in the generation of IR and are the largest leukocyte population in the adipose tissue, they provide a promising therapeutic target. Indeed, there are several strategies currently being studied to induce a 'switch' in macrophages associated with obese adipose tissue, towards the phenotype of those associated with lean adipose tissue, with arguably the most promising being those strategies designed to target the metabolic pathways within the macrophages. This chapter will discuss the polarisation and activation of macrophages within lean and obese adipose tissue and how these cells can be targeted therapeutically.

Keywords: macrophage, obesity, metabolism, inflammation

1. Introduction

Obesity is defined as abnormal or excessive fat accumulation and is linked with increased risk of development of multiple co-morbidities, including cardiovascular disease, type 2 diabetes, musculoskeletal disorders and certain cancers. Obesity and its associated co-morbidities are a significant health concern facing the global population. Worldwide obesity has tripled since 1975, with 39% of adults considered overweight and 13% considered obese [1]. This situation is prominent in childhood, with 41 million of the global under five population overweight or obese [1].

Obesity induces a state of low-grade systemic inflammation, characterized by increased serum levels of pro-inflammatory mediators, including C Reactive Protein (CRP), Tumour Necrosis Factor (TNF)-α, Interleukin (IL)-1β and IL-6, which contributes to metabolic dysfunction and insulin resistance (IR) [2]. Although the mechanisms underlying this inflammatory response are not fully understood, activation of adipose tissue macrophages (ATM) contributes to this inflammatory state, and therefore to the development of insulin resistance (IR) [3, 4]. Conversely, in lean

individuals the immune repertoire constitutes a more anti-inflammatory phenotype, with ATM alongside regulatory T cells (Tregs) releasing cytokines such as IL-10 and transforming growth factor (TGF)-β, which increase insulin sensitivity [5]. Therefore, the role of ATM in metabolic function is clearly an area of interest, indeed transcriptional profiling has identified how quickly macrophages can respond and adapt to alterations in their microenvironment [6]. This chapter will focus on the role macrophages play in the pathogenesis of metabolic disorders and explore if re-education of these cells provides a target for therapeutic intervention in obesity and its related co-morbidities.

2. The microenvironment of the adipose tissue in lean and obese individuals

Obesity historically was believed to be due to a combination of genetic predisposition and environmental factors, however, more recently it has been recognised that immunological factors can also contribute to the pathogenesis of obesity. Indeed, while over 30 gene loci combinations have been associated with the development of obesity and metabolic disease, these loci are only associated with 2–3% of the incidence of these conditions [7]. Further the energy-dense modern Western diet combined with a sedentary lifestyle undoubtedly adds to the obesity epidemic. Recent work has identified the links between dysbiosis in the intestinal microbiome and immune cell activation, linked to the ingestion of high-fat, low-fibre diets, and the development of obesity [8].

2.1 The role of the microbiome

The intestinal microbiome is essential for processing dietary polysaccharides and has been identified as a key regulator of systemic inflammation in obesity [9]. Mouse studies are routinely used to study the mechanisms underlying obesity and metabolic disease. Due to the nature of obesity being largely related to diet, diet-induced models are often favoured over genetic models (for example, leptin deficient ob/ob mice). Indeed, studies using a high-fat diet (HFD; equivalent to 60% animal-derived fats in the diet) have been used to study the potential implications of alteration in the microbiome related to diet as well as other obesity-related pathogenesis. It has been shown that the microbiome in obese mice has an increased capacity to harvest energy from the diet compared to the microbiome from lean mice [10]. Microbiome transfer studies, in which intestinal microbiota from mice raised in conventional housing was transferred into germ-free mice, induced a 60% increase in body fat and IR within 14 days, despite a reduction in food consumption [11]. The transfer of microbiota-derived products such as lipopolysaccharides and peptidoglycans, have shown to promote metabolic endotoxemia, which induces pro-inflammation in adipose tissue [12]. In contrast, the microbiota of the gut bacterial fermentation of dietary fibre was shown to have anti-inflammatory effects [12]. Indeed, it was shown that the transfer of intestinal microbiota from lean donors increased the insulin sensitivity in individuals with metabolic syndrome [13].

2.2 Adipose tissue

Adipose tissue (AT) is an important metabolic organ, which helps orchestrates metabolic and endocrine functions as well as immune responses [12]. AT functions to store excess nutrients as triacylglycerides and releases fatty acids in the fasted

state, provide cold insulation and protection of vital organs. In the AT of obese individuals, there is significant adipocyte hyperplasia and adipose tissue hypertrophy [14]. AT consists of mature adipocytes, pre-adipocytes, fibroblasts, endothelial cells, histocytes and populations of immune cells including monocytes, macrophages, natural killer (NK) cells, innate lymphoid cells (ILCs) and lymphocytes. AT is classified into three categories, namely white (WAT), beige or 'brite' (beige/brite) and brown (BAT). WAT accounts for approximately 50% of body mass and can release free fatty acids (FFA) into circulation when glucose levels are low. Whilst BAT plays an important role in thermogenesis and the production of heat [15].

The AT of obese individuals is in a state of chronic low-grade inflammation with marked infiltration various pro-inflammatory immune cells such as CD8 cells, NK cells, ILC1, Th1 cells, neutrophils and pro-inflammatory macrophages [16]. Conversely, the immune repertoire of AT from lean individuals comprises anti-inflammatory cell populations, including eosinophils, ILC2, Tregs, Th_2 cells and anti-inflammatory macrophages [16] (**Figure 1**). In lean mice, ATM constitutes approximately 5% of cells, conversely in obese mice ATM can account for up to 50% of the cells [3]. Whilst in lean human AT, ATM comprises 4% of cells compared to 12% in excess adiposity [17]. In addition to macrophages, lymphocytes and ILCs also play roles in the regulation of AT inflammation, the roles of which seem to largely involve supporting the polarisation state of the ATM populations. For example, eosinophils provide a source of IL-4 promoting an M2 phenotype. In obese mice adipose eosinophils are decreased, whilst depletion of eosinophils results in increased M1 ATM, weight gain and systemic IR [18]. Furthermore, ILC2

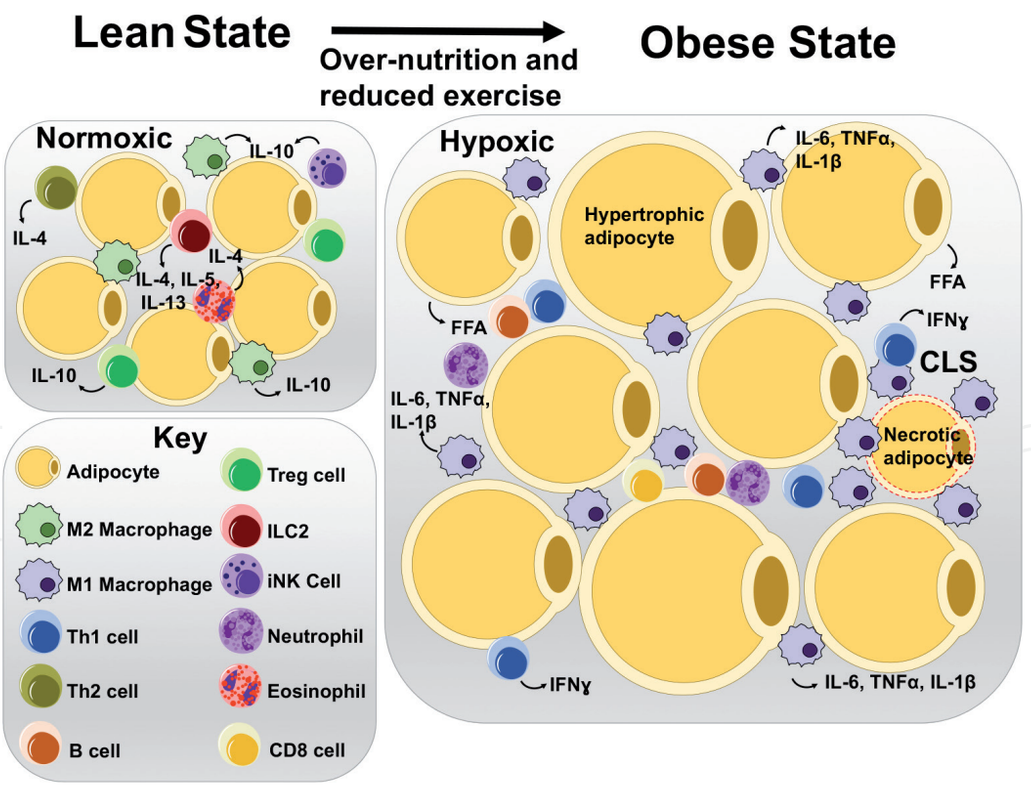

Figure 1.
Immune cell composition of adipose tissue in a lean and obese state. In the lean state, eosinophils and type 2 innate lymphoid cells (ILC2s) produce Th_2 cytokines (IL-4, IL-5 and IL-13), which promotes eosinophil recruitment and anti-inflammatory polarisation of macrophages towards an M2 phenotype, which is supported in the normoxic state of lean adipose tissue. In turn, M2 macrophages secrete anti-inflammatory cytokines such as IL-10. In the obese state, adipocyte hypertrophy, hyperplasia and hypoxia cause necrotic adipocytes, resulting in pro-inflammatory state and macrophage recruitment, forming crown like structures (CLS) surrounding the adipocytes. These macrophages are polarised towards an M1 phenotype and secrete the pro-inflammatory cytokines IL-6, IL-1β and TNF-α.

have been identified as a source of IL-5, a key cytokine in eosinophil recruitment. Thus, accumulation of eosinophils and maintenance of M2 ATM relies on ILC2 [19].

2.3 The role of the adaptive immune system in obesity

The adaptive immune system also plays an important role in obesity and metabolic disease. B cells have been shown to be involved in obesity induced inflammation and IR [20]. In obese mice, there was an identified increased in IgG^+B cells and IgG production, associated with activation of M1 ATM, increased Th1 cells and conversely, attrition of Treg cells [21]. In addition, transferring B cells into B cell deficient mice induced IR [20]. Furthermore, in obese mice, there is increased $CD8^+$ effector T cell recruitment in epididymal AT. Interestingly, it is reported that $CD8^+$ T cells precede macrophage infiltration and deletion of $CD8^+$ T cells resulted in reduced macrophage infiltration and AT inflammation whilst improving IR [22]. Conversely, both Treg cells and iNKT cells are negatively associated with obesity-induced inflammation and are enriched in lean AT. Indeed, both these immune cells are known to secrete IL-10 which promotes M2 macrophage polarisation [23, 24].

2.4 Adipose tissue macrophages

ATMs appear to play a major role in the regulation of obesity-related inflammation, with different macrophage phenotypes associated with divergent roles in the AT. In lean animals, ATM function to maintain the homeostatic micro-environment in AT by taking up excess lipids and phagocytosing dead adipocytes. Broadly speaking macrophages present in lean AT are of an M2 phenotype, which have been shown to suppress inflammation in AT [25]. Furthermore, M2 macrophages in lean AT have been associated with brown fat activation and 'beiging' of WAT in mouse models of obesity, via expression of tyrosine hydroxylase, which induces thermogenesis [26, 27]. However, this process has recently been queried, with IL-4-stimulated macrophages failing to generate sufficient levels of catecholamines to contribute to adipose tissue adaptive thermogenesis [28]. Conversely, excess lipid uptake in obese AT, induces M1 polarisation and along with excess lipid droplets, immune cells and necrotic adipocytes this forms a component called 'crown-like' structures (CLS) [29, 30]. Indeed, it has been shown that more than 90% of all macrophages in WAT of obese mice and humans are localized to dead adipocytes [31]. This metabolic activation of M1 macrophages in obese AT is associated with increased pro-inflammatory cytokines in the AT and recruitment and activation of M1 macrophages in the AT [32].

ATM in lean AT is considered a resident macrophage population, which originates from yolk-sac progenitors and self-renews via proliferation under homeostatic conditions. Over time into adulthood resident ATMs are replaced with circulating monocytes derived from bone marrow [33]. Using mouse bone marrow chimera experiments, following transplanting donor $CD45.1^+$ bone marrow into recipient $CD45.2^+$ mice, and maintenance on obesity-inducing HFD, 85% of the ATM were donor-derived compared to 15% that were recipient-derived [3]. Interestingly, the polarization of macrophages in obesity from an M2 to an M1 phenotype has been mainly attributed to the recruitment of monocytes to AT, rather than the conversion of tissue resident M2 macrophages [34]. Murine monocytes can be classified through the expression of Ly6C, with $Ly6C^{hi}$ monocytes considered inflammatory. In the steady state $Ly6C^{hi}$ monocytes differentiate into $Ly6C^{lo}$ monocytes in the circulation, which are believed to differentiate into M2 macrophages in the tissue. However, in obese AT in response to inflammatory stimuli such as the monocyte chemoattractant CCL2, $Ly6C^{hi}$ macrophages are recruited to the AT where they

differentiate to M1-like ATM [25]. Indeed, in absence of Ccl_2 expression macrophages expressed an M2 gene profile [35].

ATM represents the largest population of leukocytes within the AT and plays many vital homeostatic roles including tissue remodelling and insulin sensitivity. However, with progressive obesity ATM are the key mediators of inflammation, IR and the impairment of adipocyte function.

3. Polarization of ATM and the link to IR

Macrophages are extremely heterogenic in function and phenotype, and have historically been characterized into two phenotypes; M1 and M2. M1 macrophages are often defined as 'classically activated' and are generally pro-inflammatory in function, with a vital role in eliminating pathogens and virus-infected cells. Whereas, M2 macrophages and termed 'alternatively activated' are anti-inflammatory in function and promote tissue repair and wound healing. This is very simplified and dated model however, evidence now suggests that ATMs include highly plastic cell populations, with their phenotype largely dependent on the microenvironment of the AT. Whilst the exact number and function of ATM in the AT is evolving, it is clear there are distinct populations in the lean and obese AT, with unique tissue distribution, marker expression, transcriptional profiles and functions. Indeed, obese ATM display markers that are largely induced by their metabolic state rather than cytokine stimuli that classically polarise M1 and M2 cells [32].

3.1 M1 and M2 macrophage phenotypes

M1 macrophages are activated by signals associated with infection such as IFN-ɣ as well as bacterial-derived products such lipopolysaccharide (LPS) and free fatty acids (FFA). M1 macrophages are loosely identified by surface expression of $F4/80^+CD11C^+$ with high levels of MHC-II, CD68, CD80 and CD86 costimulatory molecules in addition to release of TNF-α and inducible nitric oxide synthase (iNOS) [36]. M2 macrophages are activated via Th_2 cytokines, IL-4 and IL-13 as well as by parasitic products. M2 macrophages are loosely identified as $F4/80^+CD206^+CD301^+CD11C^-$ and express genes encoding anti-inflammatory proteins such as *Chil3*, *Arg1* and *Il10* in mice [34]. A crucial transcription factor in M2 macrophage polarisation is peroxisome proliferator-activated receptor (PPAR-γ/δ), which can be driven by adipocyte derived IL-4 and IL-13 [37–39] (**Figure 2**). Some markers vary between mice and human macrophages. For example, there are no human homologues of the M2-associated genes *Chil3*, *Arg1 and Fizz1,* with human M2 identified based on expression of tranglutaminase-2 (TGM2) and CD68 [40].

3.2 MMe and Mox macrophage phenotypes

Macrophages with a phenotype associated with obesity are induced by several metabolic stimuli such as FFA, high insulin and glucose, oxidised phospholipids and low-density lipoproteins. These macrophages display surface markers that are neither representative of typical M1 or M2 macrophages and give rise to a population of metabolic activated (MMe) and oxidised (Mox) macrophages [41, 42] (**Figure 2**). Both these macrophage phenotypes are associated with a state of IR. MMe macrophages cell-surface markers express ABCA1, CD36 and PLIN2 and are involved in the clearance of dead adipocytes through lysosomal exocytosis as well as potentiating inflammation. NADPH-oxidase-2 (NOX2) has been identified as a key driver of the functions of MMe macrophages, with *Nox2*-deficient mice displaying attenuated ATM inflammation

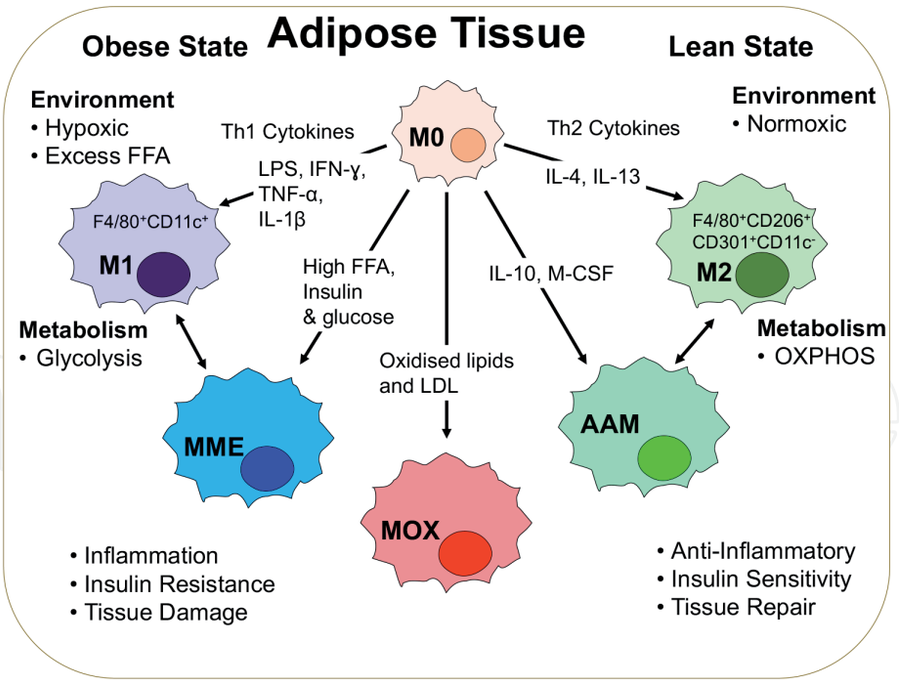

Figure 2.
Adipose tissue macrophage (ATM) polarisation in lean and obese state. ATM originate from polarisation of 'Mo' macrophages. Depending on the stimuli and local environment facilitates the macrophage polarisation. Th1 cytokines, LPS, IFN-γ, TNF-α and IL-1β promote a M1 macrophage that is characterised by F4/80^{+}CD11c^{+}. In contrast, Th$_2$ cytokines, IL-4 and IL-13 promote an M2 macrophage that is characterised by F4/80^{+}CD206^{+}CD301^{+}CD11c^{-}. Interestingly, in an obese state, the hypoxic environment and metabolic cues such as excess free fatty acids, high insulin and glucose, oxidised phospholipids and low-density lipoprotein, which promotes a metabolic activated (MME) or oxidised (MOX) macrophage. In the obese state, macrophages promote inflammation, IR and tissue damage. In contrast, in the lean state, macrophages promote ant inflammation, insulin sensitivity and tissue repair.

and improved glucose sensitivity in a model of diet-induced obesity, when compared to WT animals [41]. Mox macrophages are driven by oxidised phospholipids derived from oxidised low density lipoproteins (LDL) [43] and express surface markers Srnx-1 and Txnrd-1 [42]. Mox macrophages have been studied primarily in the context of atherosclerosis, where the oxidation of accumulated LDL leads to enrichment of the tissue with oxidised lipids, causing the polarisation of macrophages towards a phenotype dependent on the transcription factor Nrf2 [43]. However, a recent paper identified that ATM with a Mox phenotype (CX3CR1^{neg}F4/80^{low}Txnrd1^{+}HO1^{+}) are the predominant phenotype present the AT of lean mice, driven by individual oxidised phospholipids in the AT [44]. It will be an interesting further area study to appraise the role of these novel macrophage phenotypes within the context of obesity and IR.

3.3 Regulators of macrophage polarization

Macrophage polarization has been well studied over the past decade leading to the discovery of several key regulators which orchestrate macrophage polarization, such as the Signal Transducer and Activator of Transcription (STAT) family, interferons, regulators of lipid metabolism, transcription factor families, microRNAs (miRNAs) and long non-coding RNAs [45] (**Figure 3**).

3.3.1 STAT family members

The Janus Kinase (JAK)/STAT signalling pathway transmits signals from extracellular cytokines into the nucleus. Indeed, JAK/STATs are arguably the most widely studied pathway within the context of macrophage polarisation, with IFNγ binding

to its receptor triggering activation of JAK1/2-mediated tyrosine phosphorylation and subsequent dimerization of STAT1 one of the first pathways to M1 polarisation identified [46]. In addition, LPS binding to TLR4 induces autocrine production of IFN-ß that activates the type 1 IFN receptor triggering STAT1 and STAT2 phosphorylation and heterodimerisation [47]. STAT3 has a dichotomous role in macrophage polarisation, it is the key transcriptional regulator in the production of the anti-inflammatory cytokine IL-10, which can drive an anti-inflammatory macrophage phenotype, however, STAT3 can also be activated by IL-6 and IFN-ß, inducing a pro-inflammatory phenotype [48]. Conversely, IL-4 and IL-13 induces M2 macrophage polarisation largely through induction of STAT6 and KLF4 via the dual catalytic activities of MCP-1-induced protein and inducing PPAR-ɣ [49–51].

3.3.2 Interferon regulatory factors

Interferon regulatory factors (IRFs) are intracellular proteins that regulate immune cell maturation and play a pivotal role in macrophage polarization. Two key and opposing IRFs in macrophage polarisation are IRF4 and IRF5, which directly compete for binding to MyD88 and subsequent transcription factors such as NFκB. Interestingly, IRF5 was shown to promote M1 macrophages, while IRF5 expression was upregulated in obese individuals compared to lean individuals at both the mRNA and protein levels and is negatively associated with insulin sensitivity [52–54]. It was also shown that IRF5 promotes inflammatory macrophage polarization by activating the transcription of IL-12 and repressing IL-10 [55]. Conversely, IRF4 acts as an antagonist of M1 macrophage polarisation, promoting M2 macrophages [53, 54, 56]. In the context of obesity, macrophage-specific knockout of IRF4 resulted in significant IR and an increase in expression of pro-inflammatory genes [57]. Furthermore, IRF6 has also been implicated in

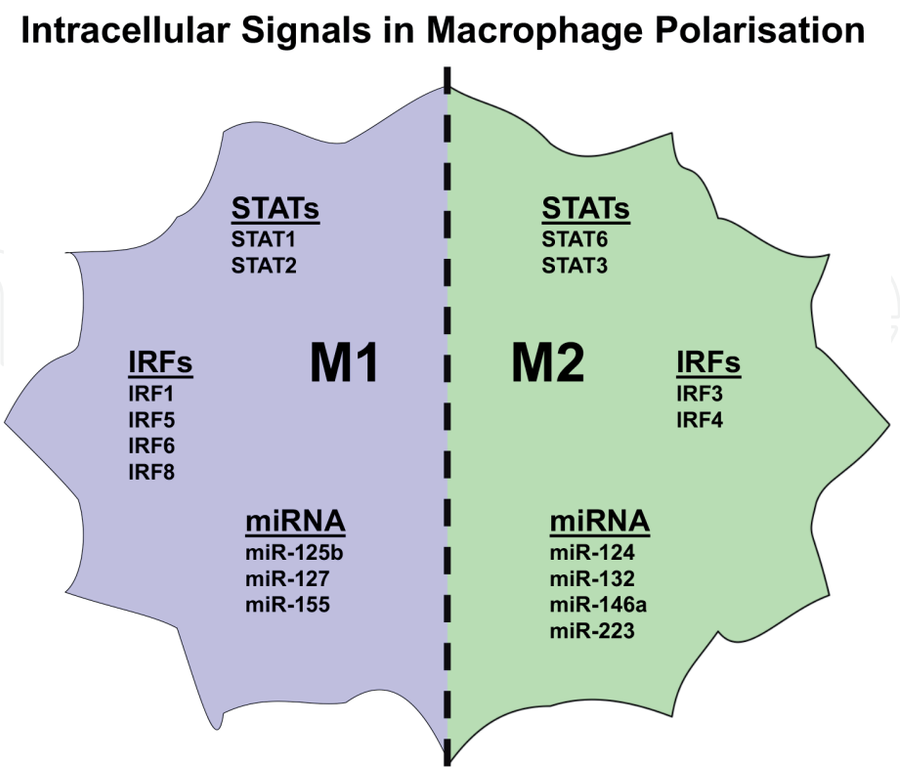

Figure 3.
Intracellular signalling of M1 and M2 macrophage polarisation. Respective intracellular key regulators of M1 and M2 macrophage polarisation. Signal transducer and activator of transcription (STAT) family, interferons (IRFs) and microRNA (miRNAs).

macrophage polarisation, promoting M1 macrophages due to suppression of PPARɣ expression, a critical regulator of M2 macrophages. Overexpression of IRF6 reduced M2 activation, whilst IRF6 knockdown enhanced M2 macrophage activation [58]. The impact of IRFs on macrophage polarisation and plasticity is clearly quite complex and further studies will hopefully provide information on how IRFs function in different microenvironments, for example, in lean versus obese AT.

3.3.3 MicroRNAs

miRNA are short inhibitory non-coding RNAs (~22 nucleotides) that degrade specific mRNA targets or block RNA translation, and have also been implicated in driving macrophage polarisation. Indeed, miR-125b expression was shown to be upregulated in murine macrophages following IFN-ɣ stimulation and was identified to promote M1 macrophage polarisation, while suppressing IRF4, an important M2 transcription factor [59]. Additionally, miR-155 was also shown to promote M1 polarisation with expression in murine macrophages increased upon TLR activation or stimulation with pro-inflammatory cytokines (TNF-α, IFN-β or IFN-ɣ) [45]. Whilst in human macrophages, miR-155 was shown to target IL-13Rα1 and inhibit STAT6 activation, thus inhibiting M2 macrophage polarisation [60]. Additionally, miR-9 enhances M1 macrophage polarisation by suppressing PPARδ [61]. Whilst miR-127 suppresses B-Cell lymphoma protein (Bc6), which promotes M1 polarisation [62]. Conversely, miR-124 promotes M2 polarisation via LPS-induced cytokine production by targeting STAT3 to decrease IL-6 production and reduce TNF-α [63]. Furthermore, miR-132, miR-146a and miR-223 induce M2 macrophages by inhibiting NF-κB [64–66].

3.4 Hypoxia and macrophage polarisation

Of interest in the context of obesity is the potential for hypoxia to influence macrophage polarisation of macrophages (**Figure 2**). Indeed, hypoxic areas in adipose tissue occur in obese individuals when rapid tissue expansion occurs without sufficient accompanying blood flow to these areas. M1 macrophages display high expression levels of Hypoxia-related genes including *Hif1α*, which has been shown to induce a pro-inflammatory phenotype in macrophages via TLR4 activation, involving the PI3K/Akt signalling pathway [67, 68]. TLR4 expression in macrophages was shown to increase in a hypoxic environment [69]. *Hif1α* enhances the transcriptional activity of NF-κB, driving production of pro-inflammatory cytokines and decreasing the induction of immune regulatory mediators [70]. In contrast, M2-like macrophages in obese individuals express *Hif2α* [71]. Indeed, HIF-2α is also upregulated under low oxygen levels [5]. It was shown that HIF-2α overexpressing macrophages suppressed pro-inflammatory responses and improved IR. Whilst knockdown of HIF-2α in macrophages induced pro-inflammatory gene expression in adipocytes [71]. Thus, it was suggested that HIF-2α counteracts the pro-inflammatory responses to relieve obesity induced IR in AT [71].

3.5 IR and macrophage polarisation

Insulin acts in the adipose tissue to promote uptake and storage of fatty acids, stored as triglycerides, and inhibits the lipolysis of stored triglycerides. IR is a reduced response to insulin in the liver, muscle and AT, due to an impairment in the insulin-signalling pathway, leading to hyperglycaemia [72]. In obesity, the increased recruitment of macrophages to AT positively correlates with IR [4]. Indeed, obese mice show increased macrophage-mediated inflammation that resulted in

long-term IR [73], with elevated levels of FFA which activated CD36 expressing macrophages and adipocytes, inducing inflammation and impairing insulin signalling [74]. In AT from lean states, the presence of M2 macrophages maintains insulin sensitivity via anti-inflammatory actions of IL-10 and STAT3 [25]. In obese individuals, there is a change in adipocyte metabolism and gene expression. Consequently, there is increased lipolysis and release of FFA which can lead to TLR4 activation of M1 macrophages [75]. Indeed, TLR4 expression is increased on macrophages in obese individuals. In co-culture studies, it was shown that macrophage activation through TLR4 signalling increased secretion of pro-inflammatory cytokines, blocking the insulin signalling cascade [76, 77]. In obese individuals, the IκB kinase (IKK) complex is activated in macrophages resulting in phosphorylation of IκBα on Ser32 and 36, degrading IκBα and allowing NF-κB to translocate to the nucleus and upregulated target genes such as pro-inflammatory cytokines including TNF-α, IL-1β and IL-6 [78].

Increased expression of pro-inflammatory cytokines negatively affects insulin signalling pathways [79]. The effect of these cytokines on IR are seen locally in AT, but also systemically as they are released into circulation [76]. TNF-α phosphorylates insulin receptor substrates (IRS), consequently preventing downstream signalling via inhibiting IKK, c-Jun N-terminal kinase (JNK) and atypical protein kinase C (aPKC) [80]. Interestingly, males have been shown to have increased TNF-α plasma concentration compared to females, leading to the suggestion that obese males are more susceptible to develop IR [81]. IL-6 is increased in the serum of obese individuals and mice, with weight loss reducing circulating IL-6, which improves insulin sensitivity [82]. Systemic IR has also observed during pregnancy, puberty and during infection such as sepsis driven by TNF-α and IL-6 [83]. It is clear from such studies that the presence of pro-inflammatory cells and the release of pro-inflammatory cytokines lead to a loss of sensitivity to insulin and a state of IR. This is possibly the biggest incentive to therapeutically target the macrophages in cases of metabolic disease.

4. The metabolic signature of macrophages

Metabolism is a series of highly interconnected pathways that generate metabolic products such as energy and macromolecules from nutrients in the microenvironment. Whilst the metabolic pathways are plastic, cells, in particular macrophages, tend to utilise a pathway that suits their immediate energy requirements. M1 macrophages have huge metabolic demands, and rely largely on glycolysis, conversely, M2 macrophages meet their energy requirements using oxidative phosphorylation (OXPHOS) pathways. During glycolysis, extracellular glucose is taken up by the cell and converted to two molecules of pyruvate and ATP; NAD^+ is converted to $NADH^+H^+$ regenerated through the breakdown of pyruvate to lactate. Glycolysis also provides the first molecule in the pentose-phosphate pathway, glucose-6-phosphate, which provides NADPH to maintain the cellular redox balance and the production of fatty acids. In M1 macrophages, the increased glucose consumption is associated with the capacity for rapid cytokine production and antimicrobial activity through ROS generation [84, 85]. Conversely, in the presence of oxygen cells produce ATP via the electron transport chain (ETC), which is linked to the tricarboxylic acid (TCA) cycle. The TCA cycle uses carbon sources, such as Acetyl CoA, glutamine or fatty acids to fuel a cycle which generates the reducing agents NADH and FADH2 that serve as electron carriers for the ETC for OXPHOS. Glycolysis is a poor producer of energy, with only two molecules of ATP per glucose molecule, compared to 36 molecules produced by OXPHOS. However,

the use of each pathway will depend on the environment and functional requirements of the cells, as glycolysis provides energy rapidly.

The TCA cycle is truncated in M1 macrophages, resulting in a reduced production of alpha-ketoglutarate (α-KG) and accumulation of citrate and succinate metabolites [86]. The accumulation of citrate leads to the production of the macrophage specific metabolite itaconic acid, which is a major feature of LPS stimulated macrophages [86]. The build-up of itaconic acid has been identified as a driver for succinate accumulation, through its ability to inhibit succinate dehydrogenase [87, 88]. The excess succinate leads to the induction of IL-1β through the stabilization of HIF-1a further enhancing inflammation in the macrophages [89]. Indeed, blocking glycolysis reduces release of CCL2 from TNF-a or LPS stimulated adipocytes, providing further evidence for a link between metabolism and inflammation [90] (**Figure 4**).

Macrophage polarization is also influenced by the metabolism of arginine. M1 macrophages upregulated nitric oxide synthase (iNOS), which catabolize arginine to citrulline and nitric oxide (NO). This NO is important for intracellular killing of pathogens. In addition, M1 macrophages use the pentose phosphate pathway, which generates NADPH for the NADPH oxidase, which produces ROS and NO. Consequently, these metabolic pathways provide M1 macrophages with rapid energy. Conversely, in M2 macrophages, arginase-1 (Arg1) is induced which produces urea, ornithine and polyamines which are key in tissue repair [36, 91].

The metabolic profile of ATM alters dependent on the microenvironment of the AT. In mice, transcriptome and extracellular flux analysis have shown that in lean AT fatty acid oxidation, glycolysis and glutaminolysis all participate in cytokine release by ATM [92]. In obese AT, both glycolysis and OXPHOS are utilised, however glycolysis takes precedence, potentially due to the hypoxic environment in the AT [92].

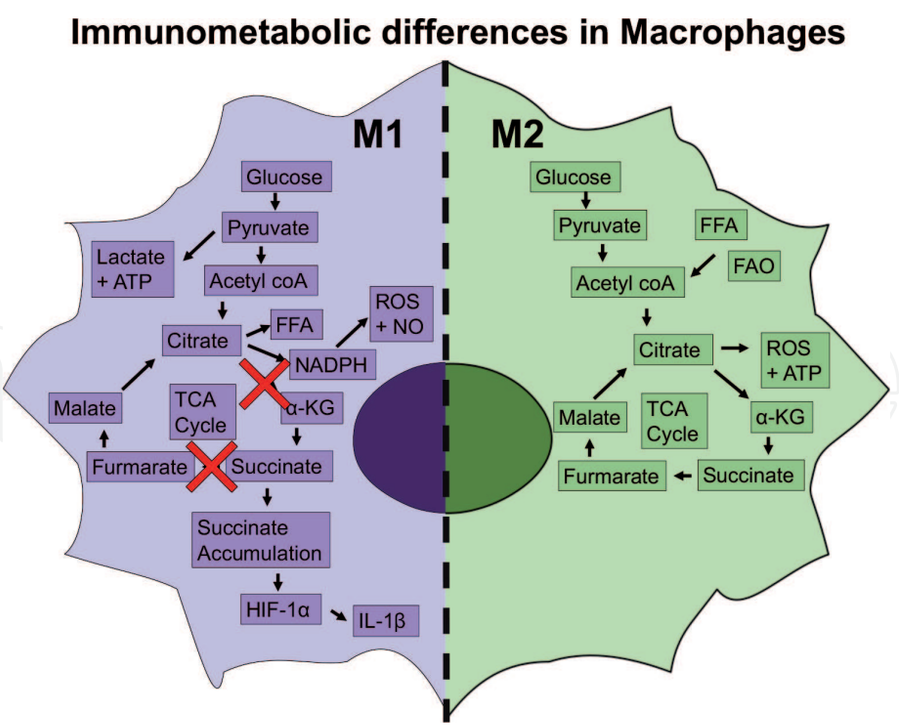

Figure 4.
Immunometabolic differences in M1 and M2 macrophages. M2 macrophages use OXPHOS and the TCA cycle to produce energy. They have increased ability to uptake free fatty acid (FFA) and fatty acid oxidation (FAO) to facilitate the TCA cycle. In contrast, M1 macrophages increase energy production oxidative glycolysis. M1 macrophages also have a 'broken' TCA cycle, resulting in accumulate of citrate and succinate. Increased succinate results in secretion of pro-inflammatory cytokine IL-1β via HIF-1α. TCA, tricarboxylic acid; HIF-1a, hypoxia-inducible factor-1a; a-KG, alpha-ketoglutarate.

5. Could targeting macrophages provide a therapeutic strategy in metabolic disorders?

As described above, as macrophages play a significant role in obesity and other metabolic disorders they are an attractive therapeutic target. The therapeutic strategies that target macrophages look to re-educate polarized macrophages, depletion of polarized macrophages or silencing macrophages. Additionally, the link between macrophage polarization and cellular metabolism suggests a potential therapeutic strategy by modulating the macrophage metabolic state. There are several therapeutic strategies commonly used to target macrophages such as depletion, proliferation, inflammation and gene silencing.

5.1 Macrophage depletion

It was shown that macrophages could be depleted *in vivo* by inducing apoptosis following accumulation of toxic particles [93]. Interesting, it was shown that by depletion of pro-inflammatory macrophages resulted in normalizing insulin sensitivity in IR obese mice [94]. Furthermore, in obese mice, the depletion of visceral adipose tissue macrophages (VATMs) by Intraperitoneal injection of clodronate liposomes, results in improved systemic insulin sensitivity, glucose homeostasis and further blocked high-fat diet-induced weight gain [95, 96]. Consequently, depletion of VATMs also resulted in prevention of CLS in WAT and a low level of blood TNF-α [96]. However, liposomes treatment as a therapy is prone to degradation and significant risks of potential off-target effects. An alternative approach is altering macrophage proliferation, such as using a nanoparticle-based delivery of simvastatin, which may provide therapeutic benefit for atherosclerosis [97]. However, as previously mentioned, in obesity, macrophages are recruited from circulating monocytes, so reducing proliferation may not provide therapeutic benefit for IR [98].

5.2 Biological therapeutics

There are several orally active synthetic ligands for PPARɣ which are used to treat IR in patients with T2D. It has been shown *in vivo* that pioglitazone, belonging to the chemical class thiazolidinediones, reduces LPS induced TLR2 and TLR4 expression on peritoneal macrophages. Whilst *in vitro*, pioglitazone reduces the synthesis and gene expression of TLR2, TLR4, IL-1β, TNF-α, IL-6 and MCP-1 in human blood monocytes [99]. However, the use of thiazolidinediones like pioglitazone in clinical studies to treat T2D has resulted in increased cardiovascular events and death [100].

Clinical studies have shown that anti-inflammatories are efficacious in patients with systemic IR. Members of the interferon family have been used to suppress the release of pro-inflammatory cytokines, however, the use of type 1 interferons, as well as other anti-inflammatory strategies, is associated with cell toxicity in long-term use. Recently studies using interferon tau (IFNT), an alternative member of the type 1 interferon family, in mice with diet-induced obesity show enhanced insulin sensitivity when compared to untreated mice. There was also a significant decrease in secretion of pro-inflammatory cytokines and increased M2 macrophages in AT, suggesting IFNT as a novel bio-therapeutic agent for treating obesity-associated disorders [101].

Interestingly, yeast-derived β-glucans (Y-BGs) have been shown to be beneficial in models for obesity. In obese humans, Y-GBs administered orally increased AT

expression of anti-inflammatory cytokine IL-10 and serum IL-10 [102]. In addition, macrophages uptake of Y-GBs increased reactive oxygen species (ROS) formation, phagosomal maturation and induction of autophagy [103].

5.3 RNA interference

Another attractive therapeutic approach in targeting macrophage polarisation would be to use RNA interference (RNAi), which reduces gene expression. This approach could target the inflammatory mediators such as TNF-α, IL-6 and IL-1β [98]. Indeed, it was shown that intraperitoneal (i.p.) administration of small interfering RNA (siRNA) selectively silenced genes such as TNF-α in epididymal ATM of obese mice and improved glucose tolerance [7]. Additionally, it was shown that i.p. administration of a rabies virus glycoprotein-derived acetylcholine receptor-binding peptide delivers siRNA into ATM and peritoneal macrophages in HFD mice. This resulted in inhibition of ATM infiltration and reduced pro-inflammatory cytokines, thus improving glucose tolerance and insulin sensitivity [104].

5.4 Metabolic reprogramming

As stated previously, macrophage phenotypes have distinct metabolism pathways. Therefore, altering the metabolic state of macrophages provides a potential therapeutic approach to metabolic disorders. Indeed, the strong link between macrophage polarization and cellular metabolism makes altering the metabolic state of the cells an attractive therapeutic prospect. To prove this principle, inducing oxidative metabolism in M1 macrophages has been shown to shift the phenotype to an M2 profile [105], while blocking oxidative metabolism in macrophages inhibits the M2 phenotype and drives the M1 macrophage phenotype. Furthermore, it was shown that by driving macrophage metabolism with glucose, insulin and fatty acids resulted in an increased pro-inflammatory ATM phenotype in obese mice [32].

Modulation of the metabolic pathways in macrophages has been studied extensively in recent years to assess the extent to which inflammatory status can be influenced by the metabolic profile of the cells. Glucose transporter (GLUT)-1 is upregulated in macrophages localised to the CLS in inflamed obese AT. *In vitro* studies show that overexpression of GLUT1 increases glucose uptake in the cells and induces release of pro-inflammatory cytokines, linking the metabolic phenotype with the inflammatory function of the cells [106]. Furthermore, knockout of fatty acid transporter protein (FATP)-1 expression, which is elevated in M2 macrophages, is associated with priming of macrophages towards an M1 phenotype, upregulating expression of NOS1 [107]. Further pathways of current interest to therapeutically target include Notch, carbohydrate kinase-like protein (CARKL), mammalian target of rapamycin (mTOR), IL-4 and IL-10, all of which show intricate links between the metabolic and inflammatory pathways in macrophages [108–112].

6. Conclusions

Obesity has long been considered a low-grade systemic inflammatory condition, which appears to be mediated largely through the prominent populations of ATM. Alterations in environmental cues, including changes in metabolites, the microbiota and inflammatory stimuli act to influence the ATM, coordinating the recruitment of pro-inflammatory monocytes and altering the metabolic state of ATM. While in lean individuals the resident ATM function to clear dead adipocytes and sequester excess lipids from the AT to maintain homeostasis within the AT,

the recruited inflammatory ATM release pro-inflammatory cytokines to induce inflammation within the AT and are involved in the pathogenic remodelling of the AT. This state of inflammation within the ATM is largely associated with IR and metabolic dysfunction through interference with insulin signalling pathways. Macrophages are heterogenous and extremely plastic and as such it has historically been difficult to define subsets. With the use of transcriptional and metabolic profiling it is now becoming possible to appraise the full role of ATM in obesity. This knowledge will aid the search for novel therapeutics targeting the metabolic capacity and inflammatory potential of ATM, restoring the homeostatic functions of resident lean ATM, to modulate obesity.

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Conflict of interest

The authors declare no conflict of interest.

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