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Pharmacological targets of metabolism in disease: Opportunities from macrophages

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ABSTRACT

From advances in the knowledge of the immune system, it is emerging that the specialized functions displayed by macrophages during the course of an immune response are supported by specific and dynamically-connected metabolic programs. The study of immunometabolism is demonstrating that metabolic adaptations play a critical role in modulating inflammation and, conversely, inflammation deeply influences the acquisition of specific metabolic settings.This strict connection has been proven to be crucial for the execution of defined immune functional programs and it is now under investigation with respect to several human disorders, such as diabetes, sepsis, cancer, and autoimmunity. The abnormal remodelling of the metabolic pathways in macrophages is now emerging as both marker of disease and potential target of therapeutic intervention. By focusing on key pathological conditions, namely obesity and diabetes, rheumatoid arthritis, atherosclerosis and cancer, we will review the metabolic targets suitable for therapeutic intervention in macrophages. In addition, we will discuss the major obstacles and challenges related to the development of therapeutic strategies for a pharmacological targeting of macrophage's metabolism.

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Abbreviations: 15(S)-HETE, 15-hydroxyeicosatetraenoic acid; 15-LOX-2, 15-lipoxygenase-2; 2-OG, 2-oxoglutarate; AcCoA, acetyl-CoA; ACLY, ATP-Citrate lyase; ACOD1, aconitate decarboxylase 1; ACPA, anti-citrullinated protein/peptide antibody; ATM, adipose tissue macrophage; ANGPTL4, angiopoietin-like 4; AP1, activator protein 1; ARG1, arginase 1; bDMARDs, biological Disease-modifying Antirheumatic Drugs; BMDMs, bone marrow-derived macrophages; CAN, canagliflozin; CARKL, carbohydrate kinase-like protein; CCL2, CC chemokine ligand 2; CCR2, chemokine receptor 2; CD206, mannose receptor; CIC, citrate carrier; CLSs, crown-like structures; COX, Cyclooxygenase; CPT, carnitine palmitoyltransferase; CSF1, Colony stimulation factor 1; DM-2-OG, dimethyl-2-oxoglutarate; EAE, experimental autoimmune encephalomyelitis; E-FABP, epidermal fatty acid binding protein; EGF, endothelial growth factor; F2,6BP, fructose-6-phosphate to fructose-2,6-bis-phosphate; FAO, fatty acid oxidation; FAS, fatty acid synthesis; FATP1, fatty acid transport protein 1; FIZZ1, found in inflammatory zone 1; FOXO3, Forkhead box O3; G6PDH, glucose 6 phosphate dehydrogenase; GATA3, GATA binding protein 3; GLUT, glucose transporter; GS, glutamine synthetase; HDCA, histone deacetylase; HIF1α, hypoxia induced factor 1 alpha; HK2, hexokinase 2; JAK, janus kinases; IDH, isocitrate dehydrogenase; IDO, indoleamine 2,3 dioxygenase; IFN-β, interferon beta; IFN-γ, interferon gamma; IGF-1, insulin-like growth factor 1; IKK, inhibitor of NF-κB kinase; iNOS, inducible nitric oxide synthase; IRF4, IFN-γ regulatory factor 4; IRF5, interferon regulatory factor 5; IRG1, immune-responsive gene 1 protein; JNKs, c-Jun N-terminal kinases; LAL, lysosomal acid lipase; LDH, lactate dehydrogenase; LDL, low density lipoproteins; LDs, lipid droplets; LPS, lipopolysaccharide; LRP5, LDL receptor-related protein 5; LXRs, liver X receptors; MAPK8, mitogen-activated protein kinase 8; MCP-1, macrophage chemoattractant protein 1; MDH, malate dehydrogenase; MHC-II, major histocompatibility complex class II receptor; MIF, migration inhibitory factor; MMPs, metalloproteases; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; NF-κB, nuclear factor kappa-light-chain enhancer of B-cell; NK, natural killer; NO, nitric oxide; NPs, nanoparticles; NRF2, nuclear factor erythroid 2-related factor 2; ODC, ornithine decarboxylase; PDGF, platelet-derived growth factor; PDH, pyruvate dehydrogenase; PD-L1, Programmed death-ligand 1; PDK4, pyruvate dehydrogenase kinase 4; PFK, phosphofructokinase; PFK-1, phosphofructokinase-1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PGE2, prostaglandin E2; PHDs, prolyl hydroxylases; PI3Ks, phosphatidylinositol 3 kinases; PK, pyruvate kinase; PKM2, pyruvate kinase M2; PLGA, poly(lactic-co-glycolic acid); PPAR, peroxisome proliferator-activated receptor; PPARγ, peroxisome proliferator-activated receptor gamma; PPP, pentose phosphate pathway; PRRs, pattern recognition receptors; RA, rheumatoid arthritis; RET, reverse electron transport; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SGLT2, sodium-glucose transporter protein 2; SLC25A1, solute carrier family 25 member 1; SOCS1, suppressor of cytokine signalling 1; STAT, signal transducer and activator of transcription; T2D, type 2 diabetes; TAMs, tumour associated macrophages; TCA, Tricarboxylic acid cycle; TCR, T cell receptor; TGF-β, transforming growth factor beta; TLRs, Toll-like receptors; TME, tumour microenvironment; TNF-α, tumour necrosis factor alpha; TRAIL, TNF-related apoptosis inducing ligand; TSC, tuberous sclerosis complex; UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; UQ, ubiquinone; UQH2, ubiquinol; VSIG4, V-set Ig domain-containing 4; VEGF, vascular endothelial growth factor; VLDL, very low density lipoproteins..

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1. Introduction

The immune system relies on the activity of cells specialized to respond rapidly to "danger" signals, such as pathogens or inflammatory stimuli. Among these cell types, macrophages play a pivotal role in sustaining the inflammatory response but also in promoting tissue homeostasis regeneration after injury. Macrophages have been known for a long time to undergo deep metabolic changes during activation (Newsholme, Costa Rosa, Newsholme, & Curi, 1996). Particular attention has been devoted to the respiratory burst associated to phagocytosis and the metabolic changes linked to production of reactive oxygen species (ROS) and recycling of NADPH and glutathione (Newsholme et al., 1996). Generally speaking, it is known that cells can tune their metabolism to adjust to alterations in nutrient levels, oxygen concentrations and signals deriving from growth factor, in order to maintain existing functions or acquiring new ones in the rapidly changing environmental conditions. During activation, macrophages undergo profound metabolic changes that are fundamental for the acquisition of their specific functional programs. Indeed, these cells are known to acquire specific metabolic profiles depending on their task. The relationship between the metabolic and the functional profiles is very specific though flexible. Indeed, pharmacological or genetic targeting of key enzymatic activities demonstrates that the associated function of macrophages can be blocked or rewired. Additionally, in inflammatory diseases, macrophages are characterized by specific metabolic shifts that impact on disease progression (Mazzone, Menga, & Castegna, 2018). On this basis, macrophage targeting by modulating key metabolic checkpoints at the intersection of different functional programs may represent a promising therapeutic strategy to treat pathological inflammation. To do so, it is necessary to define and obtain deep insights into the key metabolic mechanisms underpinning macrophage function. The aim of this review is to define the metabolic signatures of macrophages in physiological conditions as well as in selected pathologies, and to highlight possible pharmacological strategies to rewire their metabolism in order to acquire the desired homeostatic function, with particular attention to the cell-specific drug delivery.

2. Origin and function of macrophages

The origin of macrophages is dual: they can either terminally differentiate within a specific tissue from blood-derived monocytes (Hashimoto et al., 2013) or belong to the pool of resident tissue macrophages that are established during embryonic development. The latter retain self-renewal potential (Hashimoto et al., 2013) and persist into adulthood independently of blood monocyte input in the steady state (Epelman, Lavine, & Randolph, 2014). Perturbation of tissue homeostasis through the release of pro-inflammatory chemokines triggers migration of bone-marrow derived circulating monocytes to the site of inflammation, where they differentiate into macrophages, to sustain immunity and resolution of inflammation and tissue remodelling (Ley, Laudanna, Cybulsky, & Nourshargh, 2007). Additionally, tissue resident macrophages participate in the physiological tissue cellular turnover with the removal of apoptotic cells, through the process of efferocytosis (Fadok et al., 1998; Han & Ravichandran, 2011; Voll et al., 1997). Due to their immune surveillance role, macrophages sense different stimuli and respond with complex mechanisms of activation that can be recapitulated *in vitro* by the pro-inflammatory M1 or classical (Nathan, 1983; Pace, Russell, Schreiber, Altman, & Katz, 1983) and the anti-inflammatory M2 or alternatively activation (Doyle et al., 1994; Stein, 1992).

Pro-inflammatory macrophages are involved in killing pathogens and triggering initiation of adaptive response by interaction with T lymphocytes. Classical M1 polarization occurs through stimulation by microbial components, such as the lipopolysaccharide (LPS) and other Toll-like receptors (TLRs) ligands, or by cytokines secreted by T helper-1 (Th-1) lymphocytes, such as tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). The polarization program occurs through activation and nuclear translocation of specific transcription factors, such as nuclear factor kappa-light-chain enhancer of B-cell (NF-κB) (Chen et al., 1995; Chen, Parent, & Maniatis, 1996), the signal transducer and activator of transcription (STAT) 1 and 3 (Bode, Ehlting, & Häussinger, 2012; Darnell, Kerr, & Stark, 1994; Shuai et al., 1993), the IFN-γ regulatory factor 4 (IRF4) (Huang et al., 2016), the Hypoxia induced factor 1 alpha (HIF1α) and the activator protein 1 (AP1) (von Knethen, Callsen, & Brüne, 1999) (Fig. 1). This transcriptional activation leads to the expression of specific cellular markers, such as CD80, CD86, major histocompatibility complex class II receptor (MHC-II), together with cyclooxygenase 2 (COX-2), and inducible nitric oxide synthase (iNOS). This is accompanied by the release of pro-inflammatory cytokines, such as TNF-α, IL1-β, IL-6, IL-12 and IL-23, and the activation of the Th-1 responses (extensively reviewed in Martinez & Gordon, 2014; Mosser & Edwards, 2008).

Alternative or M2 macrophages are generally characterized by an anti-inflammatory gene expression profile, which favours inflammation resolution and tissue repair. M2 macrophages are induced by interleukin 4 (IL-4) or interleukin 13 (IL-13), which are secreted by innate and adaptive immune cells, such T helper-2 (Th-2) lymphocytes, mast cells and basophils (Doyle et al., 1994; Stein, 1992). Specific markers and effectors associated to this programming are STAT6, GATA binding protein 3 (GATA3), suppressor of cytokine signalling 1 (SOCS1), the peroxisome proliferator-activated receptor gamma (PPARγ), found in inflammatory zone 1 (FIZZ1), CD163 and CD36 (Fig. 1) (Murray, 2017; Viola, Munari, Sánchez-Rodríguez, Scolaro, & Castegna, 2019). The typical markers associated to the M2 profile are the mannose receptor (CD206), the decoy receptor IL-1R as well as the IL-1R antagonist. Based on this programming, these cells accomplish the task of resolving inflammation, aiding the healing and repair of the tissue. This occurs through the release of pro-fibrotic factors, such as transforming growth factor beta (TGF-β) and insulin-like growth factor 1 (IGF-1) (Mantovani, Biswas, Galdiero, Sica, & Locati, 2013). The expression and activity of metalloproteases (MMPs) and arginase 1 (ARG1) are increased (de Groot & Pienta, 2018), to favour tissue remodelling and production of polyamines and collagen (Mantovani et al., 2013). Other important functions of M2 macrophages are angiogenesis and lymphangiogenesis, which occur through vascular endothelial growth factor (VEGF)-A, endothelial growth factor (EGF), platelet-derived

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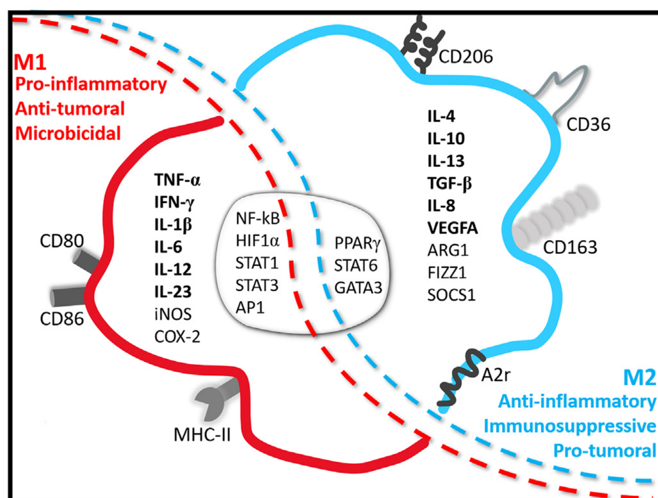

Fig. 1. Pathways associated to M1 or M2 macrophages. Proinflammatory stimuli activate transcription factors, such as NF-kB, HIF1α, STAT1, STAT3 leading to the M1-like inflammatory phenotype, with the expression of markers like iNOS, COX-2, CD80, CD86, and MHC-II and the release of IL-1β, TNF-α, IFN-γ, IL-6, IL-12, and IL-23. The M2-like anti-inflammatory phenotype is characterized by the expression of CD206, ARG1, SOCS1, FIZZ1, adenosine receptor (A2R), and by the production of cytokines such as TGF-β, IL-10, IL-4, IL-13, IL-8, and VEGFA as a consequence of the transcription factors PPARγ, STAT6 and GATA3 activation. Inducers are indicated in bold. AP1, activator protein 1; ARG1, Arginase 1; COX2, cicloxygenase 2; FIZZ1, Found in inflammatory zone 1; iNOS, inducible Nitric Oxide Synthase; GATA3, GATA binding protein 3; HIF1α, Hypoxia-inducible factor 1-alpha; IFN-γ, Interferon gamma; MHC-II, major histocompatibility complex class 2; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPARγ, Peroxisome proliferator-activated receptor gamma; SOCS1, Suppressor of cytokine signaling 1; STAT, Signal transducer and activator of transcription; TNF-α, Tumor necrosis factor alpha; TGF-β, transforming growth factor beta; VEGFA, Vascular endothelial growth factor A.

growth factor (PDGF), and IL-8 release (Corliss, Azimi, Munson, Peirce, & Murfee, 2016).

M2 alternative activation can produce specific functional responses tailored to the specific tasks. For this reason, a more detailed subtype classification has been proposed, depending on the applied stimulus. The M2a subtype is the one induced by IL-4/IL-13. The M2b subtype is induced by stimulation with immune complexes and TLR ligands or by IL-1R agonists, and is thought to be involved in the regulation of both immune and inflammatory reactions, as it produces both pro- and anti-inflammatory cytokines, such as IL-10, IL-1β, and TNF-α (Rőszer, 2015). The M2c subtype, induced by IL-10 or glucocorticoids, is mainly involved in the anti-inflammatory function. The M2d subtype corresponds to the macrophages present in the tumour microenvironment (TME), namely the tumour associated macrophages (TAMs), which will be extensively described in Section 4.4.

3. Metabolic features of M1 and M2 macrophages

The metabolism of immune cells acquires peculiar features, described below, to respond to different microenvironments. The main features of M1 and M2 macrophages are summarized in Table 1.

Glucose utilization. The diversity of the metabolic assets found in M1 and M2 macrophages are evident in many central pathways of cellular metabolism, such as those involving glucose utilization. Glycolysis is a series of reactions converting glucose to pyruvate. It not only provides energy in the form of 2 molecules of ATP but also many intermediates fundamental for anabolic processes (Lunt & Vander Heiden, 2011).

Mitogenic stimulations drive quiescent macrophages into the cell cycle, sustaining glycolysis and glutaminolysis for cell growth (Cairns, Harris, & Mak, 2011). This suggests a role for the cytosolic myelocytomatosis oncogene (c-Myc) transcription factor in this process, since it is known to both sustain cell cycle entry and drive the upregulation of glucose and glutamine catabolism upon mitogenic

Table 1
Metabolic features of M1 and M2 macrophages.

M1		M2
Glycolysis is strongly upregulated The non-oxidative branch of PPP is downregulated Lactate accumulation	Glucose utilization	Glycolytic flux is slowered PFKFB1 isoform is highly expressed compared to PFKFB3 The non-oxidative branch of PPP is upregulated
TCA cycle is broken Intermediates of the TCA cycle (citrate, succinate and itaconate) accumulation SDH is inhibited	TCA and OXPHOS	TCA cycle is intact OXPHOS is active 2-OG accumulation
iNOS is upregulated NO and citrulline synthesis Increased glutamine anaplerotic flux	Aminoacid metabolism	GS and ARG1 are upregulated Increased glutamine synthesis Ornithine and polyamine synthesis
FAS is active	Lipid metabolism	FAO is active

2-OG, 2-oxoglutarate; ARG1, arginase 1; FAS, fatty acid synthesis; FAO, fatty acid oxidation; GS, glutamine synthetase; iNOS, inducible nitric oxide synthase; NO, nitric oxide; OXPHOS, oxidative phosphorylation; PFKFB1, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3; PPP, pentose phosphate pathway; SDH, Succinate dehydrogenase; TCA, tricarboxylic acid.

stimulation (Cairns et al., 2011). This is not the case for M1 macrophages. Indeed, pro-inflammatory stimulations support glycolysis and the pentose phosphate pathway (PPP) to sustain macrophage function without enhancing proliferation, which would be bioenergetically costly. A switch between c-Myc and HIF1α activation takes place in M1 macrophages, with the activation of a transcriptional program that ensures (via HIF1α) the maintenance of metabolic capacity to support their pro-inflammatory functions, without wasting the energy required for cell proliferation (Liu et al., 2016; Palazon, Goldrath, Nizet, & Johnson, 2014). Interestingly, c-Myc transcriptional programs are executed in M2-macrophages (Pello et al., 2012) and its inhibition impairs TAM maturation and pro-tumoral activities (Pello et al., 2012)

Glycolysis is strongly upregulated in M1 macrophages (Fig. 2) and crucial for their function, as glycolysis inhibition hampers phagocytosis, and reduces ROS and proinflammatory cytokine release (Freemerman et al., 2014; Michl, 1976; Pavlou, Wang, Xu, & Chen, 2017). Different M1 signalling programs concur to the metabolic preference for glycolysis, such as TLR/NF-κB (van Uden, Kenneth, & Rocha, 2008), triggered by pathogen recognition through pattern recognition receptors (PRRs) or pro-inflammatory cytokines, and AKT/mTOR complex (Cheng et al., 2014; Joshi, Singh, Zulcic, & Durden, 2014), triggered by growth factors and pathogen-sensing receptors (Cheng et al., 2014; Kelley et al., 1999; Vergadi, Ieronymaki, Lyroni, Vaporidi, & Tsatsanis, 2017), both regulating HIF1α transcription factor (Wang et al., 2017). In the case of AKT/mTOR, it should be noticed that this axis does not seem to convey a linear signal once activated. Indeed, its activation integrates different stimuli of both intracellular and extracellular origin and balances their effect to allow the cell to adapt to diverse conditions by promoting diverse basic biological processes. For instance mTORC1 controls inflammatory modulators, regulating NF-κB activity and IL-10, TGF-β, and PD-L1 expression (Katholnig, Linke, Pham, Hengstschläger, & Weichhart, 2013). However, AKT and mTORC1 signalling also drives glucose metabolism to sustain IL-4 mediated M2 activation of macrophages, hence suggesting that alternative activation might be mediated by mTORC1 in a context-dependent manner (Covarrubias, Aksoylar, & Horng, 2015). Indeed, that loss of tuberous sclerosis complex (TSC) 1, a mTORC1 inhibitor, allows enhanced M1 and diminished M2 activation (Byles et al., 2013). Similarly, AKT kinases seem to regulate macrophage polarization in an isoform-specific manner. Indeed AKT1 ablation promotes the M1 profile, whereas AKT2 ablation has opposite effects, resulting in amplification of M2 responses (Arranz et al., 2012).

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As mentioned before, the regulation of glycolysis in M1 macrophages is related to HIF1α activation, which not only promotes the expression of inflammatory mediators (Rius et al., 2008; van Uden et al., 2008; Wang, Ma, Zhao, & Zhu, 2017) but also mediates the expression of genes encoding for glycolytic enzymes (i.e. Hexokinase 2, HK2) and the glucose transporter GLUT1 (Freemerman et al., 2014). As a signal promoting adaptation to hypoxia, HIF1α supports anaerobic glycolysis by upregulating lactate dehydrogenase (LDH) (Semenza et al., 1996), which produces lactate from pyruvate, and pyruvate dehydrogenase kinase (Kim, Tchernyshyov, Semenza, & Dang, 2006; Palsson-Mcdermott et al., 2015), that prevents pyruvate channelling into the TCA cycle by inhibiting pyruvate dehydrogenase (PDH) (Fig. 2). LDH activity also supports NADH oxidation to NAD^+ necessary to support glycolytic flux. Glycolysis is furtherly enhanced by the expression of the inducible 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and the pyruvate kinase M2 (PKM2). PFKFB3 converts fructose-6-phosphate to fructose-2,6-bis-phosphate (F2,6BP), which allosterically activates phosphofructokinase-1 (PFK-1), stimulating glycolysis (Palsson-Mcdermott et al., 2015). In M1 macrophages PKM2 is present in two different forms, as a dimer and as a tetramer. The former is less active and it translocates to the nucleus to potentiate HIF1α transcriptional activity (Mazurek, Boschek, Hugo, & Eigenbrodt, 2005; Palsson-Mcdermott et al., 2015), whereas the latter is highly active and is located in the cytosol, supporting glycolysis (Palsson-Mcdermott et al., 2015).

In M1 macrophages the oxidative steps of the PPP are upregulated, thereby resulting in ribose-5-phosphate synthesis and $NADP^+$ reduction to NADPH (Tannahill et al., 2013). The flux through the oxidative steps of PPP is crucial for function (Fig. 2). Impairment of the oxidative branch of the PPP depotentiates the pro-inflammatory function of M1 macrophages (Viola et al., 2019). In line with this finding, in macrophages overexpression of sedoheptulose kinase, also known as carbohydrate kinase-like protein (CARKL) involved in the conversion of sedoheptulose into sedoheptulose-7-phosphate, results in defective M1 polarization and dampened inflammatory response (Baardman et al., 2018; Haschemi et al., 2012). The reason for the role of the PPP oxidative branch in sustaining M1 function relies on NADPH production. The reduced form of $NADP^+$ is fundamental to support macrophage function in different ways: (I) it sustains NADPH oxidase activity, which, among other roles is the main ROS generator against pathogens and plays a crucial role in macrophage responses (Jackson, 1995; Yi et al., 2012); (II) it concurs to the endogenous antioxidant defence by favouring reduction of oxidized glutathione (Winkler, DeSantis, & Solomon, 1986); (III) it is a necessary molecule for fatty acid synthesis (FAS), which is another peculiar metabolic feature of M1 macrophages that, among other things, is required to synthesize prostaglandins.

In M2 macrophages the glycolytic metabolism plays a minor role (Wang et al., 2018), whereas OXPHOS appears to be crucial. In the absence of glucose, energy production can be sustained by glutamine channelling into the TCA cycle (Wang et al., 2018). Specific control points regulate both glycolysis and PPP. In M2 macrophages, the PFKFB1 isoform is highly expressed compared to PFKFB3, resulting in a reduction of the glycolytic rate through a much faster conversion of fructose-2-6-phosphate to fructose-6-phosphate (Mills & O'Neill, 2016; Rodríguez-Prados et al., 2010; Takeda et al., 2011). Furthermore, CARKL is upregulated and this allows the products of the oxidative steps of PPP to be channelled into the non-oxidative steps of PPP

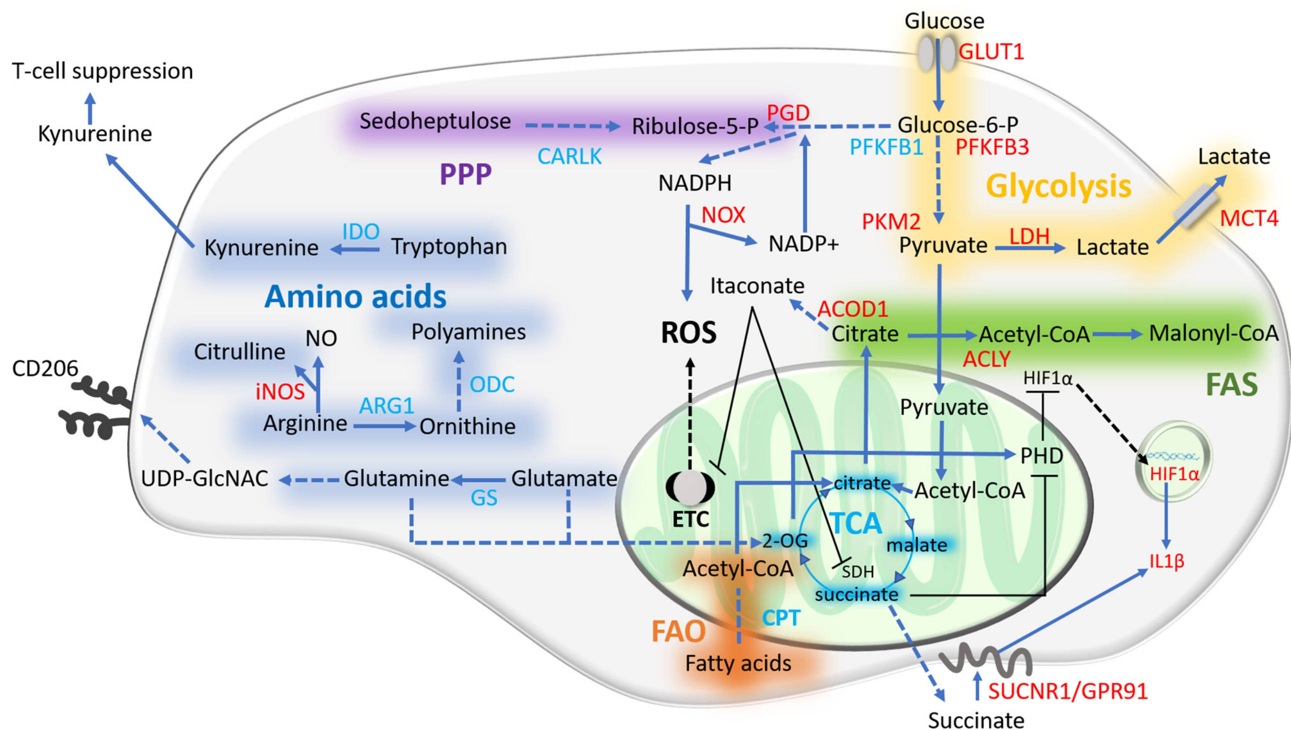

Fig. 2. Metabolic signatures of M1 and M2 macrophages. The pro-inflammatory programming (M1, in red) is characterized by the increased flux through glycolysis, the oxidative steps of the pentose-phosphate pathway, and fatty acid synthesis. Moreover, M1 cells display TCA cycle interruption, ROS formation and citrate efflux from mitochondria, which guides NADPH synthesis, and succinate efflux, that stabilizes HIF-1α. Itaconate, produced from citrate, has antibacterial function. Arginine is channelled into NO production. Anti-inflammatory macrophages (M2, in blue) display enhanced OXPHOS, fatty acid oxidation, glutaminolysis, tryptophan catabolism with release of kynurenine, and synthesis of polyamines from arginine. 2-oxoglutarate acid produced by glutaminolysis inhibits PHD, leading to HIF1α destabilization, and promotes a M2 phenotype through epigenetic reprogramming. 2-OG, 2-oxoglutarate acid; ACLY, ATP citrate lyase; ARG1, arginase1; CARLK, carbohydrate kinase-like protein; CPT, carnitine palmitoyl transferase; ETC, Electron Transport Chain; FAO, Fatty acid oxidation; FAS, Fatty acid synthesis; GS, glutamine synthetase; GLUT1, glucose transporter 1; IDH, Isocitrate dehydrogenase; IDO, indoleamine dioxygenase; iNOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; NO, nitric oxide; NOX, NADPH oxidase; ODC, ornithine decarboxylase; PGD, phosphogluconate dehydrogenase; PHD, prolyl hydroxylase; PPP, Pentose phosphate pathway; PFKFB3, phosphofructokinase fructose 2,6-biphosphatase B3; PKM2, pyruvate kinase M2; ROS, Reactive Oxygen Species; SDH, Succinate dehydrogenase; SUCNR1, succinate receptor 1; TCA, Tricarboxylic acid cycle or Krebs cycle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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(Haschemi et al., 2012) (Fig. 2). NADPH synthesis is not crucial for M2 cell functions, whereas ribose-5P production has to be sustained for nucleotide and uridine diphosphate N-acetylglucosamine (UDP-GlcNAC) synthesis (Haschemi et al., 2012). UDP-GlcNAC is required for N-glycosylation, which is essential for the modification of different cell surface protein (i.e. CD206) abundantly expressed in M2 macrophages (Tannahill et al., 2013).

The TCA cycle. The Tricarboxylic acid cycle (TCA) is another metabolic control point for M1/M2 polarization. As said above, M2 macrophages display a more flexible metabolism involving both glycolysis and OXPHOS, due to the high ATP demand of these cells to support biosynthetic processes, such as receptor glycosylation (Jha et al., 2015). On the contrary, M1 macrophages mainly rely on glycolysis rather than OXPHOS for ATP production. Indeed, M1 metabolism is associated to cytosolic accumulation of intermediates of the TCA cycle, such as citrate, succinate and itaconate, which are drained from mitochondria due to the so-called TCA cycle break (Fig. 2). At variance with M1 macrophages, under M2 stimuli macrophages increase 2-oxoglutarate (2-OG) levels, which also play important metabolic and signalling roles to sustain M2 polarization.

Citrate is produced in the TCA cycle by condensation of oxaloacetate and acetyl-CoA (AcCoA), and then converted to isocitrate and then to 2-OG, through the activity of mitochondrial isocitrate dehydrogenase (IDH). However, cytosolic demand of citrate is high for different reasons. Citrate is converted into AcCoA and oxaloacetate by ATP-citrate lyase (ACLY) (Palmieri, 2004). Oxaloacetate is converted into malate by malate dehydrogenase (MDH) and this intermediate can follow two different routes. It is recycled back into the TCA cycle, process that is facilitated by the transport activity of the mitochondrial citrate carrier (CIC), also known as solute carrier family 25 member 1 (SLC25A1), which exports citrate from mitochondria in exchange with malate (Infantino, Iacobazzi, Menga, Avantaggiati, & Palmieri, 2014; Palmieri, 2004). Additionally, malate is converted in pyruvate through the NADPH producing-malic enzyme (Newsholme, Gordon, & Newsholme, 1987) and pyruvate can enter mitochondria. AcCoA enrichment is fundamental to sustain fatty acid synthesis, and to regulate protein and histone acetylation (Pietrocola, Galluzzi, Bravo-San Pedro, Madeo, & Kroemer, 2015). Citrate itself modulates the cytosolic metabolism, by positively regulating fatty acid synthesis (Martin & Vagelos, 1962), and gluconeogenesis. Concomitantly, it inhibits directly phosphofructokinase (PFK) 1 and 2 and, indirectly, pyruvate kinase (PK) (Yalcin, Telang, Clem, & Chesney, 2009) leading to reduction of the glycolytic flux. Citrate metabolism is central in M1 macrophages (Fig. 2). LPS, TNF-α or IFN-γ stimulation induces upregulation of the mitochondrial citrate carrier CIC (Infantino et al., 2014), as well as downregulation of IDH (Tannahill et al., 2013). Increased cytosolic flux of citrate from mitochondria is required for NO, ROS and prostaglandin E2 (PGE2) production (Infantino et al., 2011, 2014; Infantino, Iacobazzi, Palmieri, & Menga, 2013), suggesting that citrate not only supports fatty acid synthesis for the production of inflammatory mediators, but also contributes to the reduction of $NADP^+$ to NADPH. CIC is known to be regulated by acetylation, which increases the transport activity of CIC (Palmieri et al., 2015). M1 macrophages display a higher level of CIC acetylation in glucose limiting conditions (Palmieri et al., 2015). By increasing the efflux of citrate, macrophages can rely on the citrate to 2-OG conversion catalyzed by the $NADP^+$-dependent IDH1 as alternative routes to produce NADPH when glucose is limiting (Palmieri et al., 2015). Besides CIC, other proteins are known to be regulated by acetylation, such as NF-κB (Greene & Chen, 2004), IL-6 and IL-10 (Hu et al., 2017; Wang, Wang, Rabinovitch, & Tabas, 2014). AcCoA is provided by ACLY, which is also upregulated in M1 macrophages (Infantino et al., 2013) whereas it is not required for M2 polarization (Namgaladze et al., 2018), although it is known to mediate the expression of some M2 markers due to histone acetylation (Covarrubias et al., 2016). Conversely, deacetylation of putative enhancers of IL-4-induced M2 genes interferes with M2 polarization (Ivashkiv, 2013; Mullican et al., 2011).

Another way citrate escapes the TCA cycle is through conversion to itaconate, which takes place in mitochondria due to the LPS-mediated upregulation of aconitate decarboxylase 1 (ACOD1) (Strelko et al., 2011; Sugimoto et al., 2012), previously known as immune-responsive gene 1 protein (IRG1) (Michelucci et al., 2013) (Fig. 2). The role of itaconate is far from being fully understood: it is classically known to display anti-bacterial properties (Berg, Filatova, & Ivanovsky, 2002; Naujoks et al., 2016), but is also involved in immunomodulation, suppression of inflammation and tolerance (Lampropoulou et al., 2016). The significance of itaconate during M1 polarization relies on its ability to induce succinate accumulation through succinate dehydrogenase (SDH) inhibition (Feingold et al., 2012; Warburn & Dickens, 1931) (Fig. 2), which is accompanied to OXPHOS reduction, ROS production and inflammasome activation (Lampropoulou et al., 2016). The underlining mechanism is apparently linked to anti-inflammatory transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) stabilization, which targets genes involved in protecting against stress-induced cell death and oxidative stress (Mills et al., 2018). Itaconate accumulates into macrophages when M2 polarization is impaired, through IRG1 upregulation (Ganta et al., 2017). Furthermore, targeting itaconate accumulation into macrophages skews their phenotype toward the M2 one (Puchalska et al., 2018).

The third control point in the TCA cycle of M1 macrophages is the SDH mediated conversion of succinate into fumarate (Fig. 2). Succinate is the substrate of SDH, also known as Complex II of the mitochondrial respiratory chain. SDH-mediated oxidation of succinate into fumarate is coupled to reduction of ubiquinone (UQ) to ubiquinol (UQH2). In the absence of ATP production high oxidation rates of succinate leads to the so called reverse electron transport (RET), characterized by electrons flux in the opposite direction toward complex I. Succinate accumulation into the cytosol concurs to M1 function by targeting prolyl hydroxylases (PHDs), thus blocking HIF1α degradation even in normoxic conditions (Tannahill et al., 2013) (Fig. 2). This potentiates the hypoxic response in M1 macrophages, which is already activated by the RET-mediated ROS production (Benmoussa, Garaude, & Acín-Pérez, 2018; Mills et al., 2016).

Similarly to citrate, succinate can induce post-translational modification on proteins (Park et al., 2013; Xie et al., 2012). Although this mechanism is much less known than protein acetylation, evidence is emerging with this respect. Succinylation of PKM2 promotes its translocation into the nucleus, where it potentiates HIF1α transcriptional activity (Wang, Wang, Wang, Tall, & Tabas, 2017). Another emerging mechanism of succinate regulatory effects stems from the discovery of succinate receptor SUCNR1/GPR91, a G-protein-coupled cell surface sensor for extracellular succinate (Doyle et al., 1994) expressed in many cell types, and activated in pathological conditions (He et al., 2004; Macaulay et al., 2007; Peti-Peterdi, Kang, & Toma, 2008; Sadagopan et al., 2007; Toma et al., 2008). LPS activates a GPR91-mediated signal transduction that sustains the pro-inflammatory function (Kelly & O'Neill, 2015; Littlewood-Evans et al., 2016) (Fig. 2). This is probably linked to a significant release of succinate from M1 macrophages as well as in pathological conditions associated to inflammation (Kim et al., 2014; Toma et al., 2008), which sustains and amplifies inflammation in an autocrine way. Interestingly, in the experimental autoimmune encephalomyelitis (EAE) murine model, transplanted neural stem cells protect against neuroinflammation through the GPR91-mediated uptake of extracellular succinate (Peruzzotti-Jametti et al., 2018).

2-OG derived from glutaminolysis is known to promote M2 macrophage polarization (Fig. 2). Inhibition of glutaminase 1 (which produces 2-OG) decreases M2 polarization in IL-4-treated mouse bone marrow-derived macrophages (BMDMs). This change is rescued by dimethyl-2-OG (DM-2-OG), a cell-permeable analogous of 2-OG, suggesting that the one generated from glutaminolysis promotes the M2 phenotype. 2-OG is known to favour the M2 phenotype through Jumonji domain containing-3 (Jmjd3) protein-dependent demethylation of histone H3

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lysine-27 (H3K27) at the promoter region of M2-specific marker genes (Liu et al., 2017) and this represents an important mechanism of metabolic control of epigenetics. In line with its key role in sustaining M2 polarization, 2-OG in LPS-stimulated mouse macrophages restricts M1 activation by suppressing IKKβ activation, and this mechanism is regulated by PHD-mediated prolyl hydroxylation of IKKβ (Liu et al., 2017; Takeda et al., 2011). As stated above, 2-OG levels in M1 macrophages are lower due to downregulation of IDH1, which concurs to the higher isocitrate/2-OG ratio of M1 macrophages (Jha et al., 2015) to support citrate escape from mitochondria. From a strictly biochemical point of view, 2-OG sustains the M2 phenotype by supporting OXPHOS and fatty acid oxidation (FAO). Indeed 2-OG feeds the TCA cycle flux, thereby providing the NADH required for OXPHOS. Furthermore, the activity of the ATP-dependent acyl-CoA synthetase leads to mitochondrial AMP accumulation, which would eventually feedback inhibit the enzyme and block FAO. The substrate-level phosphorylation sustained by 2-OG metabolism provides the nucleoside triphosphates that contribute to reduce AMP levels through adenylate kinase, thus preventing FAO inhibition (Rossi, Alexandre, Carignani, & Siliprandi, 1971).

Aminoacid metabolism. Amino acid metabolism represents another control point of macrophage function. In macrophages, arginine metabolism is modified depending on the context (Fig. 2). LPS, TNF-α or IFN-γ induce iNOS expression, that converts arginine into citrulline and nitric oxide (NO), the latter being fundamental to sustain production of anti-microbial reactive species (Schairer, Chouake, Nosanchuk, & Friedman, 2012) (Fig. 2). This pathway is self-sustained by the conversion of citrulline into argininosuccinate, which is a precursor of arginine (Qualls et al., 2012). Recently, NO has been recognized as a major regulator of macrophage metabolism, since its production is responsible for TCA cycle alterations and the loss of mitochondrial electron transport chain (ETC) complexes, similarly to what was previously observed in dendritic cells (Everts et al., 2012). Additionally, NO reroutes pyruvate away from PDH, promoting glutamine anaplerosis. This means that in a NO-rich environment, the molecule could drive the profound metabolic changes described in M1-like macrophages (Palmieri et al., 2020).

M2 stimuli induce expression of arginase 1 (ARG1), that channels arginine into ornithine, a precursor of putrescine, spermidine, and spermine, polyamines involved in tissue repair (Fig. 2). In line with this finding, ornithine decarboxylase (ODC) expression impairs inflammatory and anti-microbial function of M1 macrophages (Hardbower et al., 2017). Furthermore ARG1 activity in macrophages triggers an anti-inflammatory phenotype and reduces T-cell proliferation and cytokine production (Wu & Morris, 1998; Molon et al., 2011).

Tryptophan metabolism is regulated in immune cells by the activity of indoleamine 2,3-dioxygenase (IDO), which converts tryptophan into kynurenine (Fig. 2). IDO expression is sensitive to IFN-γ and TNF-α (Babcock & Carlin, 2000; Robinson, Hale, & Carlin, 2005), but its activity skews macrophages toward a "M2-like" state (Wang et al., 2014). IDO activity consumes tryptophan, thus limiting its availability for T cells (Fig. 2) (O'Neill, Kishton, & Rathmell, 2016; Platten, von Knebel Doeberitz, Oezen, Wick, & Ochs, 2015), which in turn impairs T cell activation (Yue et al., 2015). In addition, kynurenine itself suppresses T cell activation since it can interfere with T cell receptor (TCR) and induce regulatory T cells (T_{reg}) (Stephens et al., 2013).

Glutamine metabolism plays a crucial role in polarizing macrophages and this depends on how the glutamine flux is channelled. In macrophages triggered by IL-10, glutamine synthesis is enhanced through upregulation of glutamine synthetase (GS) (Palmieri et al., 2017) and this is responsible for the acquisition of the M2 polarization features and functions (Fig. 2). The mechanism mediating GS upregulation following IL10 stimulation has not been completely clarified in macrophages. Since GS gene transcription responds to Class O Forkhead Transcription Factor 3 (FOXO3) (Van Der Vos & Coffer, 2012), the nuclear localization of which is regulated by STAT3 (Oh, Yu, Dambuza, Marrero, & Egwuagu, 2012), it is conceivable that the STAT3 axis mediates GS expression following IL10 stimulus. Glutamine supports nucleotide and UDP-GlcNAc synthesis, which is critical for M2 macrophage polarization because it mediates glycosylation of M2 protein markers. Inhibition of N-glycosylation in IL-4-stimulated macrophages impairs the expression of Relmα, CD206, and CD301 with almost no effect on iNOS or M1-specific cytokines (Jha et al., 2015). Glutamine is the main nitrogen donor for UDP-GlcNAc generation.

Since glutamine synthesis promotes "M2-like" features in macrophages, glutaminolysis is expected to be enhanced in a more "M1-like" polarization status. This is not always the case. Through glutamine-dependent anaplerosis, LPS promotes the accumulation of succinate in macrophages, which stabilizes HIF1α, resulting in the acquisition of a M1 phenotype (Tannahill et al., 2013). However, glutamine is the precursor of 2-OG that, as stated above, is important for the engagement of FAO and the epigenetic reprogramming of M2 genes (Liu et al., 2017). Indeed inhibition of glutaminase 1 decreases expression of ARG1 and this phenotype is rescued by dimethyl-OG (DM-OG), a cell-permeable analogous of 2-OG (Liu, Yi, et al., 2017)

Lipid metabolism. Intracellular lipid metabolism includes the processes of lipid degradation and synthesis. Lipids are intracellularly compartmentalized as lipid droplets (LDs), storage organelles formed by a phospholipid monolayer decorated by proteins surrounding a core of di/triacylglycerols and sterol esters. Besides adipocytes, other cells store lipids as LDs, including macrophages and hepatocytes. LDs are now recognized as dynamic organelles, which can modulate metabolism in health and disease. Indeed, the presence of a large number and variety of proteins, including membrane-trafficking GTPases, enzymes of lipid metabolism and proteins associated with the immune system (den Brok, Raaijmakers, Collado-Camps, & Adema, 2018) suggests multiple functions for LDs, most of which are still unknown. LDs can associate with other cellular organelles through membrane contact sites, thereby promoting the communication between organelles and acting as crucial core of cell metabolism (Olzmann & Carvalho, 2019). The physiological role of LDs in the control of storage and release of fatty acids has been well characterized, while the relevance of the stored components of signalling molecules are largely unknown. The role of LDs in immune cells has been mostly characterized in macrophages and polymorphonuclear cells, and more recently in dendritic cells. In macrophages LDs regulate the production of inflammatory mediators and play a role in the proinflammatory amplification loop in sepsis (reviewed in Vallochi et al., 2018). As expected FAS is activated in M1 macrophages and, biochemically, is required for prostaglandin biosynthesis. On the other side, M2 macrophages rely on FAO (also known as β-oxidation) (Vats et al., 2006) and glutamine metabolism (Jha et al., 2015) as a way to sustain the oxidative TCA cycle. Preferentially oxidized macromolecules are triacylglycerol-rich lipoproteins, such as low density and very low density lipoproteins (LDL and VLDL), that are uptaken by the scavenger receptor CD36 and processed in lysosomes by the lysosomal acid lipase (LAL) under the control of STAT6, PPARγ (Kerner & Hoppel, 2000) and its co-activator 1 (PGC1) (Malandrino et al., 2015). Indeed targeting of CD36 or LAL in mice leads to a defective M2 activation (Huang et al., 2014). Carnitine palmitoyl transferase (CPT)-1a is also important for M2 function since it concurs to the transports of long-chain fatty acids to mitochondria. Indeed, a CPT-1a mutant form that is permanently active was found to promote FAO and reduce inflammation (Malandrino et al., 2015), although FAO is unnecessary for M2 polarization (Nomura et al., 2016). In line with studies previously described, 2-OG, which accumulates in M2 macrophages, is known to support FAO (Chawla, Nguyen, & Goh, 2011).

4. Tracing metabolic signatures of macrophages in disease

Increasing evidence on inflammation-related diseases demonstrates that macrophage function plays a significant role in the progression of

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disease. Since specific metabolic programs underline the acquisition of specific macrophage functions, it is conceivable that targeting metabolism might be an effective strategy to revert macrophage function driving pathology. In this section we will focus on different pathologies, in which macrophages are known to play a role, to dissect the role of macrophage metabolism in driving inflammation. Additionally, we will describe evidence on metabolic targets suitable for therapeutic intervention.

4.1. Obesity and diabetes

The rise in obesity worldwide has promoted the diffusion of obesity-related health issues, such as insulin resistance, type 2 diabetes (T2D), coronary artery disease, fatty liver disease, and some types of cancer (Berrington de Gonzalez et al., 2010; Flegal, Graubard, Williamson, & Gail, 2007). Besides the emphasis on embracing healthy dietary and life style habits (Leibel, 2008), the scientific community is now putting a great effort to understand the relationship between obesity and chronic metabolic diseases in which a major key pathogenic role is occupied by the chronic, low-grade inflammation, primarily mediated by innate and adaptive immune cells (Hotamisligil, 2006; Odegaard & Chawla, 2008; Olefsky & Glass, 2010; Shoelson, Lee, & Goldfine, 2006).

It is clearly recognized that the macrophage population resident in adipose tissue and other sites of metabolic regulation plays a role in disease progression, not only through the number of infiltrating cells but also due to their acquired functional state (Appari, Channon, & McNeill, 2018). Identification of the mechanisms altering macrophage biology toward a M1 or M2-like state is crucial to understand the macrophages role in obesity and insulin resistance.

Macrophage recruitment to the adipose tissue from blood monocytes is fundamental to sustain inflammation, although adipose tissue macrophage (ATM) proliferation is emerging as key event in the early stages of obesity and in promoting inflammation (for a review see Russo & Lumeng, 2018). ATMs acquire a CD11c expressing M1-like phenotype, which associates to the typical crown-like structures (CLSs) surrounding the adipocytes (Ferrante, 2007; Gericke, Weyer, Braune, Bechmann, & Eilers, 2015). The M1 inflammatory mediators, such as TNF-α, IL-6, and NO, induce insulin resistance in obese mice (Lumeng, Bodzin, & Saltiel, 2007). Macrophagic FAS has been shown to be fundamental to sustain inflammation. Targeting FAS in macrophages prevents diet-induced insulin resistance, recruitment of macrophages to adipose tissue and chronic inflammation in mice (Wei et al., 2016). Mechanistically, FAS deficiency in mice alters plasma membrane composition and disrupts Rho GTPase trafficking, which is required for cell adhesion, migration and activation (Wei et al., 2016). On the contrary, macrophages in lean adipose tissue display a $CD11c^{-}$ M2-like phenotype, are sparsely distributed and maintain insulin sensitivity by the anti-inflammatory actions of IL-10 and STAT3 activation (Lumeng et al., 2007).

Macrophage polarization toward a M1-like state seems to be mediated by the c-Jun N-terminal kinases (JNKs), also referred as stress activated-kinases, that associate with obesity and insulin resistance. JNKs are activated by fatty acids and interfere with insulin signalling through tyrosine kinase c-Src activation (Holzer et al., 2011). Genetic targeting of JNK in mice protects against insulin resistance and the switch toward a M1-like state (Han et al., 2013). Micro RNA-155 expressed in adipocyte-derived microvesicles from obese mice is also involved in inducing M1 macrophages polarization, leading to chronic inflammation and local insulin resistance (Zhang et al., 2016). Protective mechanisms against ATM M1 polarization and insulin resistance involve STAT6 and PPAR-γ and PPAR-δ in mice (Odegaard et al., 2008; Olefsky & Glass, 2010). Similarly, IL-33 produced by adipose tissue (Wood, Wang, & Trayhurn, 2009) is emerging as a major M2 polarizing cytokine, leading to decreased inflammation and protection against the effects of obesity in mice (Miller et al., 2010).

Modulation of macrophage metabolism is considered a possible therapeutic strategy against the harmful effects of ATMs. A suitable target is GLUT1 (*Slc2a1*), which in macrophages is associated to high glycolytic rate and, under LPS stimulation, enhanced release of inflammatory mediators. Additionally, it is upregulated in adipose tissue and colocalizes with ATMs in rodents (Freemerman et al., 2014). GLUT1 appears to be the ideal pharmacological target, although few studies question the role of its expression modulation in altering inflammation (Nishizawa et al., 2014). This is further substantiated by the finding that myeloid-specific GLUT1 deficient mice are not protected against the development of obesity-associated metabolic dysregulation, although activated BMDMs from $Slc2a1^{M-/-}$ mice display a reduced glycolysis and PPP rate, with an increase of alternative M2-like metabolism and activation marker mannose receptor CD206, also in adipose tissue (Freemerman et al., 2019) (Fig. 3A). An interesting emerging target is the sodium-glucose transporter protein 2 (SGLT2), for which registered inhibitors as hypoglycemic drugs in adults with T2D are available (Vivian, 2014). These drugs inhibit the absorption of glucose in the proximal tubule of the kidney. Among these, canagliflozin (CAN) was also tested for its ability to modulate inflammation both *in vitro* and *in vivo*. CAN significantly reduces inflammation by inhibiting intracellular glucose metabolism and PFK2 expression and promoting autophagy through a AMPK phosphorylation-mediated *in vitro* mechanism (Xu et al., 2018). This suggests that CAN might represent a promising anti-inflammatory drug for acute or chronic inflammatory diseases via independent mechanisms of reduction in glucose uptake.

A second metabolic relevant checkpoint in macrophages for treating this complex disease is fatty acid metabolism. Indeed, in obese and T2D patients the flux through FAO is lower (Fig. 3A). A first metabolic target is CPT-1a, which is highly expressed in human ATMs (Malandrino et al., 2015). Its expression levels positively correlate to FAO rates, which in macrophages reduce inflammation (Malandrino et al., 2015). Its overexpression reduces inflammation of macrophages exposed to palmitate (Malandrino et al., 2015).

Another target associated to fatty acid metabolism is fatty acid transport protein 1 (FATP1). FATP1 genetic ablation in leucocytes of high-fat diet mice induces increased adiposity and insulin resistance, which associates to increased M1 ATMs (Johnson et al., 2016); on the contrary, its *in vitro* overexpression decreases GLUT1 expression and reduces inflammation (Johnson et al., 2016).

Recently, evidence is growing regarding abnormal glutamine metabolism in patients with obesity or diabetes, as they display lower serum levels of glutamine and 2-OG but higher levels of succinate (Cheng et al., 2012; Wahl et al., 2012). These metabolic abnormalities associate with accumulation of M1 macrophages, which display a typical metabolic signature characterized by higher succinate but lower intracellular levels of 2-OG and glutamine (see Section 3). Evidence shows that targeting GPR91 protects mice fed with a high-fat diet from obesity (McCreath et al., 2015) and limits macrophage infiltration in mouse adipose tissue (van Diepen et al., 2017). Conversely, 2-OG supplementation reduces adipocyte inflammation and increases the M2 /M1 ratio of white ATMs (Liu, Gan, Zhang, Ren, & Sun, 2018). Incidentally, GS activity is a metabolic checkpoint for M2 function (Palmieri et al., 2017) and importantly, GS inhibition sensitizes adipocytes to proinflammatory stimuli (Palmieri et al., 2014) and reduces insulin-dependent glucose uptake in microglia (Palmieri, Menga, Lebrun, et al., 2017). These findings indicate that modulation of the succinate/2-OG ratio could represent a valid metabolic strategy to limit the obesity- or diabetes-associated pathology. In this scenario, glutamine metabolism might represent a crucial metabolic checkpoint since it may control the partitioning of the glutamine to succinate versus the glutamine to 2-OG fluxes.

4.2. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic condition associated to damage and loss of function of the joints due to a chronic inflammation. Pain is a prominent symptom of RA and contributes to the disability that

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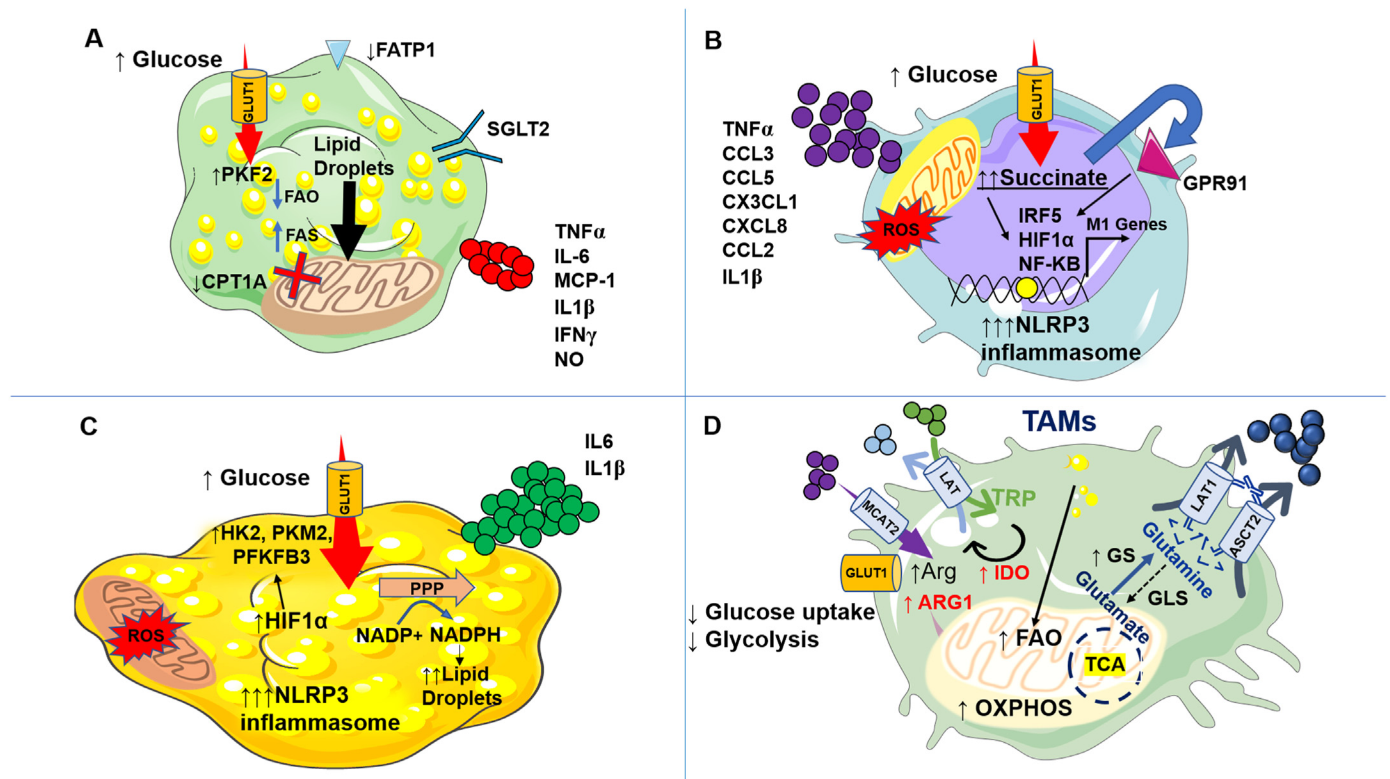

Fig. 3. Metabolic alterations of macrophages in diseases. A) Macrophages in obesity and diabetes display increased glucose uptake through GLUT1 and stimulation of the glycolytic pathway due to PFK2 activation. Fatty acid synthesis is enhanced. On the contrary, fatty acid uptake through FATP1 and CPT1A is markedly impaired, thereby preventing their oxidation and causing accumulation of lipid droplets; B) Macrophages in rheumatoid arthritis are also characterized by high glycolytic flux through GLUT transporters. Succinate drives M1 reprogramming through HIF1α stabilization but also through GPR91 activation; C) In atherosclerosis, macrophages display increased glycolysis and PPP pathway, high levels of mitochondrial ROS and overactivation of the NLRP3 inflammasome. Overall, this drives to lipid droplets accumulation and cytokine release, including IL1β and IL6; D) In cancer, TAM phenotype is characterized by increased fatty acid oxidation and activity of the TCA cycle, reduced glucose uptake and glycolysis along with up-regulation of enzymes involved in amino acid catabolism, ARG1 and IDO for arginine and tryptophan respectively. Notably, up-regulation of GS causes a rise in glutamine levels, both intra- and extracellular. The increased levels were also allowed by the decreased activity of GLS. GLUT1, glucose transport 1; PFK2, phosphofructose kinase 2; FATP1, fatty acid transport protein 1; CTP-1a, carnitine palmitoyl transferase 1a; FAO, fatty acid oxidation; GPR91, G-protein-coupled succinate receptor; HK2, hexokinase2; PKM2, pyruvate kinase M2; NLRP3, NLR family pyrin domain containing 3; TCA, tricarboxylic acid; ARG1, arginase 1; IDO, indoleamine 2,3 dioxygenase; MCAT2, amino acid transporter type 2; LAT, L-aminoacid transporter; GS, glutamine synthetase; GLS, glutaminase, ASCT-2, glutamine transporter.

associates to the disease progression (Walsh & McWilliams, 2014). Monocytes and macrophages play a fundamental role in the disease pathogenesis (Udalova, Mantovani, & Feldmann, 2016). Their infiltration in the inflamed synovial membrane and cartilage junctions is significant (Kinne, Stuhlmüller, & Burmester, 2007; Mulherin, Fitzgerald, & Bresnihan, 1996) and correlates to joint damage (Udalova et al., 2016), which occurs through stimulation of T cell responses. In animal models of RA, macrophage depletion by clodronate liposomes reduces disease progression by limiting inflammation and joint damage, although it is known that circulating monocytes and other cells of the mononuclear phagocyte system can also contribute to the pathology (Richards, Williams, Goodfellow, & Williams, 1999).

Infiltrated macrophages promote inflammation by secretion of cytokines and chemokines. They sustain the main production of TNF in the synovial membrane and at the cartilage-pannus junction in RA patients (Buchan et al., 1988; Husby & Williams, 1988) and promote secretion of CC chemokine ligand 3 (CCL3), CC chemokine ligand 5 (CCL5) and CX3C chemokine ligand 1 (CX3CL1) (involved in monocyte recruitment and activation), and CXC chemokine ligand 8 (CXCL8) and CC chemokine ligand 2 (CCL2) (involved in neutrophil and monocyte recruitment) (Koch et al., 1992; Loetscher, Dewald, Baggiolini, & Seitz, 1994) which may also function in an autocrine manner (Haringman, Kraan, Smeets, Zwinderman, & Tak, 2003). In line with the secretive asset of RA macrophages, the predominant phenotype is "M1-like". Indeed the M1/M2 ratio is increased in RA patients whereas it decreases in clinical remission patients (Fukui et al., 2017; Kennedy, Fearon, Veale, & Godson, 2011). The polarization toward a M1-like state seems to be linked to different mediators. The anti-citrullinated protein/peptide antibody (ACPA), that is elevated with high specificity in RA, induces the transcription factor interferon regulatory factor 5 (IRF5) that promotes monocyte polarization to a M1-like state (Zhu et al., 2015). Several studies implicate the involvement of Notch signaling in the polarization of macrophages toward a M1-like state, since its inhibitor thapsigargin promotes a switch of M1 macrophages toward a M2-like phenotype, that *in vivo* ameliorates join damage and bone loss (Sun et al., 2017). However, as already mentioned, it is clear that *in vivo* macrophage polarization setting is more a spectrum of these two states rather than a binary separation, an observation that also holds in relation to the metabolic status of these cells. RA macrophages are highly glycolytic and produce high levels of ATP to meet their energy demands (Zeisbrich et al., 2018) with upregulation of GLUT1 and 3 and different glycolytic enzymes (Fig. 3B). The increased glycolytic flux is not accompanied by a sustained oxidative phosphorylation and this leads to ROS production and induction of inflammatory genes, such as IL-1β (Shirai et al., 2016; Weyand & Goronzy, 2017; Weyand, Zeisbrich, & Goronzy, 2017) that in RA monocytes seems to be mediated by NLRP3 (Ruscitti et al., 2015). The high expression of HIF-1α in RA synovial fluid (Hollander, Corke, Freemont, & Lewis, 2001) suggests its involvement in the metabolic abnormalities and release of IL-1β in RA macrophages (Tannahill et al., 2013) and this is substantiated by the *in vivo* protective effect of the macrophage-specific HIF-1α deletion against myeloid infiltration and disease progression (Cramer et al., 2003). Succinate is also accumulating in RA joints and might concur to HIF1α stabilization (Tannahill et al., 2013). It binds to GPR91, which in RA sustains

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macrophage activation and secretion of IL-1β (Littlewood-Evans et al., 2016) (Fig. 3B). Although central in driving macrophage activation in hypoxia, HIF-1α is known to synergistically operate with NF-κB (Bruning et al., 2012), which seems to be the main regulator of monocytes (that are insensitive to HIF1α) (Fangradt et al., 2012) in RA synovial hypoxic tissue (Fangradt et al., 2012; Oliver et al., 2009) (Fig. 3B). The importance of hypoxia in driving inflammation is also substantiated by the fact that macrophages infiltrate the low-oxygen microenvironment of the joint, and alter their metabolism and phenotype (Ng et al., 2010). Indeed monocytes from RA patients express high levels of the chemokine receptor CXCR4 (Yang, Yao, & Wang, 2018).

The current therapeutic strategies aim at rebalancing the M1/M2 ratio. However, the studies analysing the effects of RA biological Disease-modifying Antirheumatic Drugs (bDMARDs) on macrophage polarization are scarce. Anti-cytokine bDMARDs are known to reduce inflammation by limiting recruitment of monocytes/macrophages isolated from patients (Degboé et al., 2019). Anti-TNFα agents not only shift macrophage phenotype toward a M2-like state, but also inhibit the expression of inflammatory cytokines (TNF-α, IL-6, IL-12) and induce the phagocytosis of macrophages by increasing IL-10 production (Ma & Xu, 2013).

Glucocorticoids are also known to shift macrophages of RA patients to the M2-like state similarly to other DMARDs, such as methotrexate and leflunomide (Scott et al., 2001; Weinblatt, 2013). Evidence on the effect of metabolic targets on significantly affecting RA macrophage polarization is missing and needs to be explored. A new target opportunity comes from small molecules, such as janus kinases (JAK) inhibitors that block IL-6, IL-15 and IL-17 cytokine signaling. Their beneficial effect seems to act through prevention of STAT activation (Genovese et al., 2016; Kivitz et al., 2018). However, evaluation of their role on macrophage function is far from being elucidated.

4.3. Atherosclerosis

Atherosclerosis is a chronic inflammatory disease, which progressively culminates to cardiovascular pathologies that represent the main cause of death worldwide (Herrington, Lacey, Sherliker, Armitage, & Lewington, 2016). Indeed, a great number of cardiovascular events, including heart attack and stroke, are caused by the rupture of atherosclerotic plaques in arterial vessels that can be followed by thrombus formation and fragmentation.

Atherosclerosis is characterized by a chronic low-grade sterile inflammation in the artery walls, that is initiated by the retention of cholesterol-rich lipoproteins. In the arterial wall microenvironment, these lipoproteins are subjected to oxidation or enzymatic and non-enzymatic cleavage and aggregation. Their accumulation triggers the activation of resident macrophages and the recruitment of monocytes into the intima, where they differentiate in macrophages that ingest lipoprotein particles and eventually become foam cells. The latter can release molecules that further induce cholesterol deposition, proteolytic degradation of the extracellular matrix (Chinetti-Gbaguidi, Colin, & Staels, 2015) and express genes related to lipid processing (Kim et al., 2018). As the atherosclerotic plaque progresses, local hypoxia promotes neovascularization (Heikal & Ferns, 2017). However, at the advanced stages, angiogenesis becomes defective, thereby resulting in vascular leakage and hemorrhage. The surrounding macrophages play a major role also in this context, as they are able to uptake hemoglobin (Hb), iron and red blood cells.

Taken together, macrophages appear to play a pivotal role in the various stages of atherosclerosis. Due to their different functions, it is not surprising that they display a large variety of phenotypes within the plaque. Indeed, macrophages are extremely plastic and can switch from one phenotype to another depending on the environment. Cholesterol deposit into the intima results in M1 polarization and pro-inflammatory response, and its crystals were found to activate the NLRP3 inflammasome, which results in the maturation and release of the inflammatory cytokine IL-β (Chinetti-Gbaguidi et al., 2015; Duewell et al., 2010). On the other hand, IL4/IL13-activated M2-like macrophages contribute to tissue repair and inflammation resolution. M2 macrophages upregulate liver X receptors (LXRs), which mediate important athero-protective activities by modulating cholesterol metabolism (Calkin & Tontonoz, 2010). In addition, they promote efferocytosis by scavenging apoptotic cells. A third macrophage phenotype, named Mox, has been recently identified in advanced lesions in mice (Kadl et al., 2010), representing 30% of the total number of macrophages. Oxidized phospholipids promote the formation of these macrophages by inducing Nrf2-dependent gene expression (Kadl et al., 2010). Mox macrophages display reduced phagocytic and chemotactic capacities, as compared to M1 and M2. In the haemorrhagic zones of human atherosclerotic lesions, haem directs macrophage polarization towards the Mhem phenotype (Boyle et al., 2012). Intracellular accumulation of iron and Hb enhances the activity of the oxysterol- activated LXRα, thereby inducing cholesterol efflux and preventing foam cell.

There is a great interest in elucidating how changes in metabolism of macrophages affect their function, to develop therapeutic strategies that revert the inflammatory phenotype in the atherosclerotic plaque (Bories & Leitinger, 2017; Koelwyn, Corr, Erbay, & Moore, 2018). The encouraging findings of a recent clinical trial showing that anti-IL-1β antibodies decrease cardiovascular events in high-risk patients (Ridker et al., 2017) sustain this approach, although only few studies are reported.

A fundamental factor that can influence macrophage metabolism is hypoxia. Indeed, the plaques are characterized by hypoxic regions, where HIF1α is stabilized and activates glycolysis, by increasing the expression of GLUT1, HK2 and PFKFB3 (Tawakol et al., 2015). However, it is still unclear whether the increase in glucose metabolism reflects the plaque development (Tabas & Lichtman, 2017; Tawakol et al., 2015). The increased glycolysis is paralleled by an increase of PPP, that is crucial for cholesterol, lipid and nucleotide synthesis (Yamashita et al., 2014). In fact, these macrophages accumulate LDs and cholesterol (Fig. 3C). An elevated amount of PPP metabolites was determined in atherosclerotic rabbit arteries. Notably, the impairment of the PPP pathway due to glucose 6 phosphate dehydrogenase (G6PDH) deficiency lowers ROS levels and the atherosclerotic lesion size in ApoE$^{-/-}$ mice (Matsui et al., 2006). Moreover, monocytes and macrophages from patients with coronary artery disease were found to display a higher glucose uptake and glycolytic flux, as compared to those from healthy subjects (Shirai et al., 2016). This metabolic signature fuels the generation of mitochondrial ROS, which in turn promote dimerization of PKM2 and the consequent STAT3 activation, resulting in increased levels of the pro-atherogenic cytokines IL-6 and IL-1β (Shirai et al., 2016) (Fig. 3C).

Mitochondria were found to play a critical role in atherosclerosis (Madamanchi & Runge, 2007). In the first studies, atherosclerotic vascular lesions were related to mitochondrial oxidative stress, although clear evidence of causation and cell-specific proatherogenic mechanisms of mitochondrial oxidative stress was not provided. More recently, Tabas' group showed that oxidized LDL or lipoprotein(a) can induce mitochondrial oxidative damage and progressive impairment of the mitochondrial respiratory chain, thereby preventing the shift toward OXPHOS (Wang et al., 2017; Wang, Wang, Rabinovitch, & Tabas, 2014). Importantly, selective inhibition of mitochondrial oxidative stress by a murine model in which the enzyme scavenger catalase is expressed only in macrophage mitochondria reduces NF-κB p65 activation, expression of proinflammatory cytokines, and aortic lesion area (Fig. 3C). On the contrary, quenching non mitochondrial ROS by cytosolic catalase leads to enhanced LPS-induced inflammatory cytokine induction without affecting NF-κB activation (Wang, Wang, Rabinovitch, & Tabas, 2014), further highlighting the importance of mitochondrial performance.

mTOR is a key player also in the development of this disease, although as described above its role in macrophage polarization is quite

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complex. Both pharmacological and genetic mTOR inhibition significantly reduces macrophage infiltration and the size of the lesion (Ai et al., 2014). Moreover, the inhibition of mTOR promotes macrophage autophagy that is beneficial in this pathology as it enhances removal of dysfunctional components (Martinet, Verheye, & De Meyer, 2007). On the other hand, the activation of AMPK was found to have various protective function in atherosclerosis (Vasamsetti et al., 2015; Wang, Ma, Zhao, & Zhu, 2017), including the induction of autophagy in smooth muscle cells and suppression of ER stress in endothelial cells. Specifically, in macrophages AMPK promotes catabolic pathways (FAO and OXPHOS), activates the receptor LXRα that promotes cholesterol efflux by upregulating the expression of the ABCA1 and ABCG1 cholesterol transporters, thereby resulting in prevention of foam cell formation (Kemmerer, Wittig, Richter, Brüne, & Namgaladze, 2016; Li et al., 2010; Wan et al., 2013).

4.4. Cancer and tumour associated macrophages

The tumour microenvironment (TME) is composed of tumour cells as well as infiltrating immune cells, endothelial cells, fibroblasts, secreted factors and cytokines as well as extracellular matrix proteins surrounding the primary tumour. The composition of the TME strongly impacts tumour development in many different ways. It is increasingly immunosuppressive, which inevitably limits immune cell infiltration. This is known to occur also through metabolism. A typical feature of cancer cells is an abnormal metabolism that associates to a pronounced depauperation of available nutrients, such as glucose. This instates a nutrient competition that might induce tumour progression by limiting source availability for immune cells (Chang et al., 2015; Ho et al., 2015).

TAMs are part of the TME. Characterizing macrophages *in vivo* by the dichotomous M1/M2 classification might be simplistic due to their dynamic plasticity. There is evidence that during cancer progression TAMs acquire some features shared by *in vitro* skewed M2 macrophages (Condeelis & Pollard, 2006; Qian & Pollard, 2010) but their role in sustaining and regulating tumour growth, angiogenesis, invasion and metastasis (Condeelis & Pollard, 2006; Flerin, Pinioti, Menga, Castegna, & Mazzone, 2019; Franklin & Li, 2014) represents the ultimate result of different states, both pro-tumoral and anti-tumoral, concomitantly present. The developmental stage of a tumour also plays an important role: from a metabolic point of view, in the early stage TAMs display a more glycolytic metabolism, which is gradually modified toward mitochondrial metabolism and OXPHOS (Boscá et al., 2015). This is associated to a polarized state toward an anti-tumoral function in the stages of tumour initiation and progressively changes toward an immunosuppressive pro-tumoral state in advanced stages of tumour progression (Franklin & Li, 2014).

The impact of metabolism on TAM function has been deepened by studies on the mTOR pathway. The mTOR kinases are constituents of the mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which regulate cell growth and proliferation (Düvel et al., 2010; Zarogoulidis et al., 2014). Once activated by different factors, mTORC1 and mTORC2 lead to recruitment of phosphatidylinositol 3 kinases (PI3Ks) and subsequent activation of the serine/threonine kinases AKT1, AKT2 and AKT3. The physiological inhibitors of mTORC1 are TSC 1 and 2 (Düvel et al., 2010; Housden et al., 2015; Mercalli et al., 2013; Zarogoulidis et al., 2014), and rapamycin is a drug specifically targeting mTORC1. mTORC2 activates and tunes AKT substrate specificity and plays a role in modulating cytoskeleton reorganization (Weichhart, Hengstschläger, & Linke, 2015). The role of the PI3K-AKT-mTOR axis in sustaining M1/M2 macrophage polarization is not well understood, since contradictory evidence has been gathered with this respect (see for a review Weichhart et al., 2015). This is probably due to the fact that the PI3K-AKT-mTOR pathway is the collector of signalling emanating not only from growth factors and cytokines but also from environmental signals with different downstream effects (Weichhart et al., 2015). This pathway has been investigated in TAMs, obviously with discrepant results. The switch to glycolysis in TAMs is under the control of the Akt-mTOR axis. Activation of PI3K-Akt upregulates glycolysis (Smith et al., 2012) through stabilization of HIF1α with accumulation of succinate and citrate in the cytosol (Huang et al., 2014; Krawczyk et al., 2010), leading to inflammation. However, PI3Kγ-selective inhibition with IPI-549 reprograms TAMs toward an anti-tumoral function and potentiates anti-PD-1 therapy in mouse tumour models (Kaneda et al., 2016) and it is currently in Phase-1 clinical trial (Kaneda et al., 2016). Since mTOR activation overwrites that effect and polarizes macrophages towards an immunosuppressive phenotype (Byles et al., 2013), it should be expected that mTOR inhibition rescues the inflammatory phenotype in TAMs. In contrast to this logical hypothesis, TAMs lacking the mTOR inhibitory protein, REDD1, show a general anti-tumoral function. Their enhanced glucose consumption reduces nutrient availability for endothelial cells (Wenes et al., 2016), leading to vessel normalization, decreased hypoxia and inhibition of metastasis formation (Wenes et al., 2016). Glucose metabolism is also sustained by PKM2, since in its dimeric form it potentiates HIF1α activity (Palsson-Mcdermott et al., 2015). These events linked to HIF1α activation do not unidirectionally characterize the TAMs phenotype. Indeed the production of lactate, which accumulates during hypoxic conditions, skews TAMs toward immune suppressive and proangiogenic functions, promoting tumorigenesis (Colegio et al., 2014). PKM2 itself can promote a M2-like phenotype when present in an active tetrameric function (Palsson-Mcdermott et al., 2015) (Fig. 3D).

TAMs are known to produce NO from arginine, thus promoting an anti-tumoral function (Ho & Sly, 2009; Stuehr, 1989). However, one of the typical enzyme of TAMs is ARG1 that produces polyamines from arginine, leading to a pro-tumoral function (Chang, Liao, & Kuo, 2001) (Fig. 3D). By metabolizing arginine through ARG1, TAMs interfere with the anti-tumor activity of T cells as this depletes the arginine pool for NO and protein synthesis, leading to impaired TCR function (Popovic, Zeh, & Ochoa, 2007; Rath, Müller, Kropf, Closs, & Munder, 2014) and T cell differentiation (Geiger et al., 2016). Finally, glutamine metabolism, which is traditionally considered a fuel for inflammatory macrophages (Murphy & Newsholme, 1998) displays a peculiar feature in TAMs. The ability of TAMs to synthesize glutamine through GS activity promotes their immunosuppressive, pro-angiogenic and metastatic function (Palmieri et al., 2017). This is probably to be ascribed to the role of glutamine in protein glycosylation, which is a crucial event during the differentiation of macrophages towards a “M2-like” phenotype (see above) (Fig. 3D). Tryptophan metabolism is also a peculiar feature of TAMs, which express high levels of IDO, the enzyme involved in first and rate-limiting step of the kynurenine pathway (Platten et al., 2015; Wang et al., 2014). Tryptophan depletion, IDO activity and kynurenine are known to regulate T cell differentiation and activation (Fallarino et al., 2006; Munn et al., 2005; O'Neill et al., 2016; Platten et al., 2015). Products of tryptophan catabolites display an inhibitory effect on T cells (Frumento et al., 2002; Weber et al., 2006) (Fig. 3D).

Lipid metabolism is fundamental for the acquisition of the different functions of macrophages. However, very little is known about lipid metabolism and its role in shaping the functional phenotype in TAMs. TAMs express high levels of fatty acid synthase and upregulate PPAR signalling, which promotes fatty acid oxidation and tumour growth (Fig. 3D). PPARγ is known to mediate alternatively activated macrophage polarization (Deng et al., 2015), although evidence to the contrary is also present (Van Ginderachter et al., 2006). This suggests that the response of TAMs with respect to lipid metabolism is heterogeneous and far from being understood.

Lipid oxidation is important in TAMs metabolism. COX-1 is upregulated in TAMs and this associates to an enhanced release of PGE2 (Poczobutt et al., 2016), which supports immune suppression, angiogenesis, and cancer cell migration (Baxevanis et al., 1993). In the Lewis Lung carcinoma (LLC) murine model, TAMs express COX-2 (Poczobutt et al., 2013), which is also noted in human melanoma (Bianchini et al., 2007). The role of COX-2 in macrophage function is

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underlined by the finding that COX-2-expressing macrophages are a prerequisite for IL-1β-induced neovascularization and tumour growth (Nakao et al., 2005). Additionally, in TAMs from renal cell carcinoma the eicosanoid pathway is enhanced through 15-lipooxygenase-2 (15-LOX-2) activation, leading to secretion of the arachidonic acid metabolite 15-hydroxyeicosatetraenoic acid (15(S)-HETE) (Daurkin et al., 2011). The acquisition of this metabolic feature associates with CCL2 and IL-10 production, promoting immune tolerance (Daurkin et al., 2011).

In a mouse model of mammary adenocarcinoma, TAMs expressing high levels of epidermal fatty acid binding protein (E-FABP), an intracellular lipid chaperone, display a "M1-like" phenotype and anti-tumour activity (Zhang et al., 2014). In line with this finding, E-FABP expression is lower in stroma from invasive tumours and negatively correlates with cancer progression (Zhang et al., 2014). The underlining mechanism probably relies on the E-FABP ability to mobilize tumour-derived lipids to form lipid droplets that concur to upregulate interferon β (IFNβ), leading to recruitment of natural killer (NK) cells and increase of anti-tumour activity in the TME (Zhang et al., 2014). The *in vivo* administration of the E-FABP activator EI-05 in a mouse mammary tumour model significantly reduces tumour growth (Rao et al., 2015). However, in TAMs from ovarian cancer patients, the PPARβ/δ target genes are upregulated, although this transcription asset is associated to a pro-tumoral function of TAMs (Schumann et al., 2015). In this case, polyunsaturated FAs of tumour origin accumulate in TAMs as stable droplets providing a reservoir of PPARβ/δ ligands to TAMs. This contributes to a stable upregulation of PPARβ/δ target genes associated to inflammation, cell migration and tumour progression including pyruvate dehydrogenase kinase 4 (PDK4), LDL receptor-related protein 5 (LRP5), CD300A, mitogen-activated protein kinase 8 (MAPK8) and angiopoietin-like 4 (ANGPTL4) (Schumann et al., 2015). These findings contribute to the notion that, similarly to other metabolic pathways, lipid metabolism in TAMs provides different and contrasting signals and its effect on TAM function is strictly TME-dependent.

From a therapeutic point of view, efforts against TAMs pro-tumoral functions are mainly directed towards TAM depletion (reviewed in Cassetta & Pollard, 2018). TAM depletion by trabectedin-mediated apoptosis is found to successfully limit tumour growth and metastatic spread (Germano et al., 2013). Eradication of TAMs using clodronate is effective in reducing lung and lymphoma progression (Fritz et al., 2014; Wu et al., 2014) and angiogenesis in murine cancer models (Reusser et al., 2014; Zeisberger et al., 2006). However, other studies highlight potential adverse effects of TAM depletion (Kim et al., 2008; Reed et al., 2008). Acting on monocyte recruitment to the tumour site is also used as a strategy for reducing TAM expansion. This process is mediated by CCL2-CCR2 axis. CCL2, released by tumour cells, is a potent chemoattractant for monocytes, T and NK cells, that express the receptor CCR2. Inhibition of CCL2-CCR2 signalling is successful in reducing cancer progression in several experimental models of cancer (Li et al., 2013; Qian et al., 2011) and different anti-CCL2 antibodies and inhibitors of the CCL2 receptor are currently in clinical trials (Cassetta & Pollard, 2018). Targeting the Colony stimulation factor 1 (CSF1)-CSF1 receptor (CSF1R), which promotes differentiation, proliferation and survival of monocytes and macrophages, is also under evaluation. CSF1R-targeted therapies have been found to inhibit monocyte and macrophage recruitment and to improve chemotherapy and immunotherapy in preclinical models (Peranzoni et al., 2018). CFSF1R targeting induces a reprogramming of TAMs (Pyonteck et al., 2013) and is currently tested in clinics (Edwards et al., 2018; Papin et al., 2019). Finally in recent years, several molecules able of reprogramming macrophages polarization from M2-like to M1-like, such as Class IIa histone deacetylase (HDAC) inhibitors, have been identified (Guerriero, 2018; Guerriero et al., 2017).

Evidence on the effect of targeting TAM metabolism to affect cancer progression is growing. In particular, metabolic targeting of TAMs is evaluated in combination to the common PD-1 immunotherapy. One of these, IDO1 inhibition in combination with checkpoint inhibitors, has reached clinical Phase 1 and 2, but it did not always show additional benefit to the anti-PD antibody use (Soliman et al., 2018).

Studies testing the effect of ARG1 inhibition alone or in combination with anti-PD1 therapy showed a significant early effect of the ARG1 inhibitor, although the combination did not exert any additional effect (Arlauckas et al., 2018). Targeting of the COX2/mPGES1/PGE2 axis reduces PD-L1 expression in myeloid cells infiltrating the tumour (Prima, Kaliberova, Kaliberov, Curiel, & Kusmartsev, 2017). Myeloid specific LDH-A blockade reverts immunosuppression and TAM phenotype towards an anti-tumoral one, while affecting the number of PD-L1$^+$ cancer cells (Seth et al., 2017). Glutamine and lipid metabolism are potential targets. GS is a promising pharmacological target to revert TAM phenotype, since the GS specific deletion in macrophages leads to a shift toward the "M1-like" phenotype associated with reduced angiogenesis, immunosuppression and decreased metastasis (Palmieri et al., 2017). Furthermore, ovarian cancer progression is reduced by targeting the ABC transporter responsible for cholesterol efflux from macrophages (Goossens et al., 2019). Other approaches on non-metabolic targets affecting metabolism in TAMs include activation of the Toll-like receptor 9 with a CpG oligodeoxynucleotide to promote anti-tumor activity (Liu et al., 2019), and targeting macrophage-associated V-set Ig domain-containing 4 (VSIG4) to repolarize TAMs towards a M1-like state (Liao et al., 2014).

5. Pharmacological targeting of macrophages in diseases: perspectives and challenges

It is clear that targeting metabolic checkpoints in macrophages offers the unique opportunity to revert pathological function of macrophages by selective inhibition of specific enzymes rather than ablation of general macrophage function (Beatty et al., 2011; Casazza et al., 2013). Indeed, a general depletion of macrophages might not be recommended, since macrophages can play beneficial functions. Evidence on the effects of macrophage depletion depends on the disease under study. For instance, macrophage depletion in atherosclerotic plaque can be useful only at the early stage of the disease (Martinet, Coornaert, Puylaert, & De Meyer, 2019). As a further drawback, systemic clearance of macrophages (from the whole body) has been associated with an increased risk of infection (Purnama et al., 2014), which is obviously adverse in clinical settings. However, TAM-depleting strategies have shown a significant level of efficacy in cancer (see Section 4.4).

Pharmacological targeting of metabolism might represent an innovative approach, although with significant drawbacks, such as systemic toxicity and off-target effects. With this respect, studies on glucocorticoids are enlightening. Glucocorticoids represent a very powerful way to re-polarize macrophages to an anti-inflammatory phenotype. However, their strong effect on non-macrophagic cells can be systemically harmful. Different strategies have been developed to overcome this problem and achieve a significant reduction in the amount of drug used, that is: (I) conjugation of the molecule to a ligand or antibodies against highly expressed surface receptors; (II) nanoparticle (NP)/microparticle (MP) delivery; (III) a combination of I and II.

Among the different macrophagic markers, CD163 might be a potential target for intracellular delivery of drugs to macrophages, either by using hemoglobin as ligand or targeting antibodies, due to its constitutive function as endocytic receptor (Adair, Howard, Hartley, Williams, & Chester, 2012; Harper, Mao, Strout, & Kamal, 2013). Exposure to the drug is reduced as the ligands bound to CD163 are rapidly internalized. Low-dose anti-CD163-dexamethasone conjugate effectively decreases inflammation in the hepatic acute phase response in LPS treated mice (Thomsen et al., 2016) and limits inflammation and liver fibrosis in fructose induced -severe non-alcoholic steatohepatitis (NASH)-like (Svendsen et al., 2017), demonstrating the anti-inflammatory potential of the conjugate *in vivo*. CD206, the mannose receptor, has been widely exploited with this respect, by using mannose and galactose as ligands,

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Table 2
Nanotechnology systems to target macrophages in disease.

	Drugs or targets	Effects	Models/Status	References
Liposome	IL-6 receptor- or CD163-targeted liposomes	Deplete macrophages	*In vitro* and *in vivo* preclinical models for AT (human freshly purified monocytes, RjOrl:SWISS; C57BL/6JRj mice)	Kelly et al., 2011; Rafique et al., 2019
	CD163 antibody-coated liposomes	Deplete macrophages	*In vitro* and *in vivo* preclinical models for AT and RA (human peripheral blood CD14^{+} monocytes; DBA/1J mice)	Etzerodt et al., 2012; Alvarado-Vazquez et al., 2017; Yang et al., 2017
	BPs-liposomes	Deplete macrophages M1 to M2 subtype polarization	*In vitro* preclinical models for cancer (murine 4T1 breast cancer cell line; 3T3 fibroblast; J774 murine macrophage cell line)	Sousa, Auriola, Mönkkönen, & Määttä, 2015
	Clodronate-loaded liposomes	M1 to M2 subtype polarization	*In vivo* preclinical models for AT (C57BL/6J mice)	Sercombe et al., 2015: Feng et al., 2011;
	Hb-changed liposome	M1 to M2 subtype polarization	*In vitro* preclinical models for cancer (THP-1)	Zhang & Palmer, 2012;
	STAT3 siRNA	M1 to M2 subtype polarization	*In vivo* preclinical models for cancer (B16-F10 murine melanoma in C57BL/6 mouse melanoma model)	Jose, Labala, Ninave, Gade, & Venuganti, 2018;
	LCL-SIM	Reduction of oxidative stress and HIF1α	*In vivo* preclinical models for cancer (B16-F10 murine melanoma in C57BL/6 mouse melanoma model)	Alupei, Licarete, Patras, & Banciu, 2015;
Microparticles	Y-BGs	Induction of autophagy	*In vitro* preclinical models of IRD (mouse macrophage cell line, J774A.1)	Fatima, Upadhyay, Sharma, & Sharma, 2017
Dendrimer	ABP	M1 to M2 subtype polarization	Preclinical model RA (human freshly purified monocytes)	Hayder et al., 2011
	2G-03NN24 carbosilane	M1 to M2 subtype polarization	Preclinical models for cancer (M1 macrophages)	Perisé-Barrios et al., 2015
Ab conjugated	(anti-TNFα mAb)-HA	M1 to M2 subtype polarization	Preclinical models of IRD (Sprague-Dawley rats)	Friedrich et al., 2014

ABP, Phosphorus-based dendrimer aminobisphosphonate; (anti-TNFα mAb)-HA, anti-TNFα antibodies conjugated to hyaluronic acid (HA); AT, atherosclerosis; BPs, Bisphosphonates; HB, hemoglobin; IRD, inflammatory related diseases; LCL-SIM, simvastatin loaded liposome; RA, rheumatoid arthritis; STAT3, signal transducer and activator of transcription 3; Y-BGs, Yeast-derived β-glucans.

or CD206 antibodies. Their specific targeting effect has been demonstrated in many different diseases including infection (Nahar & Jain, 2009; Rathore et al., 2011) inflammatory bowel disease (Huang, Guo, & Gui, 2018; Xiao et al., 2013), cancerous tumours (Lanlan Liu, Yi, et al., 2017; Niu, Valdes, Naguib, Hursting, & Cui, 2016), and atherosclerosis (He et al., 2018). Anti-CD11b integrin functionalization has been shown to promote macrophage uptake of factors in both macrophages and microglia (Cerqueira et al., 2012; Davis, Reichel, Bae, & Pennypacker, 2018). CD64 or Fcγ receptor I (FcγRI) could represent another interesting opportunity as it is substantially upregulated in

Table 3
Examples of nanodelivery strategies to target macrophages in disease.

Drugs or targets	Effects	Models/Status	References
C-type lectin family	Altering activation state	Review on preclinical models for IRD	Frenz et al., 2015
SPIONs	Reprogramming TAMs from M2 to M1-like subtype	Preclinical models (cancer murine primary IL-4-activated BMDM; human M2-like differentiated THP-1 cells)	Rojas et al., 2016
Man-HA-MnO2 NPs	M2 targeted	Review on preclinical models for AT	Song, Liu, Shi, Zhang, & Chen, 2016;
PEG-and mannose-NP	M2 targeted	Preclinical models cancer (B16-F10 murine melanoma in C57BL/6 mice)	Zhu et al., 2013
Mannosylated deliver siRNA	M2 targeted	Preclinical model RA (mouse BMDM)	Yu et al., 2013
siRNA-NPs	Notch1	Preclinical model cancer (CIA mice)	Kim et al., 2015
M2pep	M2 targeted	Preclinical models cancer (mice)	Cieslewicz et al., 2013
AM NPs	Suppress the uptake of oxLDL by macrophage	Preclinical models for AT (ApoE$^{-/-}$ mice)	Lewis et al., 2015;
DNP	LXR activation (+ macrophage cholesterol transporters, ABCA1/ABCG1), + cholesterol expression	Preclinical models for AT (LDLR$^{-/-}$ mice)	He, Ghosh, & Yang, 2017
Statin-loaded HDL	Reduced macrophage accumulation in the plaques	Preclinical models for AT (Apoe$^{-/-}$ mice)	Tang et al., 2015;
LyP-1 (CGNKRTRGC)	Reduced macrophage accumulation in the plaques	Preclinical model for AT (mouse model of macrophage-rich lesions in left common carotid arteries, LCCAs)	Song, Zhao, Zhu, & Zhao, 2019
rHDL Fluo	Reduced macrophage accumulation in the plaques	Preclinical model for AT (ApoE$^{-/-}$ mice)	Duivenvoorden et al., 2014
LT rHDL	Macrophage infiltration and expression of matrix metalloproteinase	Preclinical model for AT (mice)	Liu et al., 2014
TLR7/8 agonist-loaded ciclodextrin NPs	Reprogramming TAMs from M2 to M1-like subtype	Preclinical model cancer (orthotopic breast cancer model mice)	Rodell et al., 2018

AM NPs, sugar-based amphiphilic core-shell layered nanoparticles; Apoe−/−, apolipoprotein E–deficient mice; AT, atherosclerosis; CIA, collagen-induced arthritis; DNP, mannose-functionalized dendrimer nanoparticles; IRD, inflammatory related diseases; LDLR, low-density lipoprotein, LDL, receptor; LTrHDL, t lovastatin (LT) delivered by HA-modified rHDL; LXR, liver X receptor; LyP-1, cyclic peptide, LyP-1 (CGNKRTRGC); M2pep, peptide designed to recognize specifically M2-like macrophages; Man-HA-MnO2 NPs, mannan-conjugated MnO2 particles with hyaluronic acid (HA) modification; OxLDL, oxidized low-density lipoprotein; PEG-and mannose-NP, polyethylene glycol (PEG)-sheddable and mannose-modified nanoparticle delivery system; RA, rheumatoid arthritis; rHDL Fluo, reconstituted HDL (rHDL) nanoparticles to deliver statins to atherosclerotic plaques. rHDL labeled with Cy5.5 (lipid monolayer) and DiR (hydrophobic core); siRNA-NPs, siRNA against Notch1 (siRNA-NPs) through self-assembled poly-siRNA and thiolated-glycol chitosan nanoparticle; SPIONs, superparamagnetic iron oxide nanoparticles; TLR7/8, toll-like receptor type 7/8.

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macrophages with M1-like phenotype (Akinrinmade et al., 2017; Hristodorov et al., 2015), making it an attractive candidate for delivery in rheumatoid arthritis models (Albuquerque, Moura, Sarmento, & Reis, 2015; Moura et al., 2014). However, despite a great effort and promising results, the main challenge to be addressed is still the lack of selectivity, since these molecules are expressed also in macrophages of liver and spleen, as well as in other cells (for instance CD206 is expressed by a subpopulation of endothelial cells) (Andón et al., 2017).

Nanomedicines are anticipated to help researchers solving macrophage drug delivery issues. Drug delivery systems based on nanoparticles (NPs) have been widely used after several decades of technological developments and have been already successfully applied for delivery of antibiotics to macrophages (see for reviews: Kelly, Jefferies, & Cryan, 2011; Pei & Yeo, 2016; Visser, Van Staden, & Smith, 2019). Exploitation of nanomedicines has several advantages. Size and surface characteristics can be manipulated (comprising their size range, hydrophilic and charge characteristics, which allow them to function as carriers for the delivery of drugs). Release of the cargo at the target site can be controlled in a precise release and carrier degradation features can be regulated. Finally, site-specific targeting can be realized by attaching targeting ligands to the surface (Wahlich et al., 2019). NPs are a family of materials. Synthetic NPs with different structures have been created using a wide range of materials, including liposomes (Nguyen, Huang, Gauthier, Yang, & Wang, 2016; Ren et al., 2019), chitosan (Jiang et al., 2017), PLGA (Lavin et al., 2014), dendrimers (Hayder, Fruchon, Fournié, Poupot, & Poupot, 2011), silica (Huang, Zhao, Song, & Zhao, 2017) and metals, such as iron oxide or gold (Mastrotto et al., 2011). Examples of nanomaterials and macrophage-selective delivery are listed in Tables 2 and 3.

NPs are aimed at overcoming the issue of delivering the drug specifically to macrophages infiltrating at the disease site and not to macrophages (or other cells) present in healthy tissues. One possible strategy is exploiting nanocarriers that are sensitive to metabolic change, such as changes in the pH values. In the case of the TME, characterized by acidosis (ranging between pH 6.5 and 6.8), NPs have been designed to release the drug at acidic pH and to be stable in healthy tissues. An interesting "proof of concept" has been illustrated by Zhu and co-workers that describe the use of mannose-modified PLGA NPs coated with a pH-sensitive PEG layer. In healthy tissues (pH 7.4) PEG shields mannose recognition by CD206 macrophages/cells, whereas in TME (pH 6.8) PEG cleavage exposes mannose to CD206 recognition by TAMs, promoting a TAM specific uptake (Zhu, Niu, O'Mary, & Cui, 2013).

This collected evidence suggests that macrophage targeting and reprogramming is an effective strategy to treat diseases, particularly cancer. Research on macrophage-specific delivery is extensive with several strategies available. However, metabolism is currently very scarcely exploited for macrophage reprogramming. Technological advances in

Table 4
Approved metabolic drugs repositionable to modulate macrophage polarization.

Drugs	Indication	Macrophage effects	References
bDMARDs	RA: they affect the body's biological response to various cytokines	Modulation of macrophage polarization; decreased inflammatory burden	Degboé et al., 2019;
anti-TNF agents (ETA, ADA)	RA: they inhibit TNF-α	Modulation of macrophage polarization from M1 to M2 status	Paoletti et al., 2019;
Fingolimod (FTY720)	SM: it downregulates sphingosine-1 phosphate receptor (S1PR)	It increases the amount of M2 polarized macrophages in atherosclerotic plaques	Sun et al., 2018; Keul et al., 2007; Huang et al., 2012; Keul et al., 2011;
Pravastatin	Hypercholesterolemia: it inhibits 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase	It promotes M2 phenotype	Zhang, Xiao, & Li, 2018
Metformin	Type 2 diabetes: it inhibits Complex I, it activates AMP-activated protein kinase (AMPK), it inhibits glucagon-induced cAMP, it blocks mitochondrial glycerophosphate dehydrogenase	It polarizes macrophages toward the M2 phenotype, partially dependent on the activation of AMPK	Jing et al., 2018;
Glucocorticoids	Anti-inflammatory and immunosuppressive drug: Glucocorticoid Receptor agonist	It displays an anti-apoptotic effect on macrophages mediated through ERK1/2 phosphorylation in an adenosine receptor A3-dependent-manner	Barczyk et al., 2010; Ehrchen et al., 2007;
LND (low dose naltrexone)	Fibromyalgia, Crohn's disease, multiple sclerosis and localized pain syndromes: competitive opioid receptor antagonist	It shifts the macrophage phenotype toward the M1 type	Yi et al., 2012
Alpha-lipoic acid	Supplement antioxidant may have effects on inflammatory pathways, glucose control indicators, blood pressure, lipid profiles, body weight, fat mass, and food intake regulation.	It polarizes microglial cells from a M1 to a M2 state by inhibiting NF-κB nuclear translocation	Wang et al., 2018;
Omeprazole	Lysosomal disorders: inhibition of lysosomal enzymes (proton pump inhibitors)	It may influence macrophage polarization by modulating lysosomal function	Liu et al., 2013;
Bafilomycin A1	Lysosomal disorders: inhibition of lysosomal enzymes (V-ATPase inhibitor)	It suppresses cancer cell growth It may influence macrophage polarization by modulating lysosomal function	Yan et al., 2016;
Chloroquine	Malaria: inhibition of the parasytic vacuolar activity	It switches TAMs toward a M1-like phenotyp It facilitates macrophage nanodelivery	Chen et al., 2018; Wolfram et al., 2017;
Carglumic acid	Urea cycle disorders (UCDs): it ameliorates hyperammonemia in *N*-acetylglutamate synthase deficiency	It might influence macrophage polarization by modulating the citrulline/ornithine ratio	Haberle & McCandless, 2014;
4-Phenylbutyrate 4-PBA	Urea cycle disorders: it facilitates glutamine elimination through urine	AT: it increases the expression of HSP25 in macrophages, preventing macrophages cell death and monocyte-macrophage differentiation	Lynn et al., 2019;
Fenofibrate, gemfibrozil	AT: PPARα agonists, they reduce secretion of CXCL2, TNF-α, IL-6, activation of p65 of NF-κB, ERK, and TLR4 expression	They inhibit M1 polarization by a β-defensin mediated mechanism	Ann, S. jin, Chung, J. H., Park, B. H., Kim, S. H., Jang, J., Park, S., … Lee, S. H., 2015
Aspirin (acetylsalicylic acid, ASA)	Non-steroidal anti-inflammatory drug (NSAID), inhibiting cyclooxygenases (COX), including COX1 and COX2	It inhibits the activation of LPS-induced macrophages via the IκK/IκB/NF-κB pathway and the COX2/PGE2/EP2/NF-κB positive feedback loop	Liu, Yi, et al., 2017;

ADA, adalimumab; AMPK, AMP-activated protein kinase; ASA, acetylsalicylic acid; AT, atherosclerosis; bDMARDs, biological Disease-modifying Antirheumatic Drugs; COX, cyclooxygenases; ETA, etanercept; ERK, extracellular signal-regulated kinase; FTY720, fingolimod; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme-A; HSP25, heat shock protein 25; LND, low dose naltrexone; NSAID, non-steroidal anti-inflammatory drug; RA, rheumatoid arthritis; S1PR, sphingosine-1 phosphate receptor; SM, multiple sclerosis; UCD, urea cycle disorders.

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drug delivery might boost the targeting of metabolic reactions in such a way to modulate metabolic checkpoint of macrophage function. With this respect, NPs sensitive to the metabolic changes occurring in the disease environment are strongly awaited.

6. Conclusions

The research in inflammation-linked diseases is now opening new perspectives based on the growing knowledge of the metabolic changes that macrophages undergo during the different polarization processes. Understanding the role of metabolic pathways in the balance between pro and anti-inflammatory properties of macrophages is fundamental to achieve their rewiring, based on selective inhibition of specific enzymes rather than unspecific ablation of macrophage function, which is not always beneficial. Exploitation of small molecules as enzyme inhibitors, rather than antibodies, might produce important consequences with respect to both costs and efficacy. Specifically, preferential targeting to diploid cells, such as macrophages, is awaited in cancer since it would circumvent drug resistance that inevitably accompanies rapidly transforming neoplastic cells.

Inhibition of enzymatic activity raises concern about the issue of systemic toxicity, as most enzymes are ubiquitously present. More effort on design and development of effective inhibitors is awaited. Nanomedicines can offer innovative tools to bypass this issue by cell-specific delivery, with particular attention to delivery strategies sensitive to the metabolic status at the disease site. In spite of the benefits that nanomedicine has to propose, much research is still essential to estimate the safety/ toxicity associated with many NPs (Galvin et al., 2012). Nanotechnology research has focused on drug delivery, with relatively insufficient studies addressing NPs toxicity (Bhaskar et al., 2010). Testing NP pharmacokinetics, pharmacodynamics, and potential chronic toxicity *in vivo* is crucial for monitoring the effects of NPs on patients.

Another important issue to overcome is the discrepancy between *in vitro* and *in vivo* states, which is particularly important for macrophages. Metabolic characterization of functional states in macrophages has been mostly achieved *in vitro* or in murine models, in which polarization occurs in a defined and homogeneous way. This contrasts with the *in vivo* situation, in which, as stated above, macrophages display functions that are the ultimate result of different mediators being activated, with markers of opposite functional states being present concomitantly. Studies on TAMs often confirm that their switch towards an anti-tumoral function is not mediated by the predominance of markers classified as “M1-like”, but rather it is the result of complex mechanisms emanating from metabolic competition involving many different cells. Targeting a metabolic step within TME might produce different, or opposite effects compared to an *in vitro* setting. For these reasons, it is imperative to obtain insights on the metabolic profiles of primary macrophages isolated from *in vivo* tissues. Furthermore, evaluation of the metabolic preferences/limitations of the different cellular components is highly awaited in order to integrate the information regarding the *in vivo* cell-specific metabolic checkpoints.

Understanding how metabolism affects function in a pathological setting might benefit from the evaluation of the effects of existing therapeutic approaches on macrophage metabolism, which not always are available. More needs to be discovered on the role on macrophage metabolism of therapies not targeting macrophage metabolism, with particular attention to exercise training or diet. This is particularly true for obesity, in which exercise training is known to reduce inflammation. Furthermore, the repositioning of known metabolic drugs is particularly suitable to this purpose, since it would bypass the high costs/high overall attrition rates and timelines for the discovery and development of new drugs. A list of approved metabolic drugs with their known function and their (substantiated or speculated) role in influencing macrophage phenotype is reported in Table 4.

In conclusion, it is evident that many questions are still unsolved. However, immunometabolism is emerging now as a field and is opening an exciting route for the development of novel therapeutic strategies to treat immune disorders.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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