

Lipid-loaded macrophages as new therapeutic target in cancer

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Abstract Macrophages are main players of the innate immune system. They show great heterogeneity and play diverse functions that include support to development, sustenance of tissue homeostasis and defense against infections. Dysfunctional macrophages have been described in multiple pathologies including cancer. Indeed tumor-associated macrophages (TAMs) are abundant in most tumors and sustain cancer growth, promote invasion and mediate immune evasion. Importantly, lipid metabolism influences macrophage activation and lipid accumulation confers pathogenic features on macrophages. Notably, a subset of lipid-loaded macrophages has been recently identified in many tumor types. Lipid-loaded TAMs support tumor growth and progression and exert immune-suppressive activities. In this review, we describe the role of lipid metabolism in macrophage activation in physiology and pathology and we discuss the impact of lipid accumulation in macrophages in the context of cancer.

line, lipid accumulation impacts on metabolism and confers on macrophages a unique functional state.^{1,2} In the present review, we describe the impact of lipid metabolism on macrophage activation and we dissect the implications of lipid accumulation in TAMs.

MACROPHAGES IN PHYSIOLOGY

Macrophages show great heterogeneity in terms of origin, phenotypes and functional roles.³ Macrophages originate from the yolk sac during development and from the bone marrow post birth. Yolk sac-derived monocytic precursors relocate throughout the organism and differentiate into tissue-specific resident macrophages (F4/80^{hi}), like microglia in the brain, Kupffer cells in the liver, lung alveolar macrophages in lungs and osteoclasts in bone. The bone marrow is responsible instead for the generation of circulating monocytes (F4/80^{low}) that can infiltrate tissues and differentiate into mature phagocytic cells.^{3,4} Within different tissues, macrophages play essential and specific roles determined by particular gene-expression profiles.⁵

Overall, macrophages have a crucial function in the innate immune response, due to their strong antimicrobial and phagocytic properties, as well as physiological roles in development, tissue homeostasis and repair.^{3,6} Macrophages are characterized by a peculiar plasticity that allows them to modulate their activities in response to external activating stimuli (e.g., infective agents, developing tumors, etc). The polarization of macrophages is regulated by local concentrations of factors, including cytokines, chemokines, metabolites and lipids. According to a general view, activated macrophages can be polarized towards an inflammatory phenotype (referred to as M1 or classically activated macrophages, CAMs) or an anti-inflammatory one (M2 or alternatively activated macrophages). M1 macrophages are typically induced by cytokines like interferon

INTRODUCTION

Macrophages are phagocytic cells present in most tissues of the organism. If challenged with stimuli from the microenvironment, macrophages can acquire unique functional states that mediate a plethora of possible functions involved in development, tissue repair, killing of microbes and resolution of the inflammation. As key component of the innate immune response, macrophages represent a first defense of the body against pathogens. If challenged with pathogen-associated molecular patterns or damage-associated molecular patterns, macrophages acquire a proinflammatory state that results in the elimination of the pathogen. On the other hand, in the presence of immunomodulatory factors, macrophages switch towards a regulatory phenotype implicated in tissue homeostasis. Of note, macrophages represent the main infiltrating immune subset in most cancers and tumor-associated macrophages (TAMs) sustain tumor growth, promote invasion, mediate immune evasion and play a crucial role in cancer-related inflammation (CRI). Importantly, cellular metabolism is implicated in the education of macrophages and can dictate their phenotype. On this

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gamma (IFN- γ) (specifically produced by T helper 1-type lymphocytes), tumor necrosis factor alpha (TNF- α) or other molecules like bacterial lipopolysaccharide (LPS). Interleukin (IL)-4 and IL-13, which are secreted by T helper 2-type lymphocytes and share the same main receptor (IL-4R α) on macrophages, are common M2 polarizing agents. M1 and M2 macrophages differ for morphology, expression patterns and secretome, and they have antipodean functional roles. However, it is now clear that these two activation states cannot mirror completely a physiological condition, where these cells may instead acquire a wide set of phenotypic shades in response to the surrounding microenvironment.^{6–10}

Macrophage heterogeneity is evident when looking at their physiological activity. Developmental roles have been ascribed to macrophages in different contexts, starting from the general clearance of unnecessary cells undergoing apoptosis. Their phagocytic function is required for the removal of dismissed nuclei of erythrocytes, for bone reabsorption as osteoclasts and for hematopoietic steady-state maintenance in spleen and liver. Moreover, macrophages are crucial for angiogenesis modulation, trophic support in specific physiological locations (eg, brain microglia) and a potential regulatory activity of stem cell function and viability.³ When it comes to face an infection, macrophages are recruited to counter and eliminate pathogens. This occurs through the onset of an inflammatory condition established by CAMs through the secretion of proinflammatory cytokines (TNF- α , IL-1 α and IL-1 β , IL-6, IL-12, IL-23) and reactive oxygen species (ROS) that results in an effective antimicrobial response. Inflammation may result in tissue damage, with a consequent need for repair that is mediated by M2-like macrophages, which produce immunoregulatory cytokines including IL-10 and TNF- β that promote tissue remodeling and wound healing.^{6,8}

LIPID METABOLISM AND MACROPHAGE POLARIZATION

Activated macrophages show peculiar metabolic activities and are themselves able to influence metabolism through their products.¹¹ Lipid metabolism is able to condition macrophage functionality and is characterized by key pathways, enzymes and regulators.¹² A general categorization ascribes aerobic glycolysis and a split tricarboxylic acid (TCA) cycle to CAMs as preferred pathways to quickly generate ATP and sustain lipid biosynthesis, while stimuli that drive alternative activation favor fatty acid oxidation (FAO), complete TCA and oxidative phosphorylation (OXPHOS) pathways¹² (figure 1). Indeed, the role and distinction of these metabolic pathways in macrophage polarization might be more complex than generally described. This can be true for glucose consumption. Glucose uptake is fostered by M1-like reprogramming stimuli, and glycolysis, which also fuels an enhanced pentose phosphate pathway, is an essential and a fast source of energy for macrophages to sustain their inflammatory and phagocytic functionality.¹³ On

the other hand, recent studies support the hypothesis that also metabolism of M2-like macrophages partially relies on glycolysis. In this context, mTORC1 and mTORC2 seem to play an important role in glycolysis regulation,^{14,15} although probably not for macrophage polarization as much as the subsequent maintenance of M2-like features.^{16–18}

Fat supply in macrophages derives from endocytosis of lipids or from lipid intake mediated by scavenger receptors and lipoprotein receptors. If environmental levels are not sufficient, lipid biosynthesis takes place. M1-related inflammatory responses are sustained by fatty acid (FA) biosynthesis.¹⁹ In this process, acetyl-CoA is an essential component for the generation of compounds like cholesterol and FAs that in turn promote energy storage and production of inflammatory mediators.^{12,20} Accordingly, inhibition of lipid biosynthesis on genetic deletion of fatty acid synthase (FASN) in macrophages results in an altered plasma membrane and hinders macrophage proinflammatory activity.²¹ On this line, an interesting study by Im *et al* demonstrated in mice that genes coding for NRLP (NACHT, LRR, and PYD domains-containing protein) inflammasome components are under direct control of the transcription factor (TF) sterol regulatory element-binding protein 1a (SREBP-1a) in macrophages, thus linking lipogenesis to effective inflammatory activity of M1 macrophages on LPS stimulation.²² In contrast to M1 activation, the alternative activation of macrophages may be sustained by catabolism of lipids mediated by FAO. Inhibition of FAO by mean of the carnitine palmitoyltransferase (CPT)-1 inhibitor etomoxir hinders IL-4-mediated M2 polarization. In this context, depletion of CoA impacts on acetyl-CoA levels, with consequent negative effects on IL-4-dependent alternative activation of macrophages.²³ This comes in accordance with the potential epigenetic regulation that acetyl-CoA exerts on IL-4-responsive genes by inducing histone acetylation.¹⁴ Nevertheless, genetic ablation of CPT2 (CPT1 partner enzyme on the mitochondrial membrane) in macrophages blocked FAO while the expression of M2-related markers was retained, thus questioning the direct requirement of FAO for M2 polarization.²⁴ Notably, NOX4-mediated FAO has been reported to regulate NLRP3-dependent inflammasome activation and to modulate the release of proinflammatory factors including IL-1 β .²⁵ Interestingly, Hossain *et al* stated that FAO may represent a direct target for tumor therapy, since its inhibition reduces the immunosuppressive properties of tumor-infiltrating myeloid-derived suppressor cells.²⁶ In contrast, activation of Caspase 1 in TAMs in a murine model of breast cancer drives the upregulation of the medium-chain acyl-CoA dehydrogenase and hinders FAO. Resulting lipid droplet accumulation confers protumorigenic activity on TAMs, as discussed below. Blockade of this axis restores FAO and promotes tumor inhibition.²⁷ This conflicting evidence challenges the current knowledge on lipids and macrophages and additional research is needed to dissect the exact role of FAO in macrophage activation in health and disease.

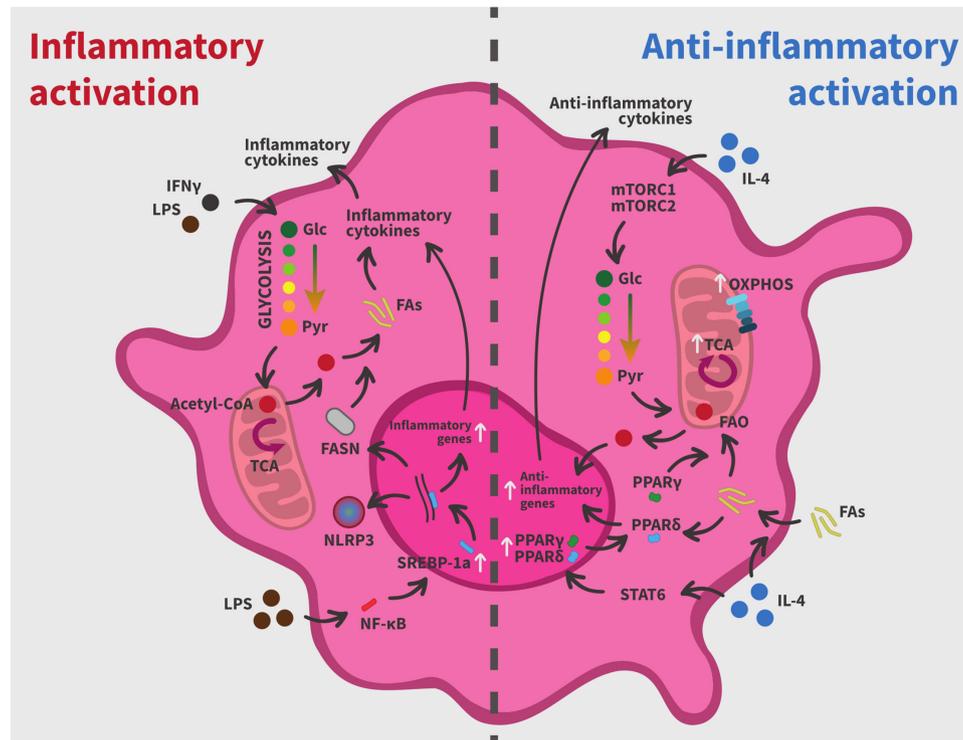


Figure 1 Macrophage metabolism and functional activation. Inflammatory activation: on exposure to stimuli like lipopolysaccharide (LPS) or interferon γ (IFN- γ), macrophages mainly use glycolysis as a fast source of energy to support their proinflammatory functions. Acetyl-CoA has a multiple role, since it fuels a split tricarboxylic acid (TCA) cycle (with the production of metabolites sustaining inflammatory functions as itaconate and succinate) and the synthesis of fatty acids (FAs), building blocks for inflammatory mediators. In parallel, LPS stimulation can induce sterol regulatory element-binding protein 1 a (SREBP-1a) expression through nuclear factor kappa B (NF- κ B) activity. SREBP-1a promotes several transcription programs, including those for components of NLRP3 inflammasome, fatty acid synthase (FASN) as well as other proinflammatory genes. Anti-inflammatory activation: on interleukin 4 (IL-4) stimulation, FAs intake increases and the transcription factor STAT6 is activated, with consequent promotion of peroxisome proliferator-activated receptors PPAR δ and PPAR γ expression. PPAR δ can sense the increased influx of FAs and subsequently induce transcription of anti-inflammatory genes, while PPAR γ mainly facilitates fatty acid oxidation (FAO). In the mitochondria, a complete TCA cycle occurs and fuels oxidative phosphorylation (OXPHOS) for energy production. This process can be supported also by products of glycolysis, which is induced at modest levels by the activity of mTORC1 and mTORC2 after IL-4 stimulation.

Regulators of lipid metabolism in macrophages

Sterol regulatory element-binding proteins

Among the regulators of lipid metabolism, SREBPs play a pivotal role. This family of nuclear TFs is composed of three isoforms, among which SREBP-1a is mainly found in immune cells, while SREBP-1c is liver-specific and SREBP-2 is expressed by different tissues. All of them are produced as inactive precursors retained in the endoplasmic reticulum (ER), and then activated by proteolytic cleavage and moved to the nucleus.²⁸ Here, SREBPs exert their function by modulating many genes involved in lipogenesis and metabolic adaptation to external stimuli. In particular, SREBP-1c is highly insulin-sensitive, and it can foster transcription of lipogenic genes such as acetyl-CoA carboxylase and FASN; its upregulation has been also associated with the metabolic syndrome.²⁹ As mentioned above, SREBP-1a is abundant in macrophages. After LPS stimulation, nuclear factor- κ B (NF- κ B) TF fosters SREBP-1a, which can in turn directly sustain M1 activation and functionality.²²

Peroxisome proliferator-activated receptors

While SREBPs activity has implications in proinflammatory macrophages, other metabolic regulators such as peroxisome proliferator-activated receptors (PPARs) have a particular influence on M2-like functionality. PPARs consist of three subtypes, PPAR α , PPAR γ and PPAR δ , which are present in different cell types and have transcriptional regulatory roles in many aspects of FA metabolism. Generally, PPAR α and PPAR δ generally regulate FAO in peripheral tissues, while PPAR γ is essential for the storage of FAs as triglycerides in adipocytes.³⁰ In macrophages, PPAR γ and PPAR δ are highly expressed and involved in both lipid metabolism and M2-like transcriptional programs. These factors are activated by and synergize with stimuli like IL-4 and IL-13 to support macrophage polarization. Specifically, these stimuli lead to tyrosine phosphorylation of STAT6, which translocates to the nucleus and promotes transcription of PPAR γ and PPAR δ . PPAR δ can sense FAs and sustain transcription of M2 essential markers like *Arg1*. PPAR γ , in turn, induces

an increase in FAO and biogenesis of mitochondria.^{12 30 31} This specific role of PPAR γ has been shown to be crucial for alternative activation of macrophages, which perform effective FA storage and oxidation, with a consequent prevention of insulin resistance and protection from effects of obesity and metabolic syndrome.³²

Mammalian target of rapamycin

The mechanistic/mammalian target of rapamycin (mTOR) is a serine/threonine kinase capable of sensing both extracellular and intracellular signals, and it is involved in a wide range of downstream pathways, from cell growth and proliferation to metabolic adaptation to stimuli. This kinase is present in the cell in two complexes, mTORC1 and mTORC2, each one with specific functions and interactors. In macrophages, mTOR emerges in the regulation of inflammation as well as lipid metabolism and glycolysis.³³ In particular, mTORC1 mainly operates in an axis culminating in SREBP activation, with subsequent transcription of lipogenesis-related genes and support to inflammation.³⁴ Interestingly, Covarrubias *et al* shows that mTORC1 can be activated also by IL-4 to enhance glycolysis and synthesis of acetyl-CoA, which regulates histone acetylation and transcription of M2-related genes.¹⁴ Likewise, mTORC2 can foster glycolysis and acetyl-CoA production in an M2-like context, to ultimately sustain FAO and OXPHOS, as well as M2-related gene expression.¹⁵

Altogether, this evidence sustains the concept of a connection between lipid metabolism and macrophage activation and supports the idea of lipid manipulation as therapeutic approach in macrophage-dependent diseases, including obesity and cancer. Still the role of metabolism in macrophages appears complex and context-dependent, and further investigation is needed to elucidate the impact of lipids on macrophage functionality. Moreover, the identity and the metabolic status of macrophages is affected by the residing tissue, and metabolic heterogeneity should be taken in consideration when considering to target macrophage metabolism for therapeutic purposes.¹³

MACROPHAGES IN CANCER

Over the last decades, increasing knowledge on tumors has unveiled their complex and multifactorial nature. To grow and proliferate, tumors require specific, variegated and consequential stimuli and support from the surrounding elements within the tissue they originate from. Indeed, the tumor microenvironment (TME) is composed of stromal cells, fibroblasts, endothelial cells, as well as immune cells related to both adaptive and innate immunity.³⁵ Among tumor-infiltrating leukocytes, macrophages often constitute the most relevant portion and their abundance at the tumor bed has been associated with a poor prognosis in many tumor types. In this context, these cells are referred to as TAMs, due to the peculiar polarization and functionality that follow

neoplastic development phases. Once re-educated by the tumor, macrophages sustain tumor growth and progression, promote invasion and metastasis and mediate immune evasion.^{36 37}

TAMs primarily originate from bone marrow-derived circulating monocytes (Ly6C⁺) that are recruited to the primary tumor site and then undergo differentiation.³⁸ It may be possible that these macrophages display a proinflammatory status at the beginning, thus promoting tumor-related inflammation and tumor initiation and are polarized towards an immunosuppressive phenotype once the tumor is established. This transition is driven by T helper 2-secreted cytokines, including IL-4 and IL-13, by interactions with B cells and fibroblasts, and by tumor-secreted products such as macrophage colony-stimulating factor, transforming growth factor β and others.¹⁰ For example, if exposed to CXCL2 (chemokine (C-X-C motif) ligand 2) released from tumor cells, TAMs acquire immunosuppressive and proangiogenic features in prostate cancer.³⁹ However, increasing evidence demonstrate that tumors are infiltrated by different subpopulations of TAMs, each one with distinct and even contrasting features. Application of single-cell RNA sequencing (scRNAseq) approaches identified a subset of TAMs that correlates with poor prognosis in liver cancer, in which the protumoral and proinflammatory polarization states coexist.⁴⁰ Accordingly, a study led by Zhang and colleagues on spinal cord tumors showed the contemporary presence of *CCL2*⁺ TAMs with high immune-response capability and *CD44*⁺ TAMs expressing angiogenesis-related genes and interacting with fibroblasts, pericytes and endothelial cells to promote tumor angiogenesis.⁴¹

A crucial role in determining the complexity and heterogeneity of TAMs phenotype is played by the metabolic features of the TME that are dictated by a low oxygen tension, nutrient deprivation, acidity and metabolic waste.^{42 43} Within the hypoxic microenvironment, metabolites like lactate are sensed by several cell types, generally suppressing immune functions.⁴⁴ Indeed, hypoxic MHC¹⁰ TAMs use extracellular lactate to fuel TCA and respiration,⁴⁵ and in particular tumor-released lactate sustains TAMs' protumor functions through hypoxia-inducible factor 1 α .⁴⁶ Interestingly, TAMs have the highest uptake of glucose within the TME, and glycolysis is thought to support the maintenance of TAMs' protumoral features in certain contexts.^{18 47} The phenotype and differentiation of TAMs is also dictated by nutrients and metabolites released by cancer cells. In sarcoma, tumor cell-derived retinoic acid has been reported to mediate the differentiation of tumor-infiltrating monocytes to macrophages that suppress T-cell-mediated antitumor immunity, rather than to antitumoral dendritic cells (DCs).⁴⁸ Furthermore, increasing relevance has been acquired by tumor-released lipids and related by-products, as it will be discussed thoroughly in the next chapters. As an example, β -glucosylceramide is reported to induce stress responses in macrophages and activate signaling pathways leading to protumor functionality.⁴⁹

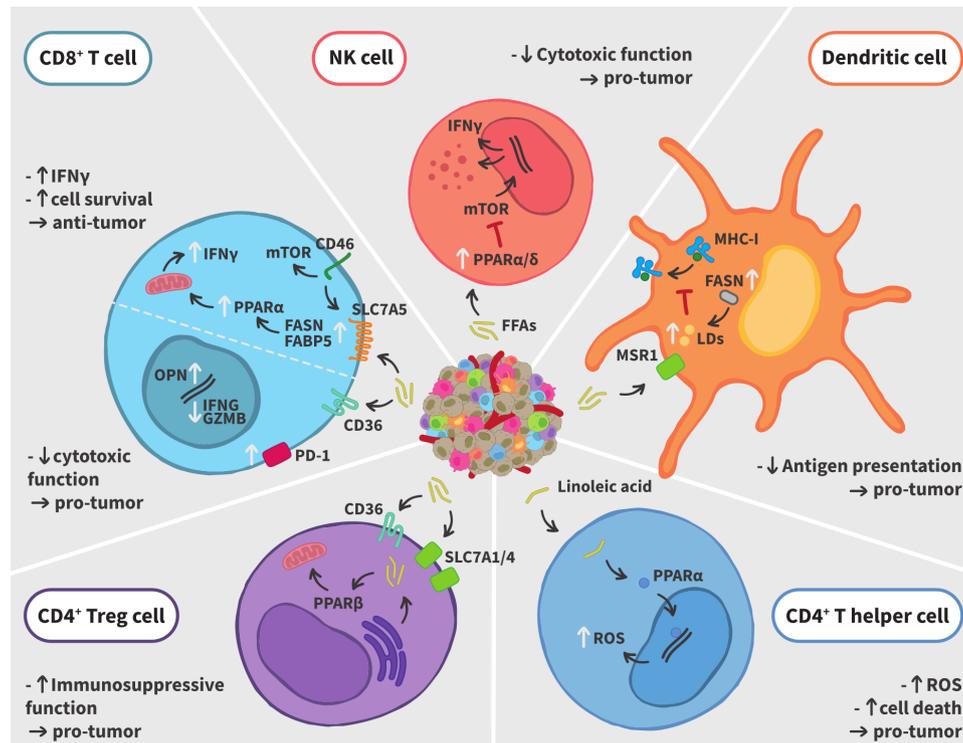


Figure 2 Influence of lipids on tumor-infiltrating immune cells. Natural killer (NK) cells: a free fatty acid (FFA)-rich environment upregulates peroxisome proliferator-activated receptors PPAR α and PPAR δ , which in turn inhibit mammalian target of rapamycin (mTOR)-mediated pathways, including transcription of cytotoxic granules and interferon γ (IFN- γ), with deriving promotion of immune tolerance. Dendritic cells (DCs): lipid uptake through Msr1 receptor leads to accumulation of lipid droplets (LD), which are responsible for the defective translocation of major histocompatibility complex class I (MHC-I) complex to the cell surface, thus impairing DC antigen presentation and subsequent priming of CD8⁺ T cells' antitumor activity. Likewise, fatty acid (FA) synthesis upon fatty acid synthase (FASN) upregulation can hamper correct DC functioning. CD4⁺ T helper cells: linoleic acid activates PPAR α , whose downstream pathways determine the accumulation of reactive oxygen species (ROS), which in turn cause CD4⁺ T cell apoptosis. CD4⁺ regulatory T (Treg) cells: lipids enter the cell through transporters like CD36 (Cluster of differentiation 36), SLC7A1 (Solute Carrier Family 7 Member 1) and SLC7A4 (Solute Carrier Family 7 Member 7). Together with synthesized ones, lipids activate PPAR β that sustains mitochondrial activity and oxidative phosphorylation (OXPHOS), with FFAs as main substrate. This supports Treg survival and immunosuppressive functions. CD8⁺ cells: FFAs can impair cytotoxic activity by inducing upregulation of tumor-tropic genes (*Opn*), downregulation of cytotoxic genes (*Ifng* and *Gzmb*) and programmed cell death protein 1 (PD-1) expression. Intake of FFAs can be mediated by CD36 with downstream pathways impairing cell functionality, like lipid peroxidation and ferroptosis. In contrast with this, CD46 transmembrane molecule can increase FASN, FABP5 (Fatty acid binding protein 5) and SLC7A5 (Solute Carrier Family 7 Member 5) levels, thus promoting FFA intake and metabolism. FFAs induce PPAR α activation, with subsequent support to mitochondrial activity that promotes release of molecules like IFN- γ . CD46 is also responsible for mTOR-mediated FA reprogramming, which is essential for CD8⁺ T cells' effector function and survival.

LIPIDS AND TME

Lipid accumulation in tumor-infiltrating immune cells

Within the TME, cells are forced to rearrange their metabolism to provide suitable conditions for tumor growth and survival. This is especially true for tumor-associated immune cells, where metabolic changes are addressed to both tumor support and immune evasion (figure 2).⁵⁰ Among the abovementioned features of TME, like acidosis, hypoxia and nutrient deprivation, dysregulated lipid metabolism strongly contributes to cancer cell survival and aggressiveness.⁵¹ In addition, the deregulation of lipid metabolism in cancer cells influences metabolic activity of infiltrating immune cells, often fostering immune-regulatory events as well as tumor growth.⁵²

CD4⁺ conventional T cells and CD4⁺ T regulatory cells

Within the TME, tumor antigen-specific CD4⁺ T cells are defined as helper T cells, for they can modulate functionality and effector activities of cytotoxic T lymphocytes as well as DCs and B cells.⁵³ In a mouse model of non-alcoholic fatty liver disease-driven hepatocarcinoma, lipid accumulation in CD4⁺ T lymphocytes caused their death.⁵⁴ Mechanistically, the intake of hepatocyte-derived linoleic acid by CD4⁺ T lymphocytes activates PPAR α and induces ROS production and apoptosis, in turn promoting hepatocarcinogenesis.⁵⁵ It is known that the increased and unbalanced levels of ROS are not only responsible for CD4⁺ T cell apoptosis but also for general immune-suppressive consequences throughout the TME.⁵⁶ Among CD4⁺ lymphocytes, regulatory T cells

(Tregs) can inhibit antitumor immunity in the context of TME, thus promoting tumor development and progression.⁵⁷ Treg's metabolic adaptation to the hostile tumor environment is a combination of glycolysis and FA synthesis and oxidation.^{58,59} In particular conditions, such as hypoxia, Tregs can rely on free fatty acids (FFAs) as a major substrate for OXPHOS.⁶⁰ More in general, intracellular lipid accumulation, mainly due to an increased rate of FA synthesis and glucose uptake, can confer a proliferative advantage on Tregs to the detriment of conventional T cells.⁵⁸ Lipid uptake can also take place by means of FA transporters. Among them CD36, SLC27A1 and SLC27A4 are specifically upregulated in intratumor Tregs.^{60,61} In particular, we uncovered a specific CD36-PPAR β pathway that is essential for Tregs survival and immunosuppressive functionality, by affecting directly their mitochondrial fitness.⁶¹ Moreover, FA supply or biosynthesis may affect the epigenetic profile of Tregs since they are employed as preferential source of acetyl groups for histone acetylation.⁶² As for lipid biosynthesis, a recent work also unveiled the crucial role of SREBPs, which are upregulated in intratumor Tregs and promote FA production, with a subsequent support to cell-mediated immunosuppression.⁶³ Interestingly, another study reported that the inhibition of the FA-binding protein FABP5 disrupts lipid metabolism and hinders OXPHOS in intratumoral Treg cells, thus provoking mitochondrial stress and mitochondrial DNA release. Consequent activation of the cGAS-STING pathway augments IL-10 production and sustains Treg's immunosuppressive function (from *in vitro* experiments in mouse and human-derived Tregs).⁶⁴ Collectively, these potentially contrasting data may indicate that a fine tuning of lipid metabolism is determinant for Tregs activation in cancer, and these cells may follow different routes to carry out their functions, depending on the specific TME context and lipid availability.

CD8⁺ T cells

CD8⁺ T cells are the major effector cells in tumor immunity. On activation by APCs (Antigen presenting cells), they migrate to the tumor tissue to kill target cells through the release of lytic granules and cytokines.⁶⁵ Cytotoxic CD8⁺ T cells exhibited an impaired antitumor activity when exposed to a FFA-enriched environment.⁶⁶ Moreover, in a murine breast cancer model, high fat diet (HFD)-fed mice accumulate at the tumor site PD-1⁺ CD8⁺ exhausted T cells, possibly because of the presence of cytokines associated with obesity-induced inflammation. These cells present higher levels of *Opm*, a tumor-trophic gene, and lower levels of *Ifng* and *Gzmb*, cytotoxic genes, compared with PD-1⁻ CD8⁺ non-exhausted T cells.⁶⁷ In contrast, in a mouse model of melanoma, FFAs determine a metabolic switch of tumor-infiltrating CD8⁺ T lymphocytes (TILs) towards FAO that contributes to energy production and promotes the secretion of effector molecules, such as IFN- γ .⁶⁸ On this line, recent evidence show that CD46 engagement in human CD8⁺ T cell (CTL) determines mTOR-mediated activation of FA reprogramming, which

is critical for proliferation, effector functions and survival of tissue-resident CD8⁺ T cells.⁶⁹ De novo lipogenesis also contributes to T cell function. Indeed, acetyl coenzyme carboxylase, which regulates both biosynthesis and breakdown of long-chain FAs, plays an indispensable role in the accumulation of antigen-specific murine CD8⁺ T cells by influencing survival of proliferating cells.⁷⁰ Of note, the microbiota-derived short-chain fatty acid (SCFA) butyrate promotes long-term survival of mouse CD8⁺ T cells as well as memory cells, through a metabolic switch towards an OXPHOS mainly fueled by glutamine utilization and FA catabolism.⁷¹ Finally, the antitumor response of CD8⁺ T cells can be improved by modulating cholesterol metabolism. Indeed, inhibition of cholesterol esterification in mouse models leads to enhanced effector functions and increase in the levels of plasma membrane cholesterol favors the formation of the immunological synapse.⁷² On the other hand, it has been shown in studies on both human samples and mouse models that uptake of tumor cholesterol by TILs causes XBP1-mediated ER stress response that promotes exhaustion and impairs antitumor immunity.⁷³ Cholesterol is also responsible for CD36 upregulation in CD8⁺ T cells, with subsequent increase in FFA uptake and activation of downstream pathways like lipid peroxidation and ferroptosis, which impair antitumor activity.^{74,75} More studies are needed to understand the differences between intracellular and extracellular cholesterol in modulating CD8⁺ T cell functions in the TME.

Dendritic cells

DCs are key regulators of T cell immunity; therefore, normal DC function is essential for achieving T cell-mediated tumor clearance. The constitutive activation of FASN in tumor cells leads to abnormal lipid accumulation in tumor-infiltrating DCs and subsequent inhibition of their ability to present antigens and prime T cells, thus hampering their antitumor functions.⁷⁶ Moreover, obesity *per se* has been associated with an impairment of DC functions and accumulation of regulatory DCs in mice.⁷⁷ This impaired cross-presentation in DCs is mainly associated with defective trafficking of peptide-major histocompatibility complex class I (MHC-I) complexes to the cell surface (in both human and mice).⁷⁸ Accordingly, lipid peroxides in the TME drive an XBP1-mediated ER stress response in intratumoral DCs, inducing triglyceride biosynthesis, lipid droplet formation and impaired antigen cross-presentation.⁷⁹ Lipid-loaded DCs are also generated on increased modified lipoprotein uptake from plasma through the scavenger receptor Msr1, specifically induced by tumor-derived factors.⁸⁰ Interestingly, previous studies reported that also depletion of lipid bodies from DCs impairs antigen presentation by MHC-I.⁸¹ Additionally, de novo synthesis of FAs after stimulation through Toll-like receptors has been identified as needed for DCs proper activation.⁸² All these results further outline the complexity of lipid metabolism in immune regulation.

Natural killer (NK) cells

NK cells are innate immune cells that show strong cytolytic function against physiologically stressed cells such as tumor cells and virus-infected cells. To date, few studies have explored the impact of lipid exposure on their function and persistence. In human and murine obesity, the PPAR α/δ target genes are highly upregulated causing the inhibition of mTOR-mediated glycolysis and transcription of cytotoxic granules and IFN- γ .⁸³ Accordingly, an alteration of NK cell compartment has been noticed in esophageal adenocarcinoma (OAC), a type of obesity-associated tumor. The omental and hepatic microenvironments of patients with OAC significantly affect number and function of NK cells, mainly shifting them towards a more IL-10⁺ regulatory and anti-inflammatory state.⁸⁴ Lastly, models of obese mice with KRAS mutation allowed to identify a severe loss of the NK cell compartment at the preneoplastic stage of pancreatic cancer, thus paving the way for pancreatic tumor progression. In this case, the specific release of IL-6 by peripancreatic adipose tissue is one of the drivers of NK cell suppression.⁸⁵

Lipid accumulation in tumor-associated macrophages

Foamy macrophages are central players in metabolic-related diseases, such as atherosclerosis, obesity and fatty liver disease. Lipid-laden macrophages lately gained the attention of cancer research as recent reports provided evidence of the infiltration of macrophages enriched in lipid droplets in tumors, including melanoma, colon, gastric and prostate cancer.^{49 86–88} Wu and colleagues reported that lipid accumulation in TAMs infiltrating colon cancer in mouse is associated with a metabolic reprogramming. Tested in vitro, macrophages exposed to unsaturated FAs augment their lipid content that in turn acts as source of FAs to sustain the mitochondrial respiration capacity of macrophages. Such metabolic switch is regulated by the mTOR signaling and induces an anti-inflammatory phenotype in macrophages, shown by an increased expression of CD206 and arginase among other protumor markers.⁸⁶ In accordance with these findings, an accumulation of foamy macrophages showing immunosuppressive features and loss of phagocytic activity has been reported recently in a model of gastric cancer. Here, macrophages were shown to accumulate lipids on tumor conditioning in vitro. These lipids upregulate phosphoinositide 3-kinase- γ (PI3K- γ) expression, which in turn leads to increased programmed death-ligand 1 exposure and protumor effects.⁸⁷ An important support to this evidence comes from scRNAseq analyses performed in different tumor contexts. For instance, Pombo Antunes *et al* unveiled the presence of a cluster of TAMs with a unique gene signature associated with phagocytosis and lipid metabolism, in both mouse models and human samples of glioblastoma.⁸⁹ Again, a population of lipid-associated TAMs was found in lung metastases in mice with orthotopic mammary tumor,⁹⁰ while Zhou and colleagues linked macrophages with a lipid-related signature to an increased expression of the innate immune receptor

TREM2 in human hepatocellular carcinoma.⁹¹ TREM2 has anti-inflammatory and immune-suppressive effect, and it had been previously associated with macrophages in the adipose tissue of obese individuals and mice.⁹² Lipid accumulation can also cause macrophage death by inducing apoptosis, as shown in a recent work on large-peritoneal macrophages (LPMs). Here, the authors showed that retinoid X receptors (RXRs) normally prevent these cells from lipid accumulation, and RXRs deficiency not only impairs LPMs survival but also impedes their early infiltration in murine ovarian tumors, with subsequent reduction of tumor progression.⁹³ If lipid accumulation has become an established feature of TAMs, the mechanisms of lipid intake and the source of lipids remain mostly unexplored. In a recent work, we uncovered a role for tumor-derived lipids in TAM activation. β -glucosylceramide released by murine melanoma cells triggers an unconventional stress response in macrophage ER: this response reshuffles the lipid composition of the ER itself and induces IRE1 (Inositol-requiring enzyme 1)-mediated activation of XBPI (X-box binding protein 1) and STAT3 (Signal transducer and activator of transcription 3) pathways, which drive macrophage protumor functions.⁴⁹ We and others contributed to unveil that scavenger receptors are upregulated in macrophages infiltrating the tumor bed and are implicated in lipid intake. Su *et al* identified lipid-laden macrophages across several tumor types. In these models, TAMs express high levels of CD36 that is implicated in lipid scavenging and consequently augmented FAO and OXPHOS. The enhanced mitochondrial OXPHOS generates energy that fuels the protumorigenic functionality of macrophages, regulated by STAT6 phosphorylation.⁹⁴ On the same line, we demonstrated that MARCO (Macrophage receptor with collagenous structure) overexpression by macrophage-infiltrating human and conditional mouse models of prostate cancer is involved in lipid intake and lipid droplet accumulation. We unveiled a heterotypic signaling between cancer cells and infiltrating macrophages that relies on IL-1 β release from cancer cells and consequent secretion of CCL6 (CC motif ligand 6) by macrophages that sustains tumor invasiveness. MARCO inhibition by means of a monoclonal antibody promotes tumor inhibition in models of prostate cancer. Importantly, we reported that exposure of tumor-bearing mice to HFA results in increased accumulation of lipids by macrophages, and consequent reprogramming at the transcriptional level.⁸⁸ Of note, lipogenesis may also be determinant in TAMs. Thanks to transcriptome and metabolome analyses on human macrophages exposed in vitro to tumor cells, Rabold and colleagues revealed the upregulation of lipid biosynthesis pathways and the increase of overall lipid content of TAMs. This metabolic switch is associated with ROS production and the release of proinflammatory mediators, which may be involved in CRI.⁹⁵ Finally, macrophages can release accumulated lipids and associated products, thus influencing back tumor progression, as shown in a model of castrate-resistant prostate cancer. In this context,

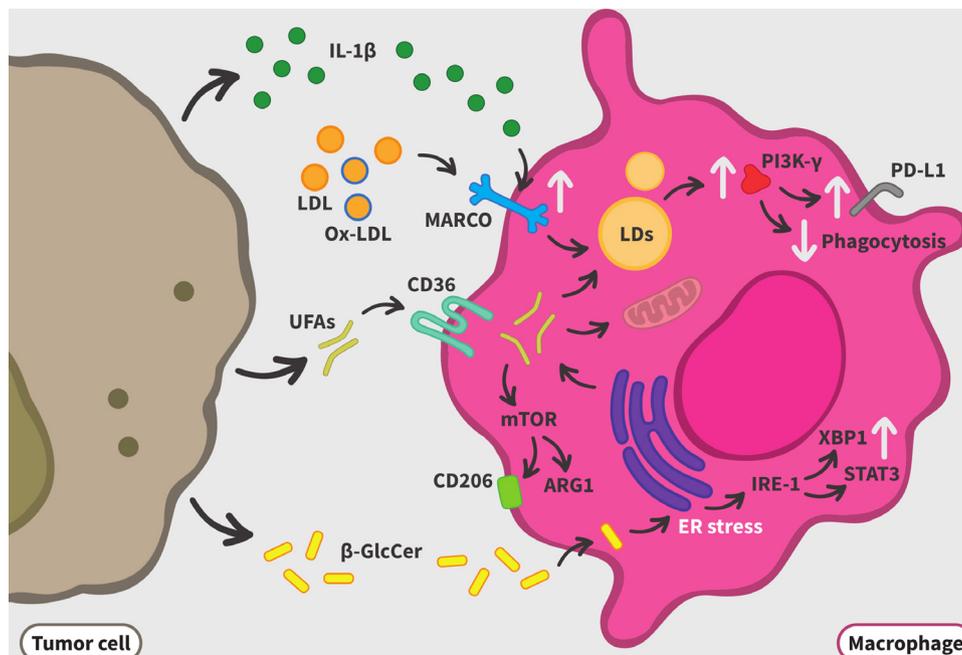


Figure 3 Mechanisms of interaction between cancer and lipid-loaded tumor-associated macrophages (TAMs).

Macrophages can assume tumor-released unsaturated fatty acids (UFAs) through specific receptors like CD36. These FAs, together with synthesized ones, sustain mitochondrial activity and determine lipid droplet (LD) formation. Lipid accumulation also supports the activation of phosphoinositide 3-kinase- γ (PI3K- γ), whose downstream pathways brings to decreased phagocytic capability and increased programmed death ligand 1 (PD-L1) expression. LDs are enriched also by the uptake of low-density lipoproteins (LDLs) and oxidized LDLs (Ox-LDLs) through MARCO scavenger receptor, whose expression is increased by tumor-secreted interleukin-1 β (IL-1 β). FAs stimulate mammalian target of rapamycin (mTOR) activation, leading to upregulation of M2-like markers like CD206 and arginase-1. Finally, tumor-released β -glucosylceramide (β -GlcCer) can be assumed by macrophages. β -GlcCer promotes endoplasmic reticulum (ER) stress, with subsequent induction of IRE-1 (Inositol-requiring enzyme 1)-mediated pathways, among which the activation XBP1 and STAT3 support protumor functions.

TAMs are particularly rich in cholesterol, precursor of steroid hormones that can be transferred to tumor cells, sustaining androgen biosynthesis and tumor resistance to therapies.⁹⁶ These findings are in accordance with previous works that confirm the favorable role of cholesterol efflux from murine macrophages for tumor progression. Nevertheless, the authors underline that this efflux affects primarily macrophage polarization, thus pointing out that its inhibition and the subsequent cholesterol accumulation can reprogram macrophages toward a proinflammatory and antitumoral activation.^{97–98} This could be in contrast with previous statements, but in fact it may confer on cholesterol and its metabolic pathway a unique function among the other lipids in TAMs.

Overall, the evidence reported above confirms the crucial role that lipid metabolism plays in macrophages within the TME, creating a vicious circle together with tumor cells (figure 3). The tumor takes advantage of lipids for its development and influences the surrounding immune cells. On the other hand, increased lipid intake or lipid-induced signaling pathways confer protumor functions on TAMs, thus supporting tumor proliferation and invasiveness.

TARGETING LIPID-RELATED PATHWAYS IN TAMs

As macrophages sustain most cancers, a strong interest exists in developing therapeutic approaches that target

TAMs to hinder their protumoral functions. Indeed many promising strategies are currently under preclinical and clinical investigation. Agents that suppress monocytes recruitment, including CCR2 (C-C Motif chemokine receptor 2), CCR5 (C-C Motif chemokine receptor 5) and CXCR4 (C-X-C chemokine receptor type 4) antagonists, have proven to be efficient in certain contexts. Moreover, direct TAMs depletion, by means of colony-stimulating factor 1 receptor (CSF1R) inhibitors or anticancer drugs such as trabectedin, showed clinical efficacy.⁹⁹ Importantly, macrophages may be exploited against cancer and therapies that can re-educate TAMs toward an antitumorigenic functional state hold strong promise. Accordingly, histone deacetylase and phosphoinositide 3-kinase (PI3K)- γ inhibitors, CXCR2 antagonists and CD40 agonists among others, that turn macrophage activation and confer antitumoral activities on TAMs, have proven to be effective in preclinical settings and may offer future therapeutic options.^{39–99} CSF1R inhibitors are currently under clinical development for their described capability of depleting or repolarizing macrophages, depending on the tumor context.¹⁰⁰ Metabolic rewiring of TAMs may represent an effective option to tune macrophage function and to confer on TAMs a protumoral role. Interestingly, blockade of CSF1R also impacts on macrophage metabolism and restores glycolysis.¹⁰¹ Accordingly,

promotion of glycolysis through glutamine synthetase inhibition switches macrophages toward a proinflammatory state and results in a marked reduction in metastasis formation in LLC (Lewis lung carcinoma) bearing mice.¹⁰² This concept though does not hold true for every cancer and glycolysis sustained by tumor-derived lactate has been described to support TAMs in certain tumors.⁴⁶ We are at the beginning of understanding the extent of the role of metabolism on the TME, and tissue macrophage metabolism is an area of intense investigation, as reviewed previously by others.¹³ In this context, lipid metabolism represents an additional promising target to rewire TAM function. As discussed above, lipid handling by macrophages can determine cancer invasion and progression and lipid accumulation confers protumoral capabilities on macrophages in certain contexts. Accordingly, administration of a specific inhibitor of DGAT (acyl CoA:diacylglycerol acyltransferase) (iDGAT), aimed at disrupting lipid droplets accumulation in cells, redirects macrophage phenotype toward an antitumor state and results in tumor inhibition in a model of colon cancer.⁸⁶ Lipid accumulation in macrophages infiltrating gastric cancer leads to the upregulated expression of PI3K- γ that in turn shifts TAMs' polarization toward alternative activation. Correspondingly, pharmacological targeting of PI3K- γ with a selective inhibitor promotes tumor regression in a preclinical gastric cancer model, associated with macrophages rewiring and T cells activation.⁸⁷ As mentioned above, lipid accumulation in TAMs infiltrating melanoma in mice triggers a IRE1-dependent ER stress response, which in turn facilitates protumorigenic polarization of macrophages. As a consequence, ER stress response and lipid reshuffling of the ER membrane represent potential targets to limit the deleterious effect of lipids on TAMs. Accordingly, administration of the LXR (Liver X receptor) agonist GW3965 to BMDMs (Bone marrow derived macrophages) results in induction of LPCAT3, an enzyme that restricts lipid-overloading-induced ER stress, with consequent inhibition of macrophage-immunosuppressive activity.⁴⁹ It has nevertheless to be noticed that DGAT and PI3K inhibition, as well as LXR activation, in the mentioned models, may target cell subsets other than TAMs and whether these approaches have a direct effect on lipid-loaded TAMs still need to be elucidated. On this regard, targeting scavenger receptors may offer a specific approach to hinder lipid accumulation in TAMs. We reported that MARCO expression mediates lipid uptake in macrophages in models of prostate cancer and MARCO blockade by mean of a monoclonal antibody promotes tumor inhibition. Importantly, we showed that MARCO expression is restricted to macrophages in the TME, and MARCO genetic and pharmacological inhibition reduces lipid accumulation in TAMs and redirects TAMs toward an antitumor profile, with consequent NK cell activation.⁸⁸ On the same line, CD36 genetic deletion stops lipid scavenging by TAMs and has shown antitumor efficacy in models of myeloma and lymphoma.¹⁰³ Importantly,

TAMs heterogeneity suggests that the approach to target lipid metabolism in macrophages should be tailored on the tumor type. As mentioned above, cholesterol efflux from macrophages confers on macrophages protumoral features. Accordingly, ABGG1 (ATP Binding Cassette Subfamily G Member 1) genetic deletion in TAMs makes them proinflammatory and improves cancer outcome in melanoma and bladder cancer models.⁹⁸ In accordance with these data, decrease of cholesterol efflux through deletion of ABCA1 (ATP Binding Cassette Subfamily A Member 1) and ABCG1 in myeloid cells impairs tumor progression in ovarian cancer models.⁹⁷

FINAL REMARKS AND FUTURE PERSPECTIVES

Lipid metabolism is acquiring considerable importance in cancer research for its role in the TME. Lipid intake and biosynthesis dictate the activation of TAMs, and lipid droplet accumulation confers protumorigenic and immunosuppressive activities on macrophages in several tumors. In parallel, lipids derived by macrophages sustain tumor progression. Still the identity and source of lipids in the TME needs to be further explored. Also, the impact of diet on lipid metabolism of TAMs remains elusive and deserves investigation. Overall, a broad range of potential targetable players in lipid metabolism has the chance to be searched for benefits in cancer treatments, and good results are on the way. Thus, being able to target and change metabolism in TAMs is a new promising strategy and a novel immunotherapy approach.

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