Journal of
Clinical & Cellular Immunology

Fraternale et al., J Clin Cell Immunol 2015, 6:2
DOI: 10.4172/2155-9899.1000319

Review Article Open Access

Polarization and Repolarization of Macrophages

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Received date: March 06, 2015, **Accepted date:** April 08, 2015, **Published date:** April 15, 2015

Abstract

Macrophages can acquire distinct phenotypes and biological functions depending on the microenvironment and the metabolic state. Macrophages classically activated by IFN-γ and/or microbial products display an M1 phenotype characterized by high expression of interleukin (IL)-12, inducible nitric oxide synthase (iNOS) and TNF-α. They trigger a Th1 immune response and are generally considered potent effector cells which kill microorganisms and tumor cells. Alternatively activated or M2 macrophages are stimulated by IL-4, IL-10, IL-13, immune complexes, or glucocorticoids. M2 phenotype is characterized by low iNOS and IL-12 production and triggers Th2 response. M2 macrophages are involved in tissue repair and angiogenesis. M1 and M2 macrophages display differences in metabolism and reduced glutathione (GSH) concentration. This review describes the main macrophage phenotypes with a major focus on the differences in protein, glucose and lipid metabolism. Moreover, repolarization of macrophages as potential therapeutic approach and the critical role of GSH in regulating repolarization are discussed.

Keywords: Macrophages; Myeloid cells; T lymphocytes; *Mycobacterium tuberculosis*

Introduction

Macrophages are a heterogeneous population that play a fundamental role in the immune system with important functions in both innate and acquired immunity. In innate immunity the main function of macrophages is to respond to pathogens and to modulate the adaptive immune response through antigen processing and presentation. Macrophages collaborate with T and B cells, through cell-to-cell interactions and through the release of cytokines, chemokines, enzymes, arachidonic acid metabolites, and reactive radicals; but macrophages also fulfill several homeostatic functions [1]. Macrophage activation can be either pro-inflammatory or anti-inflammatory, thus contributing to tissue destruction or regeneration and wound healing [2,3]. Macrophages originate from monocytes that move from the blood stream to the peripheral tissues. Here, monocytes differentiate into macrophages and dendritic cells thanks to exposure to local growth factors, pro-inflammatory cytokines and microbial compounds [4]. Recent data indicate that there are at least two other lineages of macrophages in the mouse which arise at different stages of development and persist to adulthood. The major tissue resident population of macrophages in skin, spleen, pancreas, liver, brain and lung arise from yolk sac progenitors; adult Langerhans cells are produced by fetal liver, likely through a progenitor that is derived from the yolk sac [5]. Macrophages are critical effector cells in the acute innate response for T cell mediated immunity. In 1986 two different mouse T helper (Th) cells, Th1 and Th2, were defined both by cytokine secretion and immune functions [6,7]. Th1 cells secreted interleukin (IL)-2, interferon-γ (IFN-γ) as well as lymphotoxin [LT, tumor necrosis factor (TNF)-β] and the cell-mediated immune response, indicated as "type I", was up-regulated [8,9]. Th2 cells secreted IL-4, IL-5, and IL-6 and promoted B cell proliferation and antibody secretion, indicated as "type II" immune response [7]. The role of macrophages in this balance is being increasingly appreciated and like T lymphocytes, macrophage cells differentiate into distinct effectors [10].

This review describes macrophage polarization with a major focus on differential metabolism and glutathione content which can be considered potential targets for the development of novel therapeutic tools.

Macrophage Polarization

Macrophages derived from different progenitors can acquire distinct phenotypes and biological functions depending on the microenvironment [4,5,11]. The various macrophage functions are linked to the type of receptor interaction on the macrophage and the presence of cytokines [12]. The concept of macrophage polarization was first defined in 1992 by Stein and colleagues who observed that macrophage exposure to IL-4 or IL-13 elicited an alternative type of activation with a phenotype different from the classical macrophage activation, depending on the IFN-γ [13]. Most in vitro studies display that this alternative activation was characterized by inhibition of respiratory burst of macrophages as well as enhanced expression of major histocompatibility complex class II (MHC-II) and mannose receptors (CD206) on their surface [13,14]. However, cell markers alone do not fully define the many sub populations of macrophages [15]. Over the years, several classifications of macrophage activation phenotypes were proposed [16,17], and in 2002 in vivo studies allowed to define an extended classification [16]. This included M1 polarization, also called classical activation, obtained by stimulation with pathogen-derived lipopolysaccharide (LPS) alone or in combination with IFN-γ and M2 polarization, mainly associated with anti-parasitic and tissue repair programs. The M1/M2 nomenclature is derived from the Th1 and Th2 cytokines that are associated with these macrophage phenotypes.

Classically activated or M1 macrophages differentiate in response to IFN-γ either alone or in concert with microbial stimuli (e.g. LPS) or cytokines [e.g. TNF-α and granulocyte-macrophage colony-stimulating factor (GM-CSF)] and display functional subdivisions depending on stimulation [18,19]. IFN-γ, recognized by an IFN-γ receptor (IFNGR), is mainly secreted by Th1 and CD8+ cytotoxic lymphocytes, natural killer (NK) cells and antigen-presenting cells (APC), and to a less extent by B cells. LPS is the principal component of the outer membrane of Gram-negative bacteria and is transported by the plasmatic LPS-binding protein to a cell surface receptor complex [20]. M1 macrophages can have different characteristics depending on their tissue location and are characterized by: the ability to secrete high levels of pro-inflammatory cytokines, such as IL-1β, IL-15, IL-23, TNF-α; high production of reactive oxygen intermediates (ROI) and iNOS-dependent reactive nitrogen intermediates (RNI); high capacity to present antigen and increased production of IL-12. M1 cells display enhanced endocytic functions and constitute the first line of defense against intracellular pathogens; moreover, they promote or amplify Th1 polarization of CD4+ lymphocytes by IL-12 production. Thus, M1 macrophages are generally considered potent effector cells which kill microorganisms as well as tumor cells and produce abundant pro-inflammatory cytokines [1,18] (Figure 1).

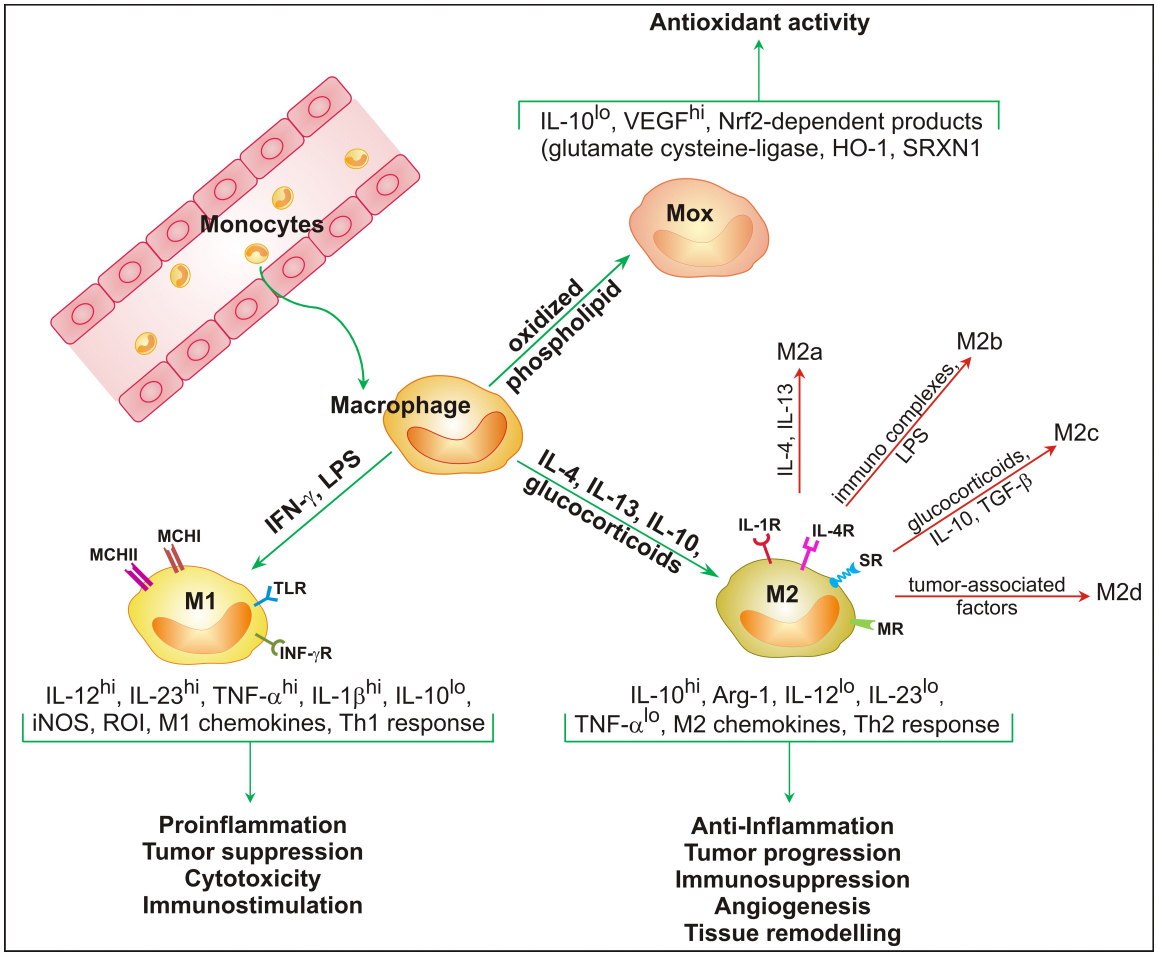

Figure 1: Macrophage polarization. Tissue macrophages acquire different phenotypes depending on microenvironmental stimuli: classical M1 phenotype, alternative M2 phenotype, and oxidized phospholipid derived macrophage phenotype (Mox). M2 phenotype can be subdivided into M2a, M2b, M2c, and M2d according to different stimuli. ROI: Reactive Oxygen Intermediates; iNOS: Inducible Nitric Oxide Synthase; Arg-1: Arginase-1; HO-1: Heme Oxigenase-1; SRXN1: Sulphiredoxin-1; VEGF: Vascular Endothelial Growth Factor

In vitro macrophage activation can be promoted directly by microbial pathogen associated molecular patterns (PAMPs), such as LPS [21,22]. This activation, known as innate, resemble the M1 activation, but different from M1 macrophages, innate activated macrophages do not possess increased phagocytic activity and cannot produce M1 biologically-active IL-12 [23]. For this reason, Martinez et al. classified classical and innate activated macrophages into M1a and M1b respectively [24].

IL-4 and IL-13 have been found to be more than simple inhibitors of classical macrophage activation; in fact, these cytokines can induce an alternative M2 form of macrophage activation [14]. Indeed, M2 macrophages can be generated by several stimuli, such as glucocorticoids and immunoglobulin complexes/Toll-like receptor (TLR) ligands. In general, M2 cells can control inflammatory responses and adaptive Th2 immunity, scavenge debris, promote angiogenesis, tissue remodeling and repair, and tumor progression [25]. Stabilin-1 is a receptor expressed on M2 macrophages which

binds the matricellular protein SPARC and mediates its clearance, thus having an important role in the orchestration of extracellular matrix remodeling in normal and tumor tissues [26]. M2 phenotype is characterized by the production of high levels of IL-10 and low expression of IL-12 and IL-23 (Figure 1). Some authors tried to simplify the nomenclature referred to M1/M2 macrophages describing these cells according to the activating condition [19] but in most papers M2 macrophages are divided into 3 subsets: M2a, M2b, and M2c based on gene expression profiles [18,24,25]. The M2a subtype is elicited by IL-4 and IL-13 which are mainly produced by Th2 cells, mast cells, and basophils. Both cytokines can down-regulate macrophage pro-inflammatory mediators such as IL-1β, TNF-α, IL-6, IL-12, IFN-γ, and superoxide anions production. Moreover, in HIV-infected individuals IL-4 decreases the expression of important membrane molecules such as CD14 and CCR5 [27]. Recent in vitro studies have reported that IL-4/IL-13 can also regulate MHC-II molecules, beta2 integrins, tissue-type plasminogen activator, and metalloproteinase (MMP)-1; moreover, M2a cells also express proteins which promote fibrogenesis, tissue repair and proliferation [28-31]. The M2b is elicited by IL-1R ligand or exposure to immune complexes plus LPS. M2b are characterized by low IL-12 and high IL-10 production, a cytokine profile which favors Th2 immune response [16]. The M2c subtype is elicited by IL-10, TGF-β, or glucocorticoids and is characterized by the downregulation of pro-inflammatory cytokines and the increased debris scavenging activity. Wang et al. described a further M2 subtype, i.e. M2d macrophage, which was characterized by low IL-12 and high IL-10 production and had some features of tumor-associated macrophages (TAMs) [32].

Most information on polarization come from studies on bone marrow macrophages; however data obtained by spleen-derived macrophages show that they are fully programmable to commit to either M1 or M2 populations and that they share several characteristics with bone marrow derived macrophages including their ability to convert from M2 to M1, but also exhibit unique features such as the high expression of CD4. These results are important because they shed more light on how spleen macrophages regulate immunity during infections [33].

Functional skewing of macrophages occurs in vivo under physiological conditions (e.g. ontogenesis and pregnancy) and in pathology (infection, cancer and allergy). The M1-M2 switch characterizes the infection by several pathogens, such as bacteria, parasites and viruses. This polarization may provide protection against inflammation and contain tissue damage; on the other hand, it can also favor pathogens that have evolved strategies to interfere with M1-associated killing, such as GSH content decrease, as discussed below ("Role of glutathione in macrophage repolarization" paragraph) [34]. Another example of polarized macrophage phenotype characterizing a pathological condition is provided by adipose tissue macrophages (ATMs) that are the main component of adipose tissue and important players in obesity-associated pathology. ATMs from obese humans are polarized towards an M1 phenotype [34]. Macrophage phenotype has an important role in tumor development: classically activated M1 macrophages contribute to the T cell-mediated elimination and equilibrium phases during tumor progression [35], while an M2-like phenotype characterizes TAMs.

Tumor Associated Macrophages (Tams)

Macrophages as well as dendritic and NK cells initiate the immune response against transformed cells. They present tumor antigens to T lymphocytes, which are then activated to kill tumor cells. IFN-γ has an important role in tumor immunity as it prepares presentation of tumor antigens by macrophages and dendritic cells to T lymphocytes; then, tumor eradication can be realized by immune cells through cytolytic molecules such as perforin [36]. However, tumor cells can often escape the immune machinery by different mechanisms. For example, they can evade from an adaptive immune response by developing deficiencies in antigen processing and presentation pathways [37]. Other tumor cells can inhibit the induction of pro-inflammatory signals promoting dendritic cell maturation [38]. Finally, overproduction of the anti-inflammatory cytokines such as IL-10 produced by both tumor cells and macrophages or T regulatory cells can favor tumor escape [39,40]. Indeed, monocyte/macrophages have an essential role in the switch from antitumor immunity to tumor growth and angiogenesis [40]. Depending on their activation, macrophages are able to secrete growth factors, cytokines, proteases, or complement components and they can acquire angiogenic (M2) or inflammatory (M1) phenotypes. The M1 macrophages induce antitumor responses as a result of secretion of IFN-γ, IL-12 or TNF-α [41]; M2 macrophages suppress immune responses as a result of secretion of transforming growth factor (TGF)-β or IL-10 and stimulate angiogenesis and tumor growth as a result of secretion of IL-17, vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), or endothelin [41]. The macrophages present in neoplastic tissues are referred to as TAMs and mainly belong to the M2 population having an IL-10^{high}, IL-12^{low} phenotype (M2d) [32,40]. TAMs stimulate tumor growth through the secretion of cytokines able to induce the proliferation of tumor or endothelial cells. TAMs are poor producers of NO and poor APC [40,42]. TAM accumulation has been associated with angiogenesis and with the production of angiogenic factors such as VEGF and platelet-derived endothelial cell growth factor [40]. Moreover, TAMs participate to the pro-angiogenic process by producing the angiogenic factor thymidine phosphorylase [43]. TAMs accumulate in hypoxic and necrotic regions within the tumors and hypoxia triggers a pro-angiogenic program in these cells, so amplificating the process of angiogenesis due to the angiogenic molecules produced by tumor cells [44]. TAMs have also a role in the dissemination of tumor cells. This can happen because of the secretion of enzymes capable of degrading the extracellular matrix, such as the MMP-9 and the urokinase-type plasminogen activator receptor [45].

Mounting data clearly point to a critical role for myeloid-derived suppressor cells (MDSCs) in tumors. These cells display predominant M2-like phenotypes with pro-tumoral and immunosuppressive activities. However, tumor-infiltrating MDSCs bear pleiotropic characteristics of M1 and M2 monocytes/macrophage in some mouse tumor models. MDSCs can change their phenotype and cannot be classified into any known monocyte/macrophage categories. MDSC polarization is accompanied by functionally changes in different pathological situations and control of M1/M2 MDSC reprogramming has emerged as a novel approach to treat cancers [46].

Mox Macrophage Phenotype Associated with Atherosclerosis

An additional macrophage phenotype is represented by oxidized phospholipid derived macrophages (Mox) (Figure 1). Atherosclerosis vascular complications are major causes of morbidity and mortality worldwide. A key role for macrophage-mediated inflammation has been described in the pathogenesis of atherosclerosis. Both M1 and M2 macrophages have been shown to be present in atherosclerotic

lesions. Recently, additional plaque-specific macrophage phenotypes have been identified, termed as Mox, heme directed macrophages (Mhem), and macrophages induced by CXCL4, formerly known as platelet factor 4 (M4A) [47]. In particular, Mox subpopulation has been described to be produced by accumulation of oxidized phospholipids in atherosclerotic lesions. Mox macrophages show different gene expression patterns and biological functions compared to M1 and M2 phenotypes. Expression of several genes in Mox macrophages is mediated by the redox-sensitive transcription factor Nrf2 [48]. It has been demonstrated that oxidative modification of phospholipids is necessary for activation of Nrf2-dependent gene expression. Nrf2 goes to the nucleus and activates the genes involved in the synthesis of antioxidant enzymes including glutamate-cysteine ligase which is the first enzyme of the cellular GSH biosynthetic pathway [48]. In fact, an increased GSH/GSSG ratio in the Mox macrophages has been described compared to M1 and M2 phenotypes, suggesting that Mox macrophages have the ability to cope better with oxidative stress. Control of redox status in macrophages by Nrf2 may be important in regulation of several cellular functions that influence tissue homeostasis and inflammation. Hence, an oxidized environment such as that of atherosclerotic lesions, induce formation of a novel macrophage phenotype (Mox) that is characterized by Nrf-dependent gene expression and may significantly contribute to pathologic processes in atherosclerotic vessels. Defective redox regulation may lead to exacerbated cell death, as seen in chronically inflamed tissue [48].

Differential Metabolism in M1 and M2 Macrophages

Metabolism is a distinctive character of the functional phenotype acquired by macrophages under the influence of signals derived from tissue microenvironment, under physiological and pathological conditions. The clear metabolic differences existing between M1 and M2 macrophages contribute to the shaping of their activation state [49]. Differences in polarized macrophages can be observed in sugar, lipid and protein profiles.

The most studied metabolism differentiating M1 and M2 macrophages is represented by arginine catabolism [50,51]. L-arginine can be a substrate for either iNOS which produces L-citrulline and nitric oxide (NO) or arginase 1 (Arg-1) which produces polyamines, L-ornithine and urea. M1-derived NO is a major effector molecule in macrophage-mediated cytotoxicity, playing an important role in controlling bacterial and parasitic infections, whilst Arg-1 expression is linked to wound healing actions of M2 macrophage population [50,51] (Figure 2).

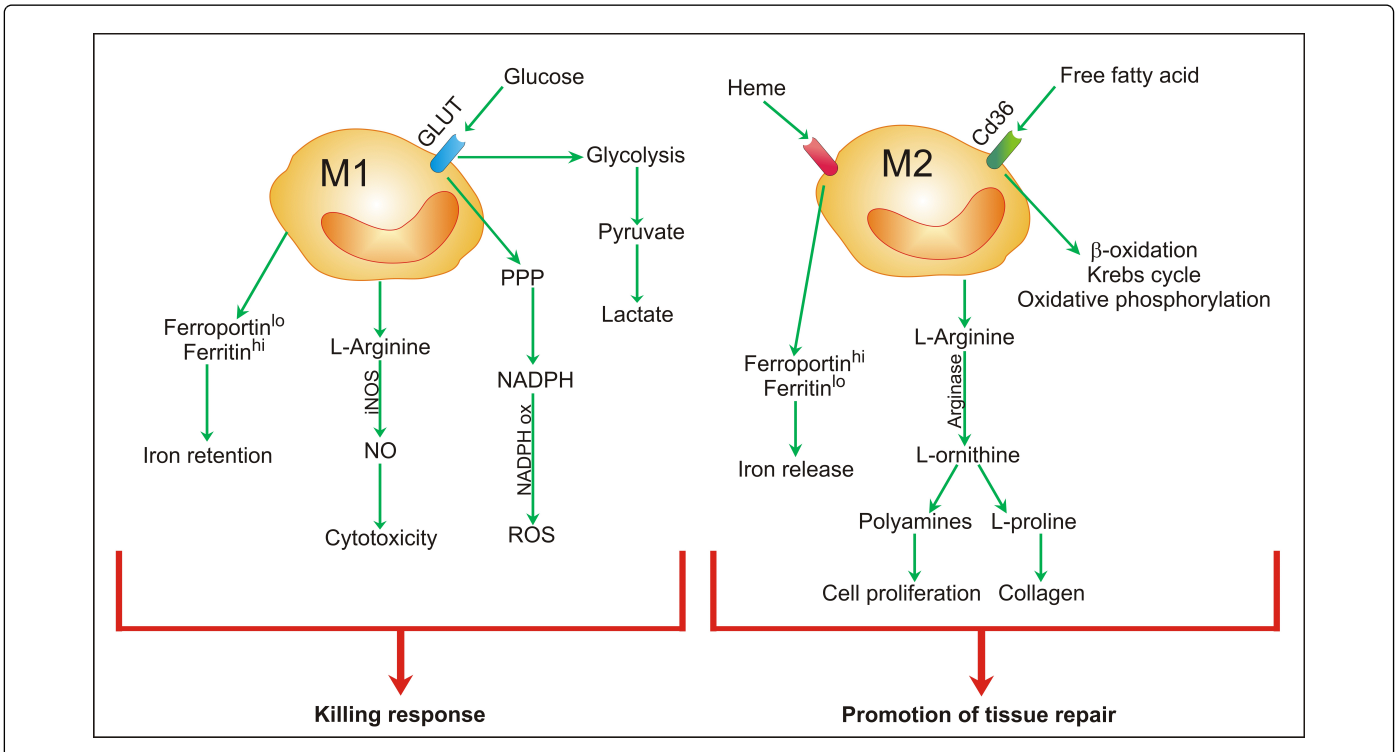

Figure 2: Main metabolic pathways in M1 and M2 macrophages. Left: classically activated macrophages (M1) induce an aerobic glycolytic program that results in lactate production. The pentose phosphate pathway (PPP) provides ROS for killing response. L-arginine is a substrate for iNOS which produces NO, the major effector molecule in M1 macrophage-mediated cytotoxicity. M1 macrophages display iron retention characterized by high ferritin and low ferroportin. Right: alternatively activated macrophages (M2) trigger a metabolic program including β-oxidation, Krebs cycle and oxidative phosphorylation. Arginase 1 (Arg-1) produces polyamines and L-ornithine responsible for tissue repair. Iron release (low ferritin and high ferroportin) favors tissue repair and cell proliferation.

With regard to sugar metabolism, in M1 macrophages anaerobic glycolytic pathway is induced upon activation, which involves an increase in glucose uptake as well as the conversion of pyruvate to lactate [52]. At the same time, the activities of the respiratory chain are attenuated. Furthermore, the pentose phosphate pathway (PPP) is also induced following IFN-γ/LPS activation. This pathway generates

NADPH for the NADPH oxidase which is important for ROS production. In macrophages, increased lactate formation and activation of the PPP after phagocytosis had been already observed, suggesting potential importance of adapted metabolism on the activation cascade [53] (Figure 2). Additionally, down-regulation of the carbohydrate kinase-like protein (CARKL), which catalyzes the production of sedoheptulose-7-phosphate, is required for the development of an M1 phenotype [54]. It has been observed that the drop of NADH levels in cells overexpressing CARKL during macrophage activation, results in redox shift. PPP activity contributes to reduction of redox couples via NADPH. Hence, increased GSH and NADH generation are observed during M1 activation while M2 activation resulted in an up-regulation of CARKL which was not followed by increased GSH or NADH formation. These findings represent a functional distinction between the two polarization states that is CARKL dependent. Hence, CARKL can be considered a sedoheptulose kinase orchestrating pro- and anti-inflammatory immune responses through metabolic control [55]. On the other hand, fatty acid oxidation and oxidative metabolism are the preferential pathways in IL-4-activated macrophages [56] (Figure 2). IL-4 activates the transcription factor STAT6 which can trigger a pathway inducing mitochondrial respiration [54]. Upon activation, M2 macrophages can drive the pyruvate into the Krebs cycle and can induce expression of components of electron transport chain. Increased glycolysis in M1-polarized macrophages permits to quickly trigger microbicidal activity and cope with a hypoxic tissue microenvironment. In contrast, oxidative glucose metabolism in M2-polarized macrophages provides sustained energy for tissue remodeling and repair [56] (Figure 2).

Lipid metabolism is also differentially regulated during macrophage activation. The pathways which are activated in M1 macrophages are generally inhibited in M2 macrophages. For example, in IFN-γ/LPS-activated M1 macrophages COX-2 is up-regulated while COX-1 is down-regulated; conversely, IL-4-activated M2 macrophages show an up-regulation of COX-1 [57].

Furthermore, the existence of tight links between expression and function of aminoacid catabolizing enzymes and M1/M2 differentiation has been reported. An example is represented by indoleamine 2,3-dioxygenase 1 (IDO1), an enzyme which catalyzes oxidative tryptophan (Trp) catabolism. Through the depletion of the essential aminoacid tryptophan, IDO1 can limit the growth of several pathogens, including viruses, bacteria, and protozoa [58-60]. A new role of IDO1 in the M1 vs M2 polarization has been described in the human acute monocyte leukemia cell line THP-1 where a forced expression of IDO1 induces an M2-like profile, characterized by high IL-10 and low IL-12 levels [56,60].

Iron metabolism is also involved in M1/M2 phenotypes. Macrophages play an important role in iron homeostasis by recycling iron through phagocytosis of senescent red blood cells and their polarization is associated with differential regulation of iron metabolism [61]. On the other hand, iron can be used by microbes for proliferation, virulence, and persistence [62]. M1 macrophages show a metabolic profile which favors iron retention characterized by low heme uptake, high ferritin (iron storage) and low ferroportin (iron export). By this way, M1 macrophages reduce the labile iron pool, the metabolically active fraction of cytosolic iron that is available for metabolic purpose [63]. On the other hand, M2 macrophages have a sustained heme uptake as well as a reduced iron storage and enhanced release of iron (low ferritin and high ferroportin) [61]. Sequestration of iron by M1 macrophages would have a bacteriostatic and tumoristatic effect; while iron release from M2 macrophages would favor tissue repair and cell proliferation (Figure 2).

Recently, metabolomic and transcriptional data were obtained on murine macrophage M1 and M2 polarization confirming previously recognized metabolic features and adding new interesting information. Two critical pathways were identified for M2 polarization: glutamine-related metabolism and the Uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) pathway. On the other hand, in M1 macrophages an important role for the aspartate-arginosuccinate shunt in coordinating the NO and tricarboxylic acid (TCA) cycles was described [64].

The data reported clearly suggest how metabolic adaptation is an integral aspect of macrophage polarization and how macrophages, through the expression of different mediators, can be an essential component in the orchestration of various aspects of metabolism and the development of pathologies.

Different Polarization of Mouse and Human Macrophages

The molecular repertoire of macrophages in health and disease can provide novel biomarkers for diagnosis, prognosis, and treatment. But most of the information come from murine studies and translation to the human can be problematic. For instance, Ym1 and Fizz1, which are important M2 murine markers, lack human homologs [65]. Both human and murine studies have shown that M1 macrophages up-regulate major enzymes involved in glycolysis; however, oxidative metabolism and fatty acid oxidation do not seem to predominate in human M2 macrophages [66]. Another important aspect that seems to differ between murine and human macrophages is the role of iNOS and Arg-1 in M1 and M2 macrophages. Only mouse M2 macrophages do not express iNOS, but express high levels of Arg-1, which synthesizes polyamine and proline that stimulate cell growth, collagen formation and tissue repair [50,51] (Figure 2). In human in vitro polarized macrophages this metabolic signature is absent [65]. Recently Martinez et al. carried out an interesting study about similarities and differences in resting and IL-4 alternatively activated mouse and human macrophages where they underlined that the enzyme transglutaminase 2 is a conserved marker which provided a consistent activation biomarker for both human and mouse M2 macrophages and monocytes [67]. Further studies will be necessary to identify human M1/M2 markers in the pathogenesis and resolution of different diseases and cancer.

Repolarization of Macrophages as a Potential Therapeutic Approach

The translation of the discoveries on the mechanisms behind macrophage activation and the different metabolic profiles to human diseases is a stimulating prospect since there are pathologies that have been associated with one particular macrophage phenotype or another. Moreover, some authors have hypothesized that gender-associated differences in macrophage polarization could play a role in the different disease incidence between males and females [54,68].

Reprogramming macrophages to switch their phenotype could provide stimulatory/destructive (M1) or suppressive/protective (M2) therapeutic strategies. Polarization of macrophages toward M1 phenotype can inhibit tumor growth, whereas undesirable immune responses, such as autoimmunity, transplant rejection and graft-

versus-host diseases can be suppressed through M2 macrophages. Therefore, understanding the molecular basis of macrophage polarization may facilitate their clinical application and drug development.

Indeed, the treatment of cancer may take advantage of therapies favoring attraction and polarization of M1 macrophages as well as reprogramming of M2 macrophages to the M1 subset. Some strategies aim to inhibit the induction of M2 macrophages. One therapeutic option can interfere with macrophage attraction and differentiation by abrogation of the prostaglandin E2, IL-6 and STAT3 activation loop [69]. This may affect tumor growth and limit the induction of tolerogenic macrophages. A second strategy consists in depriving tumors from growth factors (for example by using monoclonal antibodies) to prevent the differentiation of M2 macrophages. However, this approach is not simple because it is difficult to reach high antibody titers in poorly vascularized areas where macrophages tend to accumulate [69]. These areas are characterized by low oxygen levels that play a determinant role in driving macrophage differentiation and functions. It has been suggested that blocking hypoxia-inducible factors (HIF) could control the expression of several genes involved in tumor progression and that macrophages can be exploited to deliver HIF-regulated therapeutic genes to otherwise inaccessible areas in tumors [70].

Other anti-tumor strategies aim to reprogram M2 macrophages once they exist. In fact, in vitro macrophages can adapt to another phenotype upon a strong polarizing stimulus [71]. Reprogramming of M2 to M1 type macrophages requires receptor-mediated activation and the presence of polarizing cytokines (e.g. IFN-γ). Manipulation of macrophage polarization has already been proved to be somewhat successful clinically. For example, IFN-γ has been administered in patients with ovarian carcinoma to turn M2 macrophages into activated IL-12 producing M1 cells [72]. As NF-κB has been demonstrated to be a key regulator of macrophage polarization [34,73], conversion of M2 TAM toward a tumouricidal M1 phenotype was obtained through a TAM-targeted delivery system that combines modified liposomes and NF-κB decoy complexes [74].

Another strategy is based on forced activation of Notch signaling which can increase M1 macrophages; in fact, TAM have a lower level of Notch pathway activation in mouse tumor model and consequently low SOCS3 which is essential for classical macrophage activation [75].

A deep knowledge of molecular mechanisms of macrophage polarization can provide ideas for new therapies for cancers through the modulation of macrophage polarization.

Activin A is a pluripotent growth and differentiation factor of the TGFβ family described to regulate the growth of numerous cell types and to modulate cytokine and chemokine release from myeloid cells [76]. Recently it has been reported that activin A is important for macrophage polarization, but different effects were described in mouse and human macrophages. The ability of activin A to trigger arginase-1 expression and inhibit IFN-γ-induced NO synthase 1 expression has led to the suggestion that it functions as a Th2 cytokine that promotes alternative murine macrophage activation [77]. On the other hand, human M1 macrophages release high levels of activin A that could be used to skew macrophage polarization toward the acquisition of a proinflammatory phenotype [78].

An example of M2 induction is provided by Schistosoma mansoni-derived soluble egg antigens (SEAs) that can induce M2-driven immune responses and can counteract inflammatory responses as well as improve the outcome of chronic inflammatory diseases like atherosclerosis [79].

More and more attention has been paid on the metabolic intermediates that can be directly implicated in a particular macrophage phenotype because they could be considered important therapeutic targets. Strategies that increase glucose uptake by modulating the expression of GLUT1 transporter has been shown to be useful to impart an M1-like phenotype in macrophages and could be crucial to develop novel therapeutics for obesity and diabetes [80].

Another strategy to change macrophage phenotype is based on inhibition of mitochondrial respiration that not only blocks the M2 phenotype but also drives the macrophage into an M1 state. On the contrary, forcing oxidative metabolism in M1 macrophage potentiates the M2 phenotype [81].

Haschemi and colleagues studied the effects of manipulating CARKL expression on macrophage metabolism [55]: constitutive expression of CARKL in macrophage cell lines decreased glycolytic flux upon LPS stimulation, mimicking the metabolic profile of M2-like cells. Conversely, ablation of CARKL expression by RNAi primed macrophages to adopt an M1-like metabolic state prior to LPS stimulation.

As the examples reported suggest, manipulation of macrophage polarization could be somewhat successful clinically. But we should be more cautious. In fact, unlike parasite infections, allergy and many cancers, in which the functional phenotypes of macrophages in vivo mirror those of canonical M1-M2 or M2-like polarized states as defined in vitro, in a number of pathological conditions macrophage populations express mixed phenotypes. The contribution of coexisting cells and the impact of changes during disease evolution should be carefully considered case by case.

Role of Glutathione In Macrophage Repolarization

Reduced glutathione (GSH) is the prevalent non-protein thiol in animal cells and the most abundant antioxidant in aerobic cells. It is implicated in many cellular functions, such as degradation and synthesis of proteins and DNA or detoxification of toxins and carcinogens [82]. Moreover, in the last years GSH has been described to have an important role of in the immune response, by influencing the activity of both lymphocytes and macrophages [83-85].

The role of macrophage cytokines and the switch between Th1 and Th2 responses are crucial in several diseases such as cancer, allergy, AIDS, and other infectious diseases as well as in ageing. Many in vitro and in vivo studies demonstrated that GSH depletion in APC, such as macrophages, correlated with defective antigen processing and inhibited Th1-associated cytokine production. In murine studies, GSH depletion in APC decreases the secretion of IL-12, known to regulate IFN-γ production, and leads to switch from the typical Th1 cytokine profile towards Th2 response patterns [84-87] (Figure 3). Also in human alveolar macrophages, GSH levels play a central role in determining whether a Th1 or Th2 cytokine response will develop [88]. Hence, M1 cells have a higher ratio of reduced-to-oxidized glutathione (GSH/GSSG), with opposite effects of IFN-γ and IL-4 on the reductive status [88]. It is possible to regulate the amount of IL-12 secreted by modulating the intracellular GSH/GSSG balance and an IL-12^{low} M2-like phenotype can be changed into an IL-12^{high} M1-like phenotype. This has been demonstrated in a mouse asthma model where the release of Th2 cytokines orchestrate the recruitment and

activation of the primary effector cells of the allergic response. The same authors showed that changing glutathione redox status by γ-Glutamylcysteinylethyl ester (γ-GCE) could potentiate Th1 response through IL-12 production. [89].

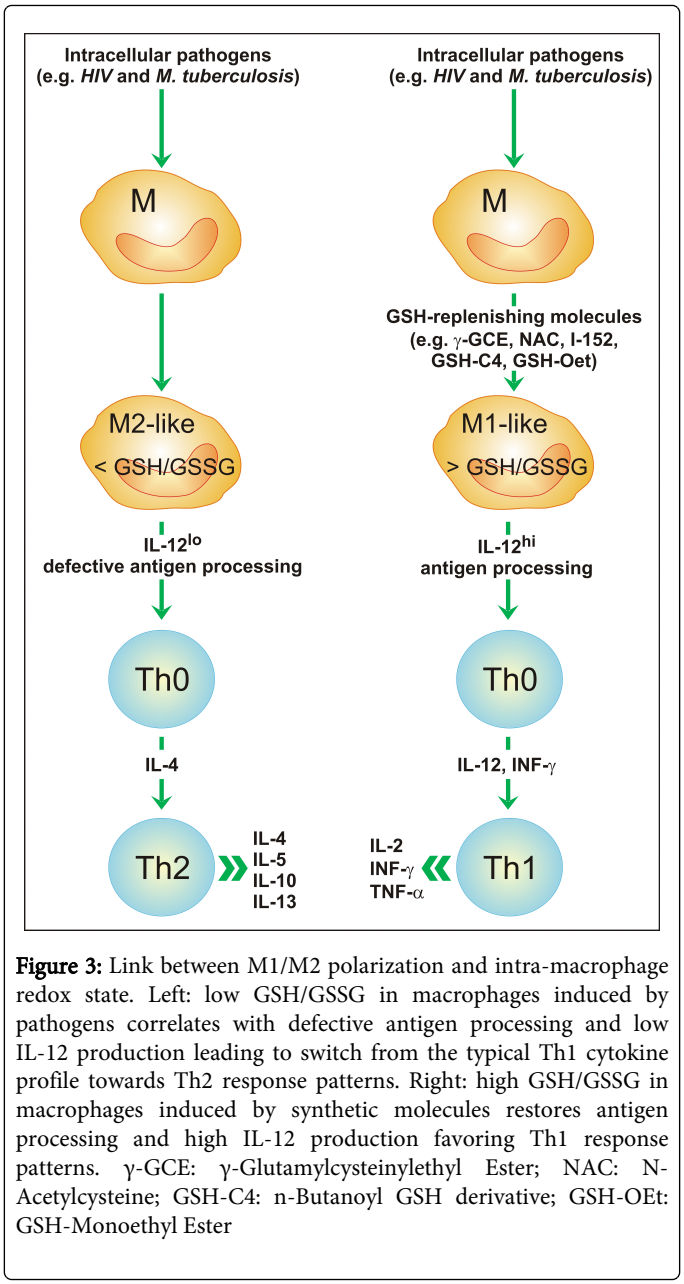

Figure 3: Link between M1/M2 polarization and intra-macrophage redox state. Left: low GSH/GSSG in macrophages induced by pathogens correlates with defective antigen processing and low IL-12 production leading to switch from the typical Th1 cytokine profile towards Th2 response patterns. Right: high GSH/GSSG in macrophages induced by synthetic molecules restores antigen processing and high IL-12 production favoring Th1 response patterns. γ-GCE: γ-Glutamylcysteinylethyl Ester; NAC: N-Acetylcysteine; GSH-C4: n-Butanoyl GSH derivative; GSH-OEt: GSH-Monoethyl Ester

Mycobacterium tuberculosis infection provides an interesting example of an intra-macrophage pathogen able to modulate the T cell responses by altering the intra-cellular redox state. In fact, Th1 immune response is down-regulated in patients with active tuberculosis infection [90], who have altered glutathione balance [91]. Alam et al. have demonstrated that IL-12 induction in native macrophages is controlled directly by the intracellular glutathione-redox and that manipulation of the macrophage redox state by N-acetylcysteine (NAC), a GSH precursor, can influence in vitro cellular immune response of peripheral blood mononuclear cells obtained from patients with active tuberculosis to pathogens [92]. These data support the critical importance of glutathione-redox status in regulating the induction of IL-12 which activates the Th1 T cell immune response crucial for inducing protection against the intracellular pathogens like *Mycobacterium tuberculosis*. Therefore, it is possible that these pathogens, modulating the GSH/GSSG balance in macrophages, transform these cells in M2-like macrophages characterized by low IL-12 production, thus polarizing the immune environment to their favor. In fact, as already discussed, M1 macrophages display a microbicidal activity against a wide range of intracellular parasites.

Other works showed that *Mycobacterium tuberculosis* was adapted to survive within macrophages expressing M2 markers and with down-modulation of bactericidal M1 response [93].

Low GSH levels have also been described in other infections characterized by an unbalance in Th1/Th2 in favor of Th2; for example, decreased GSH content has been found in lung macrophages of HIV-infected individuals even if treated with antiretroviral therapy [94].

M2-like polarization can represent an immune escape strategy for many other intracellular pathogens such as *Brucella spp.*, *Leishmania*, *Listeria monocytogenes* [63]. Indeed, some redox enzymes derived from protozoan parasites can exert effects on macrophage phenotypes. For example, *Toxoplasma gondii* peroxiredoxin can promote Th2 macrophage phenotype, enhanced parasite replication, elevated arginase-1 enzyme activity and increased IL-10 synthesis [95].

On the other hand, several molecules have been used to increase the intra-macrophage GSH content to reinforce Th1 immune response (Figure 3). Some examples have been already reported above (e.g. γ-GCE and NAC). Others are represented by: the GSH-monoethyl ester (GSH-OEt) which enhances LPS-induced IL-12 p40 protein production and mRNA expression in human macrophages [96]; the n-butanoyl GSH derivative (GSH-C4) and I-152 (a precursor of cysteine usable for GSH synthesis) increased IL-12 levels in mice immunized with Ovalbumin or HIV Tat protein strengthening Th1 immune response [97,98]. The role of these molecules in influencing the macrophage polarization has suggested to consider them as new immunomodulators [99].

It can be observed that a low GSH/GSSG ratio characterizes a M2-like phenotype both in murine and human macrophages and that GSH replenishment can represent a strategy for repolarization of both macrophage species [84-89]. Hence, it may be suggested that low macrophage GSH level can provide a consistent biomarker for human and murine M2 macrophages.

A correlation between CD4+ T cell phenotypes and macrophage phenotypes has also been described in a murine model of *Fasciola hepatica* helminth infection [100]. This and other helminth infections are characterized by a prevalence of M2 macrophages which can induce the differentiation of naïve T cells to Th2 phenotypes [101].

The functional relation between T cell phenotype and macrophage phenotype can have important implications in vaccine development. New immunomodulators activating either M1 or M2 macrophage phenotype could be employed in new vaccines able to stimulate cellular (Th1) or humoral (Th2) immune response respectively. To this regard, another important aspect to consider is the different way of antigen processing and presentation in M1 and M2 macrophages. For example, it has been recently reported that luminal pH that ensue phagocytosis is different in M1 and M2 human macrophages [102]. The elevated pH in M1 macrophages optimizes pathogen killing and

maximizes antigen presentation of microbial components; accordingly, enhanced antigen presentation is a hallmark of M1 macrophages [103]. In contrast, in M2 cells phagosomal acidification occurs rapidly for efficient hydrolysis and recycling of apoptotic cell components [102].

Conclusions

Macrophages are a heterogeneous population of innate myeloid cells involved in health and in the regulation of immune responses in various diseases, e.g. tumors, infections, allografts, autoimmune disorders, etc. Macrophages can have different functions and different transcriptional profiles.

Mounting data clearly show that macrophage polarization from one phenotype to the other is accompanied by functional and metabolic changes in different pathological situations. Moreover, metabolic intermediates and signaling pathways have an important role in defining M1/M2 polarization. The role of metabolic intermediates is much more important than expected and knowledge about the metabolic status of differentially activated macrophages holds great potential for clinical applications. Hence, control of M1/M2 macrophage reprogramming has emerged as a novel therapeutic approach to treat cancers and other diseases and metabolic pathways can be considered potential targets for manipulating macrophage polarization. However, an optimal macrophage reprogramming in the treatment of different diseases needs further research to understand the molecular events driving macrophage polarization and to discover additional subset markers used to differentiate the different macrophage subtypes.

Acknowledgements

This work was supported by PRIN (Research projects of national interest) 2010-2011-[2010PHT9NF_004] granted to Fraternale.

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