Minireview *Highlight article*

Macrophage polarization in hypoxia and ischemia/reperfusion: Insights into the role of energetic metabolism

Elmira I Yakupova[1,2], Grigoriy V Maleev[3], Andrei V Krivtsov[4] and Egor Y Plotnikov[1,5]

[1]A.N. Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow 119234, Russia; [2]Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino 142290, Russia; [3]Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka 142432, Russia; [4]Center for Pediatric Cancer Therapeutics, Dana Farber Cancer Institute, Harvard Medical School, Boston, MA 02215, USA; [5]V.I. Kulakov National Medical Research Center of Obstetrics, Gynecology, and Perinatology, Moscow 117997, Russia
Corresponding authors: Andrei V Krivtsov. Email: andrei_krivtsov@dfci.harvard.edu; Egor Y Plotnikov. Email: plotnikov@belozersky.msu.ru

Impact Statement

The review summarizes the current knowledge on macrophage phenotype alteration (also known as macrophage polarization) under conditions of hypoxia (oxygen deprivation) *in vitro* or ischemia/reperfusion in various tissues (liver, kidney, heart, brain) *in vivo*. We propose a new concept on how ischemia/reperfusion alters macrophage metabolism and how interventions in macrophage metabolism (e.g. dietary interventions) may affect macrophage polarization. The concept of macrophage phenotype shifting from M1 to M2, induced by targeting the energy metabolism, might be useful for developing treatment strategies for ischemic injuries.

Abstract

Macrophages, the key cells of innate immunity, possess wide phenotypical and functional heterogeneity. *In vitro* studies showed that microenvironment signals could induce the so-called polarization of macrophages into two phenotypes: classically activated macrophages (M1) or alternatively activated macrophages (M2). Functionally, they are considered as proinflammatory and anti-inflammatory/pro-regenerative, respectively. However, *in vivo* studies into macrophage states revealed a continuum of phenotypes from M1 to M2 state instead of the clearly distinguished extreme phenotypes. An important role in determining the type of polarization of macrophages is played by energy metabolism, including the activity of oxidative phosphorylation. In this regard, hypoxia and ischemia that affect cellular energetics can modulate macrophage polarization. Here, we overview the data on macrophage polarization during metabolic shift–associated pathologies including ischemia and ischemia/reperfusion in various organs and discuss the role of energy metabolism potentially triggering the macrophage polarization.

Keywords: Macrophages, hypoxia, ischemia, metabolism, energy metabolism, M1–M2 macrophage polarization

***Experimental Biology and Medicine* 2022; 247: 958–971. DOI: 10.1177/15353702221080130**

Introduction

Macrophages contribute to regulating tissue homeostasis, organ development, tissue remodeling, regeneration, inflammation, and various pathologies. Being an essential component of innate immunity, macrophages differentiate toward various phenotypes in response to environmental alterations.[1] They are sensitive to a wide spectrum of stimuli, including viral, microbial, and parasite antigens; immune complexes; and various mediators released by apoptotic, necrotic, or other cells.

Depending on the growth factors, cytokines, and other mediators released into the microenvironment by macrophages, they are divided into two major *in vitro* subsets with different physiological activity:[2] the proinflammatory M1-polarized macrophages and the anti-inflammatory M2-polarized macrophages.[3] Macrophage activation and differentiation *in vivo* is not a linear process; each subset has different characteristics and functions in homeostasis and plays different roles in the outcome of disease development.[4] Currently, macrophage polarization *in vivo* is considered to be a wide continuum of phenotypes between M1 and M2 states[4–6] that get activated differently based on a variety of physiological signals. However, such discrete macrophage classification is still useful for *in vitro* studies, including a description of their function in modeling pathological processes.

Hypoxia is defined as a condition of reduced oxygen saturation (pO2, 0–20 mmHg) which arises as a result of a damaged or dysfunctional vascular network and diminished blood and oxygen supply. Hypoxia accompanies many pathological conditions, including inflammation, hepatic ischemia, organ transplantation, cerebral stroke, myocardial infarction (MI), arthritic joints, atherosclerotic plaques,

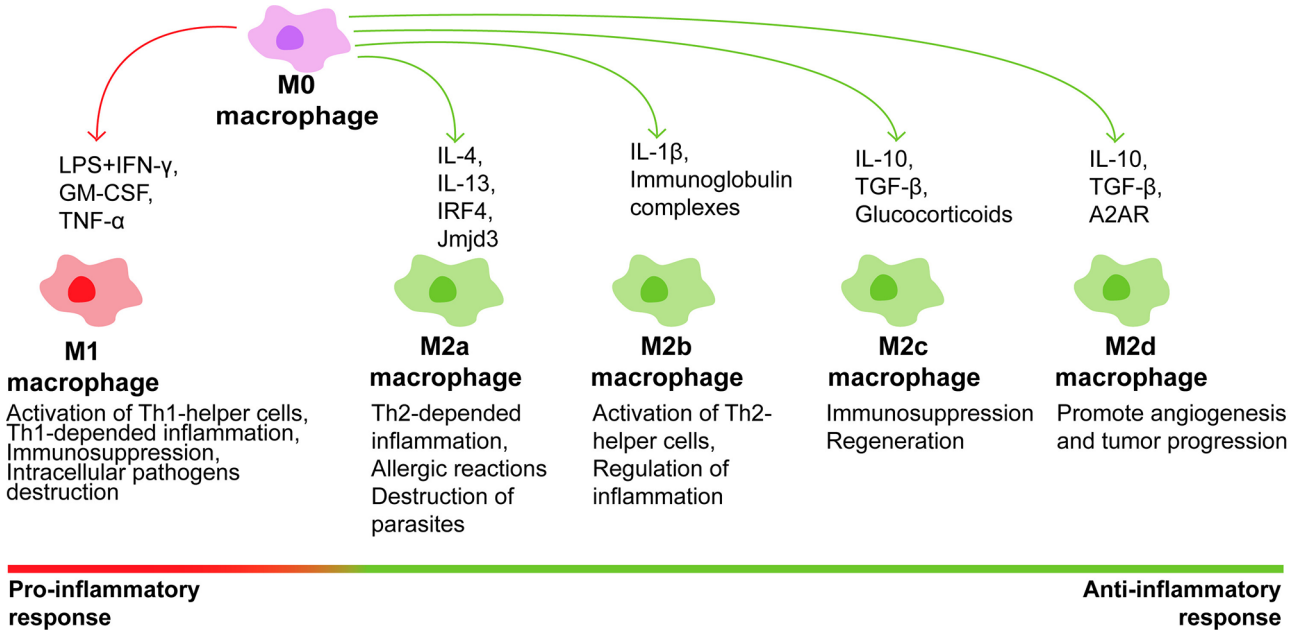

Figure 1. Macrophage polarization *in vitro* including M2 macrophage subsets (M2a, M2b, M2c, and M2d). The scheme shows activating stimuli for M1/M2 polarization and their main functions. (A color version of this figure is available in the online journal.)

and malignant tumors.[7–10] Macrophage polarization can be induced by hypoxia and is associated with pathological conditions like ischemia and ischemia/reperfusion (IR). Here, we discuss how mild alterations of energy metabolism by changing a diet (glucose-free, low-calorie, keto diets) can affect macrophage phenotype and hypoxic/ischemic damage.

M1/M2 paradigm of macrophage polarization

Currently, two major *in vitro* macrophage subsets are recognized: proinflammatory M1 macrophages and anti-inflammatory M2 macrophages.[11] They are activated by different molecules, have different surface markers, and produce different chemokines and cytokines (Figure 1; see Supplementary Table).

M1 macrophages are activated by toll-like receptor (TLR) ligands, such as lipopolysaccharides (LPS; bacterial cell wall components), interferon-γ (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor-alpha (TNF-α).[12–14] M2 macrophages are divided into four subsets (M2a, M2b, M2c, and M2d). Their activating stimuli (Figure 1) include the following:

M2a: interleukin (IL)-4 or IL-13 (secreted by mast cells, basophils, and Th2-lymphocytes), downstream involvement of jumonji domain-containing-3 (Jmjd3), and interferon regulatory factor-4 (IRF-4);[15]
M2b: immune complexes and IL-1β;[15–18]
M2c: glucocorticoid hormones, IL-10, or transforming growth factor-β (TGF-β);[17–19]
M2d: TGF-β + adenosine 2A receptor (A2AR), IL-10.

Furthermore, IL-21,[20] IL-33,[21] and IL-34[22] were shown to induce M2 polarization. Since the activation status of macrophages is regulated by suppressors of cytokine signaling (SOCS) protein isoforms, this protein was suggested to be a potential marker for macrophage phenotype: SOCS3 is associated with M1 macrophages, whereas an increase in the SOCS1/SOCS3 ratio could be a potential marker for M2 macrophages.[23–25]

M1 macrophages perform proinflammatory, microbicidal, and tumoricidal functions acting as the effective killers of pathogens due to a high antigen presentation capacity, high expression of receptors, and proinflammatory cytokines.[26–29] Apart from proinflammatory chemokines and cytokines (see Supplementary Table), they employ inducible nitric oxide synthase (iNOS) to produce NO from l-arginine[30] and express the Th1-attracting chemokines.[22,31]

M2 macrophages demonstrate high phagocytosis capacity, production of extracellular matrix components, angiogenic and chemotactic factors, and IL-10.[32,33] Activated M2 macrophages eliminate apoptotic cells, mitigate inflammatory response, and promote wound healing.[1] Moreover, M2 macrophages are thought to be involved in organ morphogenesis, tissue remodeling, and endocrine signaling.[5,34,35] M2 macrophages stimulated by IL-4 possess an increased arginase expression resulting in arginine-to-ornithine conversion.[36] Ornithine is a precursor of polyamines and collagen that promotes tissue regeneration and wound repair. Beyond that, arginase competes with iNOS for arginine, which leads to decreased NO production. However, prolonged activation of M2 macrophage activity may induce tissue damage and fibrosis.[37] In particular, M2 macrophages can promote excessive production and remodeling of the extracellular matrix, which can also cause a pathological outcome. It has been shown that fibrosis development in wound healing during chronic schistosomiasis is caused by the uncontrolled activation of M2 macrophages, while inhibiting the IL-4 receptor on M2 macrophages or using antibodies against the IL-4 receptor reduced the degree of fibrosis in the lesion.[38]

Subsets of M2 macrophages have several specific functional features. M2a macrophages recruit Th1-lymphocytes, eosinophils, basophils, and mast cells.[22,31] The primary role of M2a macrophages is to mediate the allergy response.[39–41] M2b macrophages stimulate migration of eosinophils, Th2-lymphocytes, and T-regulatory cells. M2c macrophages play crucial roles in the phagocytosis of the apoptotic cell process.[42] M2d macrophages are characterized by the increased production of IL-10 and vascular endothelial growth factor (VEGF) and low expression of TNF-α and IL-12, which eventually promotes angiogenesis.[43]

There is a huge variety of markers used for the identification of macrophage population.[44–54] Examples of surface marker detection by flow cytometry are shown below:

- M1 ($CD11b^+$ $CD80^+$) and M2 ($CD11b^+$ $CD206^+$);
- M1 ($CD64^+$ $CD80^+$) and M2 ($CD11b^+$ $CD209^+$);[50]
- M1 (CD80, CD86, TLR2, TLR4) and M2 (CD204, CD163, CD206);[51]
- M1 ($CD45^+$ $F4/80^+$ $CD86^+$) and M2 ($CD45^+$ $F4/80^+$ $CD206^+$);[52]
- M1 ($CD45^+$ $CD11b^+$ $F4/80^+$ $CD206^-$) and M2 ($CD45^+$ $CD11b^+$ $F4/80^+$ $CD206^+$);[46]
- M1 (CD86) and M2 (CD206, CD163);[53]
- M1 ($CD68^+/CD80^{high}/CD206^{low}$), M2 ($CD68^+/CD80^{low}/CD206^{high}$), and M0 phenotype ($CD68^+/CD80^{low}/CD206^{low}$).[54]

Of note, $CD45^+$, $CD14^+$, $CD11b^+$, $CD163^+$, CD19, and $CD68^+$ are common markers for human whole-population macrophages.

However, there are several flaws in the M1/M2 paradigm. (1) Dividing macrophages into M1/M2 classes is considered too simplistic as macrophage activation and differentiation are not linear, and each subset has different characteristics and functional roles in homeostasis and affects the outcome of disease development.[4,11] (2) Nahrendorf and Swirski pointed out the significant differences between the *in vitro* and *in vivo* data.[55] After a 7-day *in vitro* incubation of microglia and peritoneal macrophages, they completely lost their tissue-specific gene expression.[56] Thus, comparing the data obtained from cells in *in vivo* and *in vitro* experiments appears to be too hasty. (3) Various signals in an organism and cellular environment induce functional diversity of the macrophages.[6,57] Smith *et al*.[57] studied the macrophage response to a combined M1 and M2 activation triggered either simultaneously or sequentially. They showed that simultaneous action of LPS, IFN-γ, IL-4, and IL-13 induces both M1 marker, CD86, and M2 marker, CD206. Over time, the macrophages lost their expression of CD86 simultaneously displaying an increased expression of CD206.[57] This work also demonstrated that macrophage reprogramming to an opposite phenotype is dependent on the initial polarization state and the strength of the secondary signal.[57] Murine bone marrow–derived macrophages stimulated with LPS + IFN-γ, IL4, or both were analyzed using single-cell RNA sequencing and single-cell secretion profiling. Variability in the negative cross-regulation between certain LPS + IFN-γ-specific and IL-4-specific genes results in cell transcriptome heterogeneity. The authors suggest that increasing the functional diversity within a single population is one of the strategies employed by macrophages in response to variable environmental cues.[6]

To summarize, when interpreting *in vivo* studies, the M1/M2 paradigm should be used with caution, since it significantly simplifies the way macrophage functional activity is. Some investigators even suggest to use "pro-inflammatory" and "pro-regenerative" terms instead of M1 and M2.[49]

Macrophage polarization during hypoxia *in vitro*

Hypoxia is a condition characterized by insufficient oxygen supply in tissues. Discussing data on *in vitro* hypoxia models (Table 1), we should keep in mind that in human healthy tissues, the physiological normoxia corresponds to the oxygen concentration within the range from 4% (muscle) to 9.5% (kidney, outer cortex).[58,59] However, in cell culture models, under normoxia, cells are exposed to atmospheric oxygen concentrations (about 20% O_2),[60] while for hypoxic conditions, cells are exposed to 1% O_2.[60] O_2 saturation in solid tumors is known to be within the range of 1–2% O_2[59,61] which was suggested to induce macrophage migration into the tumor core.[62,63] Studying the macrophage polarization during hypoxia could shed a light on the mechanisms of tumor inflammation.

Two human glioblastoma (hGBM) cell lines U87 and U251 are known to develop a slightly hypoxic and a severely hypoxic solid tumor, respectively.[66] Using the cell-derived xenografts established by orthotopic inoculation of U87 or U251 cells into the right caudate-putamen, Leblond *et al*.[8] showed that hypoxia facilitated the macrophage migration to the tumor with M2 phenotype being more pronounced than M1. The same results were obtained from co-culturing the pancreatic cancer cells (PCC) and macrophages, which promoted M2 polarization.[68]

Under hypoxic conditions, macrophages were shown to express higher levels of growth and angiogenic factors such as VEGF and glucose transporter-1.[63,69,70] Hypoxia conditions in tumors induced the anti-inflammatory polarization.[54,71,72] In tumors, the macrophages tend to acquire M2 polarization promoting tumor growth due to the production of a large number of mitogenic, angiogenic, and prometastatic cytokines and enzymes, including growth factors (VEGF, fibroblast growth factors-1 and fibroblast growth factors-2, platelet-derived growth factor (PDGF), hepatocyte growth factor (HGF), placental growth factor (PGF), and angiopoietin-1).[71] The proangiogenic factor VEGF-A is produced almost exclusively in macrophages in hypoxic areas in human breast cancer,[73] which is dependent on the increase of hypoxia-inducible factors (HIF) especially HIF-1α.[73,74] There is the HIF-1α-dependent expression of additional proangiogenic molecules such as basic fibroblast growth factor (bFGF), CXCL8/IL-8, adrenomedullin, and matrix metalloproteinase-9 (MMP-9).[62] HIF-1 activity was demonstrated to increase the expression of chemokine CXCL12 and its receptor CXC4 (CXCR4) in macrophages, which enhance the adaptation to hypoxia. CXCL12 may contribute to the chemoattraction of monocytes and macrophages toward the tumor hypoxic sites facilitating angiogenesis and promoting

Table 1. Hypoxia effects on macrophage polarization *in vitro*.

Models/cells	Macrophage polarization conditions	Hypoxic conditions	Markers used	Outcome	References
Human monocytic THP-1 cells; murine bone marrow–derived macrophages	*For M1 polarization*: Macrophages were incubated for 24 h with 10 pg/mL LPS and 20 ng/mL rhIFNγ *For M2 polarization*: Macrophages were incubated for 48 h with 20 ng/mL rhIL-4 and 20 ng/mL rhIL-13	Macrophages were incubated in a CO_2-independent medium with 4 mM l-glutamine and 3.75 g/L d-glucose *Normoxia conditions*: 21% O_2 *Chronic hypoxia conditions*: 6-h cell incubation under 1% O_2, 99% N_2 *Cycling hypoxia (cyH) condition*: Four consecutive cycles of 1-h hypoxia (1% O_2) followed by 30-min reoxygenation (21% O_2) (6 h) of cell culture	mRNA expression for TNF-α, IL-1β, IL-8, human leukocyte antigen (HLA-DR), CD80, PTGS2, CD206, CCL22, iNOS, Arg-2, MARCO, IFIT1, MRC-1 Secretion of TNF-α, IL-8, IL-6, IL-1β, macrophage inflammatory protein (MIP-2) Phosphorylation of STAT1, p65	cyH induced a proinflammatory phenotype in human M0 macrophages and amplified the proinflammatory phenotype (M1); only a slight influence of hypoxia on M2 macrophages and an increase in IL-8 mRNA expression were observed cyH-induced M0 phenotype and amplified proinflammatory phenotype in M1 macrophages were suggested to be caused by nuclear factor-κB (NF-κB) and c-jun activation Similarly, cyH induced the M1 phenotype in murine M0 macrophages and promoted the proinflammatory phenotype displayed by murine M1 macrophages	Hunyor and Cook[59]
Human myeloid leukemia THP-1 cells	*For M0 phenotype*: THP-1 cells were differentiated by incubation with 10 ng/mL PMA for 24 h at 37°C *For M1 polarization*: M0 cells were incubated with 100 ng/mL LPS and 20 ng/mL IFN-γ for 48 h *For M2 polarization*: M0 cells were incubated with 20 ng/mL IL-4	*Normoxia conditions*: RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin, cultured at 37°C with 5% CO_2; O_2 not specified *Hypoxia conditions*: Incubator with oxygen control containing 1% O_2, 5% CO_2, and 94% N_2, with or without 5 μM SB203580 (an inhibitor of p38 mitogen-activated protein kinase (MAPK)) was used; after 24- and 48-h incubation, supernatants and cells were collected for further analysis	*Surface markers*: CD86, CD206 Secretion of IL-1β, TNF-α, HLA-DR, TARC, CD163, p38, HIF-1α, and β-actin VEGF (including the predominant isoforms, $VEGF_{189}$, $VEGF_{165}$, $VEGF_{145}$, $VEGF_{121}$) and CCL17	Macrophage differentiation toward M2 was observed, while the level of M2 polarization induced by IL-4/hypoxia conditions was not changed; hypoxia significantly decreased IL-1β expression in M1 macrophages and increased the VEGF mRNA levels both in M1 and M2 macrophages Hypoxia decreased the secretion of proinflammatory mediators (IL-1β, TNF-α, VEGF, and CCL17) into the supernatants from M1-polarized macrophages; the effects of hypoxia were independent of HIF-1α; the decrease in levels of proinflammatory cytokines induced by hypoxia was reverted by adding SB203580 (p38-dependent signaling inhibitor[64]) implying the involvement of hypoxia-activated p38-dependent signaling in the proinflammatory cytokine production[65]	Ke et al.[10]
Two models of hGBM: U87 and U251, known to be non-hypoxic and severely hypoxic, respectively; bone marrow–derived macrophage culture	(1) Tumor models consist of an orthotopic injection of hGBM cells into athymic rats (after that macrophage migration and polarization were investigated) (2) Macrophages were isolated from the bone marrow of femora and tibiae of *nude* mice *For M1 polarization*: Cells were incubated in the medium (1 g/L glucose Dulbecco's modified Eagle's medium (DMEM)) with 100 ng/mL LPS for 24 h *M2 polarization*: Cells were incubated in the medium (1 g/L glucose DMEM) with 50 ng/mL recombinant mouse IL-4 for 24 h	*Normoxia conditions*: 20% O_2 and 5% CO_2 in an incubator for different time periods (1 g/L glucose DMEM) *Hypoxia conditions*: A humidified 5% CO_2/balance N_2 gas mixture in a hypoxic chamber (1% or 0.2% O_2) for different periods (1 g/L glucose DMEM)	*Surface markers*: CD68, CD14 iNOS and Arg1 immunostaining Nitric oxide production	Macrophages infiltrated the tumors originated from the U251 cell line, and the accumulation of macrophages occurred predominantly in hypoxic regions; the expression of M2 polarization markers in macrophages correlated with hypoxia severity; the authors suggest that hypoxia can induce M2 polarization in M0 and even in M1 macrophages	Leblond et al.[8]

(Continued)

Table 1. (Continued)

Models/cells	Macrophage polarization conditions	Hypoxic conditions	Markers used	Outcome	References
Peripheral blood mononuclear cells are separated as $CD14^+$ (monocytes)	*For M0 phenotype*: Primary mononuclears were cultured for 6 days with RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS) in the presence of 100 ng/mL hM-CSF under normoxic or hypoxic conditions *M1 polarization*: Culturing M0 for an additional 24 h with fresh medium supplemented with 5% FCS and containing 100 ng/mL LPS from *Escherichia coli* O111: B4 *For M2 polarization*: 20 ng/mL hIL-4	*Normoxia conditions*: 20% O_2 for 24 h *Hypoxic conditions*: Cell incubation and handling in a sealed anaerobic workstation incubator, incorporating a gas mixing system and flushed with a mixture of 1% O_2, 5% CO_2, and 94% N_2 for 24 h	*Flow cytometry*: CD14, CD80, CCR7, and TREM-1; CD68, CD206, CD86, HLA-DR, CD36 *ELISA*: IL-12, TNF-α, IL-1β, CXCL8, IL-6, and IL-10; osteopontin; CCL18; CCL24; and TGF-β1	M1 macrophages exhibited a decrease in the M1 markers (CD80 and CCR7) as well as a significant increase in the expression of the M2 markers (CD206 and CD36) under hypoxic conditions, indicating that hypoxic M1 macrophages switched to the M2 type; IL-12, TNF-α, IL-6, and IL-10 secretion was significantly reduced, while production of proangiogenic mediators, CXCL8 and OPN, was elevated; hypoxia induced a significant upregulation of CD206 and CD36 in M0 and M2 macrophages; hypoxia induced a substantial downregulation of CD80 and an increase in the CXCL8 level in M2 macrophages, while it caused a decreased IL-12, IL-6, and IL-10 secretion as well as an increased release of TGF-β in M0; these results indicate an important role of the hypoxic environment as a direct trigger of human macrophage polarization toward the M2 activation state	Vishnyakova et al.[53]
Macrophages ($CD68^+$) were differentiated from human THP-1 monocytes; pancreatic cancer cell lines PANC-1 and BxPC-3	*M0 polarization*: Human THP-1 monocytes were differentiated into macrophages by an incubation (5% CO_2 with RPMI 1640 medium containing 10% FBS) in the presence of PMA *M1/M2 phenotype* was investigated after the experiment	*Normoxia conditions*: 5% CO_2 with RPMI 1640 medium containing 10% FBS; O_2 and duration are not specified *Hypoxia conditions*: Incubation in a hypoxia cell incubator with 1% O_2; duration is not specified	CD206, CD163, IL-10, TGF-β, and arginase-1; iNOS, IL-1β, CD11b, CD68 CD9 (exosome markers)	miR-301a-3p was highly expressed in hypoxic pancreatic cancer cells and enriched in hypoxic pancreatic cancer cell–derived exosomes; exosomes isolated from PANC-1 and BxPC-3 cells cultured under hypoxic conditions promote M2 activation of macrophages *in vitro*	Corroyer-Dulmont et al.[66]
Murine brain microglia (BV2 cell line); murine macrophages (RAW 264.7 cell line)	BV2 cells were cultured with RPMI 1640. RAW 264.7 cells were cultured with DMEM high-glucose cultured media; the cultured cells were incubated in a humidified atmosphere under the presence of 5% CO_2	To induce oxygen and glucose deprivation, microglia and macrophage cultures were washed with PBS and cultured in a deoxygenated glucose-free balanced solution containing 5.36 mM KCl, 0.81 mM NaH_2PO_4, 0.81 mM $MgSO_4$, and 116 mM NaCl for 4 h in an anaerobic chamber; after 4 h, the cells were washed with PBS, the culture media was replaced, and cells were incubated for 24 h with 5% CO_2	IL-1β, IL-6, TNF-α, IL-10, CD206, and CD40	H/R upregulated M1 phenotype indicated by the release of IL-6, TNF-α, and IL-1β, both in BV2 microglial cells and in BV2 microglial supernatants; furthermore, H/R injury triggered the expression of M1-related mediators such as IL-6, TNF-α, and IL-1β ASK1 was involved in macrophage polarization; ASK1 inhibition induced by the NQDI-1 drug reduced the expression and release of M1-associated factors and, vice versa, elevated the expression and release of M2-associated factors after H/R; at the transcription level, ASK1 inhibition suppressed the expression of M1-associated genes and augmented M2-associated genes	Jeong et al.[67]

LPS: lipopolysaccharides; TNF-α: tumor necrosis factor-alpha; IL: interleukin; PMA: phorbol 12-myristate 13-acetate; IFN-γ: interferon-γ; TARC: thymus and activation regulated chemokine; VEGF: vascular endothelial growth factor; hGBM: human glioblastoma; CSF: colony-stimulating factor; ELISA: enzyme-linked immunosorbent assay; TGF-β: transforming growth factor-β; PBS: phosphate-buffered saline; H/R: hypoxia/reperfusion; ASK1: apoptosis signal-regulating kinase 1.

metastasis.[74] Based on these data, inhibiting HIF-1 can be employed to enhance the efficacy of angiogenesis inhibitors during anticancer therapy. [67,75]

Meanwhile, human monocytic THP-1 cells and murine bone marrow–derived macrophages can switch to the M1 phenotype under hypoxic conditions.[60] Cycling hypoxia (see Table 1) induced a proinflammatory phenotype in human and murine M0 macrophages and amplified the proinflammatory phenotype (M1) while not affecting M2 macrophages.[60] Moreover, murine brain microglia (BV2 cell line) and murine macrophages (RAW 264.7 cell line) were shown to acquire M1 polarization upon hypoxia/reperfusion *in vitro*.[76]

Despite the uncovered differences in the macrophage response to hypoxic conditions *in vitro*, hypoxia in tumors is largely thought to cause M2 phenotype. Such macrophages are called protumoural phenotype macrophages. But the question arises: what are the reasons behind the inconsistency of the data on hypoxia effects obtained from the experiments on different macrophage cell cultures? The differences in the effects of hypoxia might lie in the cell-type-specific reactions, and therefore, no unified "hypoxic macrophage phenotype" can be defined.

Macrophage polarization upon IR

Ischemia-reperfusion injury (IRI) is organ damage caused by a limited supply of oxygen (hypoxia) and nutrients as well as the effects of metabolic by-products after the blood flow is restored. IR causes the damage of different organs, including hepatic ischemia-reperfusion (hIR), MI, kidney injury, ischemic stroke (IS), and organ transplantation.

Liver

hIR occurs in many clinical cases, including liver transplantation and vascular control techniques during liver surgery,[77] and is an important factor affecting postoperative mortality and morbidity.[37,78] The liver comprises many types of immune cells – resident macrophages (Kupffer cells [KCs]), dendritic cells (DCs), natural killer (NK) cells, and natural killer T (NKT) cells – which play a significant role in hIR.[79] Upon IR, blood monocytes can infiltrate the liver and differentiate into macrophages[79] with subsequent M1 and M2 polarization.[37]

Hypoxia and concomitant metabolic disorders are known to accompany hIR and affect macrophage polarization. There are two main phases of liver IR: the initial stages with predominant M1 type and the late stage of liver IR with predominant M2 type.[37,78]

hIR-induced tissue damage is mediated by many factors, including high levels of reactive oxygen species (ROS), imbalanced intracellular calcium ion concentrations, changes in cellular pH, and the release of danger-associated molecular patterns (DAMPs).[37] DAMPs, such as high-mobility group box 1 (HMGB1), were shown to be significantly elevated in the liver after 1-h IR (Figure 2). HMGB1 binding to TLR4 resulted in KC polarization to the M1 phenotype. Other DAMPs such as histamine, DNA fragments, and ATP could also activate KC M1 polarization through TLR-dependent signalization. M1 macrophages accumulated by

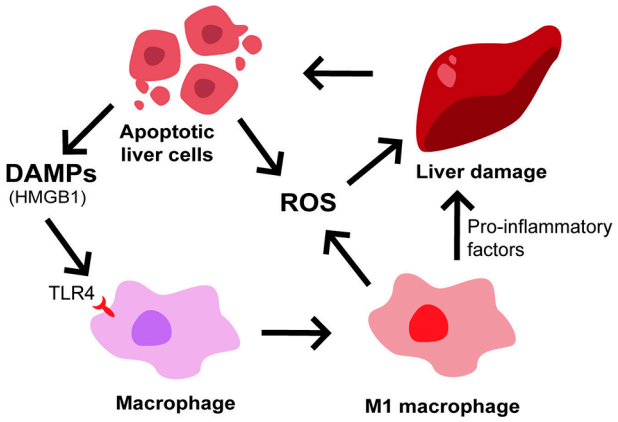

Figure 2. Macrophage polarization during the initial stages of hIR. IR damage resulted in cell apoptosis and generating high ROS levels in the liver. Apoptotic cells produced DAMPs such as HMGB1 promoting M1 polarization via stimulating TLR4 and other receptors on macrophages. M1 macrophages further produced ROS and proinflammatory factors which in turn caused liver damage.[37] (A color version of this figure is available in the online journal.)

8 to 72 h after IR[80] produced the proinflammatory cytokines, TNF-α, and ROS.[81]

By 48 to 72 h after liver IR injury, an increase in the M2 macrophage population was observed.[80] M2 macrophages released anti-inflammatory factors such as IL-10 and TNF-β to alleviate injury. IL-10 inhibited the activation of NF-κB and the proinflammatory factors such as TNF-α, IL-1β, IFN-γ, and IL-2.[82,83]

Kidney

Chronic kidney diseases and kidney transplantation are also accompanied by IR injury. As in the liver, M1-type macrophages prevail at the early stage of kidney IR for the proinflammatory cytokines producing. The peak of IL-6 production was observed by 4 h after the reperfusion followed by the expression of TNF-α, IL-1β, and MCP-1 in the injured kidney.[83] Ko *et al.*[84] showed that macrophage depletion reduced kidney damage. However, macrophages are essential for tubular epithelial cell regeneration at the reperfusion stage.[85] During the later stages of kidney IR injury, macrophages acquire the M2 phenotype and exert regenerative effects.[86–89]

These data indicate that M1/M2 shift could be harnessed in treating hepatic and kidney pathologies. However, this strategy should be used with caution as M2 polarization is involved in many abnormal repair processes such as interstitial fibrosis and crescent formation.[90] According to Tian and Chen,[90] this strategy may have the following pitfalls: (1) as macrophages are highly heterogeneous, dividing them into two groups (M1/M2) is not methodologically correct;[91] (2) it is likely that the *in vivo* expression of M1 and M2 polarization markers is independent of each other;[92] (3) *in vivo*, macrophages comprise the cells derived both from blood monocytes and resident macrophages – therefore, they can possess some additional features;[93] and (4) the polarization of M1 and M2 macrophages undergoes dynamic alterations, and adoptively transferred macrophages may undergo polarization switch *in vivo*.[94,95] Thus, no matter this

possibility seems to be promising, further research into the continuum of macrophages is needed.

Heart

Ischemic heart pathologies, particularly MI, are characterized by IR of the cardiac tissue. Using mouse model (1–3 post-MI days), M1 phenotype of macrophages was shown to prevail at the first stage of MI. The function of these macrophages included removing the cell debris and degrading the extracellular matrix. After 1 and 3 days post-MI, macrophages upregulate several anti-inflammatory genes: *Slfn4*, *Cd9*, *Tnip1*, and *Gpr132*.[96] After 5 to 7 days post-MI, the accumulation of M2 macrophages was observed. It produced anti-inflammatory, proangiogenic, and repair factors and engulfed the apoptotic cells, thus facilitating neoangiogenesis.[97] However, the long-lasting impact of M1 macrophages in the infarcted heart could cause negative effects such as an expansion of the infarct size and scar formation,[98] while M2 macrophages facilitated neoangiogenesis and scar repair.[99] Genes involved in extracellular matrix reorganization including fibroblast-specific genes like *Col1a1* and *Postn* were activated in macrophages after 7 days post-MI suggesting the impact of macrophages on the extracellular matrix proteins in forming the infarct scar.[96]

M1 to M2 shift during MI was shown to induce myocardial repair;[100–103] however, researchers still discuss the role of macrophage polarization during MI. Of note, the macrophage phenotypes in healthy and infarcted hearts are much more complex than the phenotypes defined by the M1/M2 polarization paradigm.[4] For instance, in MI heart, both pro-M1 and pro-M2 stimuli are present, so even a panel of M1/M2 markers does not reflect the *in vivo* macrophage polarization state.[96,104] Ma *et al.* proposed another classification based on post-MI condition explored day by day. They classified macrophages into cardiac macrophages at day 1 cM(MI-D1) post-MI, day 2 cM(MI-D2) post-MI, and so on.[46] *In vitro* classification of macrophages could be also based on the used stimuli: cM(IL-4) can be defined as resident cardiac macrophages stimulated with IL-4.[46] This idea was supported by the fact that proinflammatory day-1 macrophages did not display all typical M1 features, whereas day-7 macrophages did not display typical M2 features.[55,96] A summary of the macrophage polarization during MI is shown in Figure 3 (based on the study of Mouton et al.[96]).

To summarize, the data on macrophage polarization during MI are still insufficient and blurred with inconsistencies. To achieve some consensus, further and more detailed work is needed.

Brain

IS is caused by the blockage of cerebrovascular blood flow. Cerebral ischemia activates microglia and resident macrophages in the brain. After IS, the blood–brain barrier is disrupted, and immune cells such as macrophages, neutrophils, and leukocytes infiltrate the lesion area via the disrupted barrier.[105–107] Microglia being the safeguard equivalent of macrophages in central nervous system (CNS) possesses the M1/M2 dichotomy (M1 classical proinflammatory state and M2 alternative anti-inflammatory/neuroprotective state).[108,109] However, drawing this line of similarity, one should bear in mind that microglia and monocytes/macrophages have different cellular origins. Microglia originates from yolk sac progenitors in the neuroepithelium, while monocytes/macrophages originate from hematopoietic stem cells. There are differences in the expression profiles of these cells. Monocytes/macrophages express CCR2, CD11b, Ly6C, F4/80, and low levels of CX3CR1,[65,108] while microglia expresses high levels of CX3CR1, CD11b, and F4/80; low levels of CD45; and no CCR2.[64] However, both microglia and monocytes/macrophages share similar functions.[110] The main markers shared by M1/M2 microglia and macrophages were described in previous works.[111,112]

Murine models of focal transient cerebral ischemia showed changes in microglia/macrophage polarization at 1 to 14 days of reperfusion.[113] The data indicated that M2 phenotype was dominant at the first stage subsequently shifting to M1.[111,113] However, the expression of M1-type genes (iNOS, CD11b, CD16, CD32, and CD86) was found to gradually increase over time after 3 days and remain elevated for at least 14 days after brain ischemia. The expression of M2 marker mRNA (CD206, Arg1, CCL22, Ym1/2, IL-10, TGF-β) was induced from 1 to 3 days after ischemia and peaked by 3 to 5 days postinjury. The expression of most M2-type genes began to decrease at day 7 after ischemia and was restored to the preinjury levels by day 14.[113] Barakat and Redzic[111] reported that cells with both phenotypes were present in the affected area, but their relative amount changed in time (mostly due to M2 macrophages that could acquire M1 phenotype) and was probably dependent on the proximity to the ischemic core. Thus, the microglia/macrophage polarization stages in brain ischemia cannot be clearly defined as they have both M1 and M2 markers (Figure 4).[109,114]

In the murine model of the middle cerebral artery occlusion, the inoculation of IL-4-polarized BV2 promoted angiogenin expression in the brain. Therefore, microglia cell transfer performs a protective function during IS via promoting angiogenesis.[115] The cytokine IL-4 was shown to improve the long-term neurological outcomes after the stroke through inducing the M2 phenotype in microglia/macrophages.[116] The latter indicates a possible strategy to decrease the neuronal damage after IS by switching M1 phenotype to M2.[109,113,115,117]

Although the conversion of macrophage phenotypes *in vivo* during hypoxia/ischemia is very complex, the simplified model assumes the presence of two extreme phenotypes – M1 (proinflammatory) and M2 (anti-inflammatory) – and phenotype switching could represent an approach aimed at alleviating tissue damage and improve tissue repair.

Energy metabolism of M1/M2 macrophages during IRI

The ability of various agents to improve the outcome of ischemic events by targeting macrophage polarization was studied in the works.[109,112,118] As M1 and M2 macrophages have different energy metabolism, one of the approaches is to alter the macrophage metabolism.[119–121] In M1 macrophages, the glycolysis rate is elevated, while the tricarboxylic acid cycle and oxidative phosphorylation are attenuated. In M2

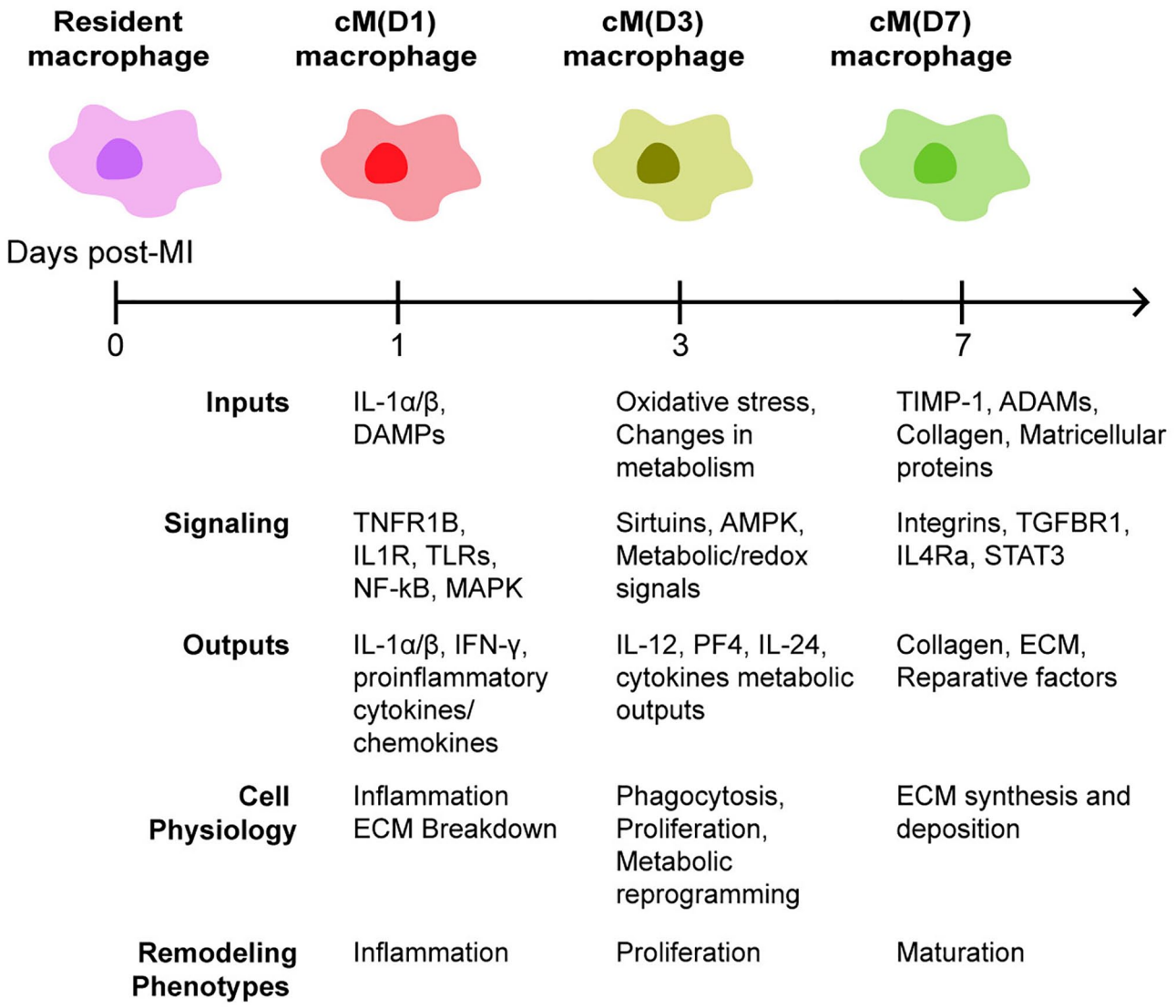

Figure 3. The macrophage polarization stages after MI (from the study of Alagesan and Griffin[95]). (A color version of this figure is available in the online journal.)

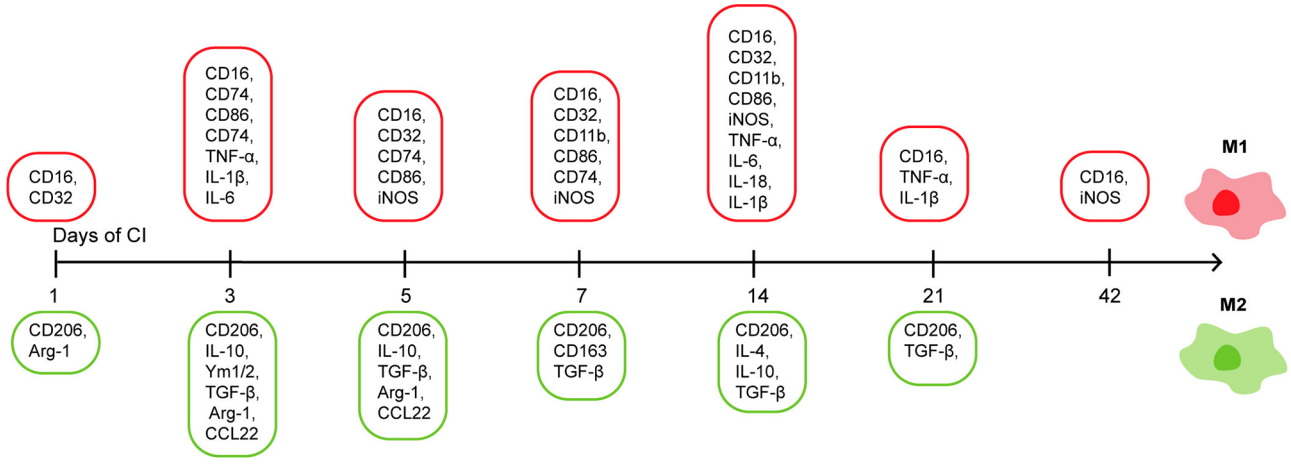

Figure 4. Dynamic changes in microglia marker levels after IS.[111] The scale indicates the time (days) after ischemia. M1 polarization markers are indicated in red (top panel), and the M2 markers are indicated in green (bottom). (A color version of this figure is available in the online journal.)

macrophages, the main energy source is oxidative phosphorylation.[119,122–124] LPS-activated macrophages upregulate the expression of the glucose transporter Glut1,[125] whereas IL-4-activated macrophages increase the expression of lipoprotein lipase and CD36 which mediate the uptake of fatty acids.[126] Therefore, M1 cells preferentially use glucose as an energy source while M2 consume fatty acids.

HIF1α is a well-known signaling molecule that induces a metabolic shift toward anaerobic glycolysis.[127] Elevated lactate production and consequent acidification of the extracellular environment[127] promote M2 macrophage polarization.[128] Consistent with these data, M2 polarization was promoted by the lactic acid produced by hypoxic tumor cells.[127,128]

By day 3 post-MI, the genes related to mitochondrial oxidative phosphorylation were shown to be upregulated, indicating metabolic reprogramming, which can also indicate a wound repair status.[96]

Chouchani *et al.* showed that selective accumulation of the Krebs cycle intermediate, succinate, is a universal metabolic signature of ischemia in a range of tissues, and is responsible for mitochondrial ROS production during reperfusion. Fumarate overflow from purine nucleotide breakdown and partial reversal of the malate/aspartate shuttle lead to the reversal of succinate dehydrogenase (SDH) and promote succinate accumulation during ischemia. After reperfusion, the accumulated succinate is rapidly oxidized, driving extensive ROS generation by reverse electron transport at mitochondrial complex I.[129]

Similarly, macrophages after LPS stimulation produce itaconate,[130] which besides its antibacterial role inhibits SDH in a dose-dependent manner, provoking succinate accumulation.[131,132] Using exogenous itaconate as well as Irg1−/− mice model, Lampropoulou *et al.* revealed itaconate-dependent modulation of macrophage activation through tricarboxylic acid (TCA) cycle regulation.[133] It was hypothesized that itaconate transiently inhibits SDH to gradually "awaken" mitochondrial function that upon reperfusion minimizes ROS production and tissue damage. Thus, itaconate acts as a mitochondrial redox regulator to improve physiological outcomes after IR.[134]

There are multiple mechanisms underlying the influence of metabolism in macrophage activation, and a number of polarizing signals affect the metabolic signaling pathways which coordinate biosynthetic and bioenergetic metabolism involved in macrophage activation.[124] Furthermore, the functions of macrophages could be modulated by targeting their metabolism.

Modulation of macrophage polarization by targeting energy metabolism

Based on the data on the role of succinate in macrophage activation, it was shown that attenuation of ischemic succinate accumulation by pharmacological agents is sufficient to ameliorate *in vivo* IR injury in murine models of heart attack and stroke.[129] A similar approach was used by Zhang *et al.*, who showed that 4-week aerobic preoperative exercise significantly attenuates liver injury and inflammation after IR in mice. Exercise resulted in the appearance of KCs favoring an anti-inflammatory phenotype via metabolic reprogramming. Mechanistically, the exercise-induced release of high-mobility group protein B1 increased the level of the above-mentioned itaconate, which shifted KCs toward an anti-inflammatory phenotype via nuclear factor-2 erythroid-related factor-2 (NRF2).[135]

Cytosolic acetyl-CoA production also has a direct effect on macrophage polarization, as shown by examining LPS-induced TLR signaling.[136] The first stage of TLR activation led to the generation of citrate and its conversion to acetyl-CoA in the cytosol. At later stages, itaconate synthesis from citrate by the LPS-inducible gene *IRG1* serves as an anti-inflammatory feedback mechanism. Itaconate synthesis in response to LPS diminishes the expression of several cytokines, including IL-12, IL-6, IL-1β, and IL-18.[133] Alterations in acetyl-CoA content also caused a shift in histone acetylation profile in various LPS-responsive genes.[136]

Pyruvate dehydrogenase (PDH) could be an additional target for translational studies to treat chronic inflammatory diseases since it oxidized pyruvate into citrate.[137] This assumption is based on the finding that LPS-activated macrophages need the stabilization of HIF1α, which induces expression of pyruvate dehydrogenase kinase 1 that inhibits PDH via phosphorylation.

Calorie restriction is one of the possible ways for modulating metabolism and reducing inflammation,[138] since obesity might induce insulin resistance and local low-grade inflammation.[139,140] In murine insulin resistance model established by 60% high-fat diet for 12 weeks, M1 macrophages accumulated in the adipose tissue.[141] A decrease in insulin resistance was associated with the reduced M1 and elevated M2 macrophage polarization.[141] Under a low-fat diet, eccentric exercise markedly inhibited M1 polarization and activated M2 macrophages in the epididymal fat tissue.[141]

Orillion *et al.* observed an increase in M1-like tumor-associated macrophages (TAMs) along with a decrease in the M2-like phenotype in the C57BL/6 mice (with subcutaneously tumor transplantation) fed a 7% protein diet[132] that enhanced the antitumor capacity of macrophages providing the rationale for clinically using this approach during immunotherapy.[142]

The elevated lactate production by tumor cells led to inhibiting the host immune response[143] and promoted M2 polarization of macrophages under hypoxia.[128] A ketogenic diet (KD: low-carbohydrate, average-protein, and high-fat diet) was shown to decrease the lactate production by glycolytic tumors and resulted in an improved antitumor immune response[143] inhibiting tumor growth.[144] KD stimulated oxidative stress in transplanted CT26+ tumor cells and induced M1 polarization of TAMs reducing the levels of HDAC3/PKM2/NF-κB 65/p-Stat3 proteins.[144]

Spinal cord injury induced the expression of TNF-α and IL-1β, whereas their levels were reduced in the rats with increased ketone levels. Under KD, iNOS expression (the marker of M1 macrophages) was inhibited while arginase-1 expression (the marker of M2 macrophages) was stimulated.[145] Increased arginase activity reroutes arginine from iNOS and, together with reduced iNOS expression, this

could decrease NO production, thus causing M2 polarization. Therefore, ketogenic metabolism promoted the macrophage polarization toward M2 polarization, inhibiting an inflammatory response, reducing the loss of gray matter, and facilitating functional recovery after spinal cord injury.[145] Note that this effect is the opposite of the anti-M2 effect of KD in tumors. Although the exact mechanism by which the KD provides neuroprotection and M2 macrophage polarization is not fully understood, it has been shown that the lactate level is increased in the CNS during KD or fasting-induced ketosis,[146–149] which is the inverse to the KD effect in tumors and could explain this controversy.

Thus, macrophage polarization can shift by the influence on energy metabolism. Such pathological process like IRI depends on macrophage polarization during different times after the damage. Considering that calorie restriction and KD induce M2 macrophage polarization that inhibits an inflammatory response, it would be a good strategy for developing a treatment for IRI.

Conclusions

The existing M1/M2 macrophage paradigm has been criticized for its being the oversimplified model of a highly complex phenomenon. However, such rough classification is still useful for studying metabolic alterations in macrophages and their role in inflammation. Like it was discussed already, macrophage polarization changing is observed during IRI at different times after the damage. The concept of macrophage phenotype shifting from M1 to M2, induced by targeting the energy metabolism, might prove useful for developing treatment strategies for IRI.

AUTHOR CONTRIBUTIONS

All authors contributed to the article and approved the submitted version.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Russian Science Foundation (21-75-30009).

ORCID ID

Egor Y Plotnikov https://orcid.org/0000-0003-2838-3704

SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

REFERENCES

1. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest* 2012;**122**:787–95
2. Bronte V, Pittet MJ. The spleen in local and systemic regulation of immunity. *Immunity* 2013;**39**:806–18
3. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 Macrophages and the Th1/Th2 Paradigm. *J Immunol* 2000;**164**:6166–73
4. Frangogiannis NG. Emerging roles for macrophages in cardiac injury: cytoprotection, repair, and regeneration. *J Clin Invest* 2015;**125**:2927–30
5. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000prime Rep* 2014;**6**:13
6. Munoz-Rojas AR, Kelsey I, Pappalardo JL, Chen M, Miller-Jensen K. Co-stimulation with opposing macrophage polarization cues leads to orthogonal secretion programs in individual cells. *Nat Commun* 2021;**12**:301
7. Ferrante CJ, Leibovich SJ. Regulation of macrophage polarization and wound healing. *Adv Wound Care* 2012;**1**:10–6
8. Leblond MM, Gerault AN, Corroyer-Dulmont A, MacKenzie ET, Petit E, Bernaudin M, Valable S. Hypoxia induces macrophage polarization and re-education toward an M2 phenotype in U87 and U251 glioblastoma models. *Oncoimmunology* 2015;**5**:e1056442
9. Wang Y, Han CC, Cui D, Li Y, Ma Y, Wei W. Is macrophage polarization important in rheumatoid arthritis? *Int Immunopharmacol* 2017;**50**:345–52
10. Ke X, Chen C, Song Y, Cai Q, Li J, Tang Y, Han X, Qu W, Chen A, Wang H, Xu G, Liu D. Hypoxia modifies the polarization of macrophages and their inflammatory microenvironment, and inhibits malignant behavior in cancer cells. *Oncol Lett* 2019;**18**:5871–8
11. Murray PJ. Macrophage Polarization. *Annu Rev Physiol* 2017;**79**:541–66
12. Abumaree MH, Al Jumah MA, Kalionis B, Jawdat D, Al Khaldi A, Abomaray FM, Fatani AS, Chamley LW, Knawy BA. Human placental mesenchymal stem cells (pMSCs) play a role as immune suppressive cells by shifting macrophage differentiation from inflammatory M1 to anti-inflammatory M2 macrophages. *Stem Cell Rev Rep* 2013;**9**:620–41
13. Barros MHM, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. *PLoS ONE* 2013;**8**:e80908
14. De Gaetano M, Crean D, Barry M, Belton O. M1- and M2-type macrophage responses are predictive of adverse outcomes in human atherosclerosis. *Front Immunol* 2016;**7**:275
15. Satoh T, Takeuchi O, Vandenbon A, Yasuda K, Tanaka Y, Kumagai Y, Miyake T, Matsushita K, Okazaki T, Saitoh T, Honma K, Matsuyama T, Yui K, Tsujimura T, Standley DM, Nakanishi K, Nakai K, Akira S. The Jmjd3-Irf4 axis regulates M2 macrophage polarization and host responses against helminth infection. *Nat Immunol* 2010;**11**:936–44
16. Ambarus CA, Santegoets KC, van Bon L, Wenink MH, Tak PP, Radstake TR, Baeten DL. Soluble immune complexes shift the TLR-induced cytokine production of distinct polarized human macrophage subsets towards IL-10. *PLoS ONE* 2012;**7**:e35994
17. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;**8**:958–69
18. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, Paruchuri K, Mahabeleshwar GH, Dalmas E, Venteclef N, Flask CA, Kim J, Doreian BW, Lu KQ, Kaestner KH, Hamik A, Clément K, Jain MK. Kruppel-like factor 4 regulates macrophage polarization. *J Clin Invest* 2011;**121**:2736–49
19. Hao NB, Lu MH, Fan YH, Cao YL, Zhang ZR, Yang SM. Macrophages in tumor microenvironments and the progression of tumors. *Clin Dev Immunol* 2012;**2012**:948098
20. Pesce J, Kaviratne M, Ramalingam TR, Thompson RW, Urban JF Jr, Cheever AW, Young DA, Collins M, Grusby MJ, Wynn TA. The IL-21 receptor augments Th2 effector function and alternative macrophage activation. *J Clin Invest* 2006;**116**:2044–55
21. Hazlett LD, McClellan SA, Barrett RP, Huang X, Zhang Y, Wu M, van Rooijen N, Szliter E. IL-33 shifts macrophage polarization, promoting resistance against Pseudomonas aeruginosa keratitis. *Invest Ophthalmol Vis Sci* 2010;**51**:1524–32
22. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006;**177**:7303–11
23. Liu Y, Stewart KN, Bishop E, Marek CJ, Kluth DC, Rees AJ, Wilson HM. Unique expression of suppressor of cytokine signaling 3 is essential for classical macrophage activation in rodents in vitro and in vivo. *J Immunol* 2008;**180**:6270–8

24. Sachithanandan N, Graham KL, Galic S, Honeyman JE, Fynch SL, Hewitt KA, Steinberg GR, Kay TW. Macrophage deletion of SOCS1 increases sensitivity to LPS and palmitic acid and results in systemic inflammation and hepatic insulin resistance. *Diabetes* 2011;**60**:2023–31
25. Lindsey ML, Saucerman JJ, DeLeon-Pennell KY. Knowledge gaps to understanding cardiac macrophage polarization following myocardial infarction. *Biochim Biophys Acta* 2016;**1862**:2288–92
26. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, Walsh MC, Choi Y, Shadel GS, Ghosh S. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 2011;**472**:476–80
27. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014;**41**:14–20
28. Mauro A, Russo V, Di Marcantonio L, Berardinelli P, Martelli A, Muttini A, Mattioli M, Barboni B. M1 and M2 macrophage recruitment during tendon regeneration induced by amniotic epithelial cell allotransplantation in ovine. *Res Vet Sci* 2016;**105**:92–102
29. Yamaguchi R, Sakamoto A, Yamamoto T, Narahara S, Sugiuchi H, Yamaguchi Y. Differential regulation of IL-23 production in M1 macrophages by TIR8/SIGIRR through TLR4- or TLR7/8-mediated signaling. *Cytokine* 2017;**99**:310–5
30. Yao Y, Xu XH, Jin L. Macrophage polarization in physiological and pathological pregnancy. *Front Immunol* 2019;**10**:792
31. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010;**11**:889–96
32. Fuentes L, Roszer T, Ricote M. Inflammatory mediators and insulin resistance in obesity: role of nuclear receptor signaling in macrophages. *Mediators Inflamm* 2010;**2010**:219583
33. Bohlson SS, O'Conner SD, Hulsebus HJ, Ho MM, Fraser DA. Complement, c1q, and c1q-related molecules regulate macrophage polarization. *Front Immunol* 2014;**5**:402
34. Pollard JW. Trophic macrophages in development and disease. *Nat Rev Immunol* 2009;**9**:259–70
35. Forbes SJ, Rosenthal N. Preparing the ground for tissue regeneration: from mechanism to therapy. *Nat Med* 2014;**20**:857–69
36. Kreider T, Anthony RM, Urban JF Jr, Gause WC. Alternatively activated macrophages in helminth infections. *Curr Opin Immunol* 2007;**19**:448–53
37. Wang H, Xi Z, Deng L, Pan Y, He K, Xia Q. Macrophage polarization and liver ischemia-reperfusion injury. *Int J Med Sci* 2021;**18**:1104–13
38. Hesse M, Modolell M, La Flamme AC, Schito M, Fuentes JM, Cheever AW, Pearce EJ, Wynn TA. Differential regulation of nitric oxide synthase-2 and arginase-1 by type 1/type 2 cytokines in vivo: granulomatous pathology is shaped by the pattern of L-arginine metabolism. *J Immunol* 2001;**167**:6533–44
39. Kim HY, DeKruyff RH, Umetsu DT. The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat Immunol* 2010;**11**:577–84
40. Melgert BN, ten Hacken NH, Rutgers B, Timens W, Postma DS, Hylkema MN. More alternative activation of macrophages in lungs of asthmatic patients. *J Allergy Clin Immunol* 2011;**127**:831–3
41. Saradna A, Do DC, Kumar S, Fu QL, Gao P. Macrophage polarization and allergic asthma. *Transl Res* 2018;**191**:1–14
42. Zizzo G, Hilliard BA, Monestier M, Cohen PL. Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction. *J Immunol* 2012;**189**:3508–20
43. Grinberg S, Hasko G, Wu D, Leibovich SJ. Suppression of PLCbeta2 by endotoxin plays a role in the adenosine A(2A) receptor-mediated switch of macrophages from an inflammatory to an angiogenic phenotype. *Am J Pathol* 2009;**175**:2439–53
44. Biswas SK, Chittezhath M, Shalova IN, Lim JY. Macrophage polarization and plasticity in health and disease. *Immunol Res* 2012;**53**:11–24
45. Atri C, Guerfali FZ, Laouini D. Role of human macrophage polarization in inflammation during infectious diseases. *Int J Mol Sci* 2018;**19**:1801
46. Ma Y, Mouton AJ, Lindsey ML. Cardiac macrophage biology in the steady-state heart, the aging heart, and following myocardial infarction. *Transl Res* 2018;**191**:15–28
47. Palma A, Jarrah AS, Tieri P, Cesareni G, Castiglione F. Gene regulatory network modeling of macrophage differentiation corroborates the continuum hypothesis of polarization states. *Front Physiol* 2018;**9**:1659
48. Wang LX, Zhang SX, Wu HJ, Rong XL, Guo J. M2b macrophage polarization and its roles in diseases. *J Leukoc Biol* 2019;**106**:345–58
49. Poltavets AS, Vishnyakova PA, Elchaninov AV, Sukhikh GT, Fatkhudinov TK. Macrophage modification strategies for efficient cell therapy. *Cells* 2020;**9**:1535
50. Tarique AA, Logan J, Thomas E, Holt PG, Sly PD, Fantino E. Phenotypic, functional, and plasticity features of classical and alternatively activated human macrophages. *Am J Respir Cell Mol Biol* 2015;**53**:676–88
51. Trombetta AC, Soldano S, Contini P, Tomatis V, Ruaro B, Paolino S, Brizzolara R, Montagna P, Sulli A, Pizzorni C, Smith V, Cutolo M. A circulating cell population showing both M1 and M2 monocyte/macrophage surface markers characterizes systemic sclerosis patients with lung involvement. *Respir Res* 2018;**19**:186
52. Lee C, Jeong H, Bae Y, Shin K, Kang S, Kim H, Oh J, Bae H. Targeting of M2-like tumor-associated macrophages with a melittin-based pro-apoptotic peptide. *J Immunother Cancer* 2019;**7**:147
53. Vishnyakova P, Poltavets A, Nikitina M, Muminova K, Potapova A, Vtorushina V, Loginova N, Midiber K, Mikhaleva L, Lokhonina A, Khodzhaeva Z, Pyregov A, Elchaninov A, Fatkhudinov T, Sukhikh G. Preeclampsia: inflammatory signature of decidual cells in early manifestation of disease. *Placenta* 2021;**104**:277–83
54. Raggi F, Pelassa S, Pierobon D, Penco F, Gattorno M, Novelli F, Eva A, Varesio L, Giovarelli M, Bosco MC. Regulation of human macrophage M1-M2 polarization balance by hypoxia and the triggering receptor expressed on myeloid cells-1. *Front Immunol* 2017;**8**:1097
55. Nahrendorf M, Swirski FK. Abandoning M1/M2 for a network model of macrophage function. *Circ Res* 2016;**119**:414–7
56. Gosselin D, Link VM, Romanoski CE, Fonseca GJ, Eichenfield DZ, Spann NJ, Stender JD, Chun HB, Garner H, Geissmann F, Glass CK. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* 2014;**159**:1327–40
57. Smith TD, Tse MJ, Read EL, Liu WF. Regulation of macrophage polarization and plasticity by complex activation signals. *Integr Biol* 2016;**8**:946–55
58. Carreau A, El Hafny-Rahbi B, Matejuk A, Grillon C, Kieda C. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. *J Cell Mol Med* 2011;**15**:1239–53
59. Hunyor I, Cook KM. Models of intermittent hypoxia and obstructive sleep apnea: molecular pathways and their contribution to cancer. *Am J Physiol Regul Integr Comp Physiol* 2018;**315**:R669–87
60. Delprat V, Tellier C, Demazy C, Raes M, Feron O, Michiels C. Cycling hypoxia promotes a pro-inflammatory phenotype in macrophages via JNK/p65 signaling pathway. *Sci Rep* 2020;**10**:882
61. Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. *Hypoxia* 2015;**3**:83–92
62. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 2004;**104**:2224–34
63. Murdoch C, Muthana M, Lewis CE. Hypoxia regulates macrophage functions in inflammation. *J Immunol* 2005;**175**:6257–63
64. Hambardzumyan D, Gutmann DH, Kettenmann H. The role of microglia and macrophages in glioma maintenance and progression. *Nat Neurosci* 2016;**19**:20–7
65. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, Strauss-Ayali D, Viukov S, Guilliams M, Misharin A, Hume DA, Perlman H, Malissen B, Zelzer E, Jung S. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. *Immunity* 2013;**38**:79–91
66. Corroyer-Dulmont A, Pérès EA, Petit E, Durand L, Marteau L, Toutain J, Divoux D, Roussel S, MacKenzie ET, BarrÃ© L, Bernaudin M,

Valable S. Noninvasive assessment of hypoxia with 3-[18F]-fluoro-1-(2-nitro-1-imidazolyl)-2-propanol ([18F]-FMISO): a PET study in two experimental models of human glioma. *Biol Chem* 2013;**394**:529–39
67. Jeong W, Park SR, Rapisarda A, Fer N, Kinders RJ, Chen A, Melillo G, Turkbey B, Steinberg SM, Choyke P, Doroshow JH, Kummar S. Weekly EZN-2208 (PEGylated SN-38) in combination with bevacizumab in patients with refractory solid tumors. *Invest New Drugs* 2014;**32**:340–6
68. Wang X, Luo G, Zhang K, Cao J, Huang C, Jiang T, Liu B, Su L, Qiu Z. Hypoxic tumor-derived exosomal miR-301a mediates M2 macrophage polarization via PTEN/PI3Kγ to promote pancreatic cancer metastasis. *Cancer Res* 2018;**78**:4586–98
69. Lewis JS, Lee JA, Underwood JC, Harris AL, Lewis CE. Macrophage responses to hypoxia: relevance to disease mechanisms. *J Leukoc Biol* 1999;**66**:889–900
70. Burke B, Giannoudis A, Corke KP, Gill D, Wells M, Ziegler-Heitbrock L, Lewis CE. Hypoxia-induced gene expression in human macrophages: implications for ischemic tissues and hypoxia-regulated gene therapy. *Am J Pathol* 2003;**163**:1233–43
71. Murdoch C, Lewis CE. Macrophage migration and gene expression in response to tumor hypoxia. *Int J Cancer* 2005;**117**:701–8
72. Egners A, Erdem M, Cramer T. The Response of macrophages and neutrophils to hypoxia in the context of cancer and other inflammatory diseases. *Mediators Inflamm* 2016;**2016**:2053646
73. Lewis JS, Landers RJ, Underwood JC, Harris AL, Lewis CE. Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. *J Pathol* 2000;**192**:150–8
74. Cramer T, Yamanishi Y, Clausen BE, Forster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, Firestein GS, Gerber HP, Ferrara N, Johnson RS. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* 2003;**112**:645–57
75. Liang Y, Zheng T, Song R, Wang J, Yin D, Wang L, Liu H, Tian L, Fang X, Meng X, Jiang H, Liu J, Liu L. Hypoxia-mediated sorafenib resistance can be overcome by EF24 through Von Hippel-Lindau tumor suppressor-dependent HIF-1α inhibition in hepatocellular carcinoma. *Hepatology* 2013;**57**:1847–57
76. Cheon SY, Kim EJ, Kim JM, Kam EH, Ko BW, Koo BN. Regulation of microglia and macrophage polarization via apoptosis signal-regulating kinase 1 silencing after ischemic/hypoxic injury. *Front Mol Neurosci* 2017;**10**:261
77. Ye L, He S, Mao X, Zhang Y, Cai Y, Li S. Effect of hepatic macrophage polarization and apoptosis on liver ischemia and reperfusion injury during liver transplantation. *Front Immunol* 2020;**11**:1193
78. Serracino-Inglott F, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. *Am J Surg* 2001;**181**:1606
79. Dong Z, Wei H, Sun R, Tian Z. The roles of innate immune cells in liver injury and regeneration. *Cell Mol Immunol* 2007;**4**:241–52
80. Dal-Secco D, Wang J, Zeng Z, Kolaczkowska E, Wong CH, Petri B, Ransohoff RM, Charo IF, Jenne CN, Kubes P. A dynamic spectrum of monocytes arising from the in situ reprogramming of CCR2+ monocytes at a site of sterile injury. *J Exp Med* 2015;**212**:447–56
81. Tsung A, Sahai R, Tanaka H, Nakao A, Fink MP, Lotze MT, Yang H, Li J, Tracey KJ, Geller DA, Billiar TR. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. *J Exp Med* 2005;**201**:1135–43
82. Yoshidome H, Kato A, Edwards MJ, Lentsch AB. Interleukin-10 suppresses hepatic ischemia/reperfusion injury in mice: implications of a central role for nuclear factor kappaB. *Hepatology* 1999;**30**:203–8
83. Lu TF, Yang TH, Zhong CP, Shen C, Lin WW, Gu GX, Xia Q, Xu N. Dual effect of hepatic macrophages on liver ischemia and reperfusion injury during liver transplantation. *Immune Netw* 2018;**18**:e24
84. Ko GJ, Boo CS, Jo SK, Cho WY, Kim HK. Macrophages contribute to the development of renal fibrosis following ischaemia/reperfusion-induced acute kidney injury. *Nephrol Dial Transplant* 2008;**23**:842–52
85. Vinuesa E, Hotter G, Jung M, Herrero-Fresneda I, Torras J, Sola A. Macrophage involvement in the kidney repair phase after ischaemia/reperfusion injury. *J Pathol* 2008;**214**:104–13
86. Lee S, Huen S, Nishio H, Nishio S, Lee HK, Choi BS, Ruhrberg C, Cantley LG. Distinct macrophage phenotypes contribute to kidney injury and repair. *J Am Soc Nephrol* 2011;**22**:317–26
87. Zhang MZ, Yao B, Yang S, Jiang L, Wang S, Fan X, Yin H, Wong K, Miyazawa T, Chen J, Chang I, Singh A, Harris RC. CSF-1 signaling mediates recovery from acute kidney injury. *J Clin Invest* 2012;**122**:4519–32
88. Li W, Zhang Q, Wang M, Wu H, Mao F, Zhang B, Ji R, Gao S, Sun Z, Zhu W, Qian H, Chen Y, Xu W. Macrophages are involved in the protective role of human umbilical cord-derived stromal cells in renal ischemia-reperfusion injury. *Stem Cell Res* 2013;**10**:405–16
89. Ranganathan PV, Jayakumar C, Ramesh G. Netrin-1-treated macrophages protect the kidney against ischemia-reperfusion injury and suppress inflammation by inducing M2 polarization. *Am J Physiol Renal Physiol* 2013;**304**:F948–57
90. Tian S, Chen SY. Macrophage polarization in kidney diseases. *Macrophage* 2015;**2**:e679
91. Novak ML, Koh TJ. Phenotypic transitions of macrophages orchestrate tissue repair. *Am J Pathol* 2013;**183**:1352–63
92. Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. *J Leukoc Biol* 2013;**93**:875–81
93. Yona S, Jung S. Monocytes: subsets, origins, fates and functions. *Curr Opin Hematol* 2010;**17**:53–9
94. Cao Q, Wang Y, Zheng D, Sun Y, Wang C, Wang XM, Lee VW, Wang Y, Zheng G, Tan TK, Wang YM, Alexander SI, Harris DC. Failed renoprotection by alternatively activated bone marrow macrophages is due to a proliferation-dependent phenotype switch in vivo. *Kidney Int* 2014;**85**:794–806
95. Alagesan S, Griffin MD. Alternatively activated macrophages as therapeutic agents for kidney disease: in vivo stability is a key factor. *Kidney Int* 2014;**85**:730–3
96. Mouton AJ, DeLeon-Pennell KY, Rivera Gonzalez OJ, Flynn ER, Freeman TC, Saucerman JJ, Garrett MR, Ma Y, Harmancey R, Lindsey ML. Mapping macrophage polarization over the myocardial infarction time continuum. *Basic Res Cardiol* 2018;**113**:26
97. Yan X, Anzai A, Katsumata Y, Matsuhashi T, Ito K, Endo J, Yamamoto T, Takeshima A, Shinmura K, Shen W, Fukuda K, Sano M. Temporal dynamics of cardiac immune cell accumulation following acute myocardial infarction. *J Mol Cell Cardiol* 2013;**62**:24–35
98. Leuschner F, Dutta P, Gorbatov R, Novobrantseva TI, Donahoe JS, Courties G, Lee KM, Kim JI, Markmann JF, Marinelli B, Panizzi P, Lee WW, Iwamoto Y, Milstein S, Epstein-Barash H, Cantley W, Wong J, Cortez-Retamozo V, Newton A, Love K, Libby P, Pittet MJ, Swirski FK, Koteliansky V, Langer R, Weissleder R, Anderson DG, Nahrendorf M. Therapeutic siRNA silencing in inflammatory monocytes in mice. *Nat Biotechnol* 2011;**29**:1005–10
99. ter Horst EN, Hakimzadeh N, van der Laan AM, Krijnen PA, Niessen HW, Piek JJ. Modulators of macrophage polarization influence healing of the infarcted myocardium. *Int J Mol Sci* 2015;**16**:29583–91
100. Harel-Adar T, Ben Mordechai T, Amsalem Y, Feinberg MS, Leor J, Cohen S. Modulation of cardiac macrophages by phosphatidylserine-presenting liposomes improves infarct repair. *Proc Natl Acad Sci U S A* 2011;**108**:1827–32
101. Ma Y, Halade GV, Zhang J, Ramirez TA, Levin D, Voorhees A, Jin YF, Han HC, Manicone AM, Lindsey ML. Matrix metalloproteinase-28 deletion exacerbates cardiac dysfunction and rupture after myocardial infarction in mice by inhibiting M2 macrophage activation. *Circ Res* 2013;**112**:675–88
102. Courties G, Heidt T, Sebas M, Iwamoto Y, Jeon D, Truelove J, Tricot B, Wojtkiewicz G, Dutta P, Sager HB, Borodovsky A, Novobrantseva T, Klebanov B, Fitzgerald K, Anderson DG, Libby P, Swirski FK, Weissleder R, Nahrendorf M. In vivo silencing of the transcription factor IRF5 reprograms the macrophage phenotype and improves infarct healing. *J Am Coll Cardiol* 2014;**63**:1556–66
103. Zhou LS, Zhao GL, Liu Q, Jiang SC, Wang Y, Zhang DM. Silencing collapsin response mediator protein-2 reprograms macrophage phenotype and improves infarct healing in experimental myocardial infarction model. *J Inflamm* 2015;**12**:11
104. Shiraishi M, Shintani Y, Shintani Y, Ishida H, Saba R, Yamaguchi A, Adachi H, Yashiro K, Suzuki K. Alternatively activated macrophages determine repair of the infarcted adult murine heart. *J Clin Invest* 2016;**126**:2151–66

105. Amantea D, Nappi G, Bernardi G, Bagetta G, Corasaniti MT. Post-ischemic brain damage: pathophysiology and role of inflammatory mediators. *FEBS J* 2009;**276**:13–26
106. Denes A, Thornton P, Rothwell NJ, Allan SM. Inflammation and brain injury: acute cerebral ischaemia, peripheral and central inflammation. *Brain Behav Immun* 2010;**24**:708–23
107. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. *Nat Med* 2011;**17**:796–808
108. Kanazawa M, Ninomiya I, Hatakeyama M, Takahashi T, Shimohata T. Microglia and monocytes/macrophages polarization reveal novel therapeutic mechanism against stroke. *Int J Mol Sci* 2017;**18**:2135
109. Wang J, Xing H, Wan L, Jiang X, Wang C, Wu Y. Treatment targets for M2 microglia polarization in ischemic stroke. *Biomed Pharmacother* 2018;**105**:518–25
110. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, Mehler MF, Conway SJ, Ng LG, Stanley ER, Samokhvalov IM, Merad M. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science* 2010;**330**:841–5
111. Barakat R, Redzic Z. The role of activated microglia and resident macrophages in the neurovascular unit during cerebral ischemia: is the jury still out? *Med Princ Pract* 2016;**25**:3–14
112. Jiang CT, Wu WF, Deng YH, Ge JW. Modulators of microglia activation and polarization in ischemic stroke (Review). *Mol Med Rep* 2020;**21**:2006–18
113. Hu X, Li P, Guo Y, Wang H, Leak RK, Chen S, Gao Y, Chen J. Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia. *Stroke* 2012;**43**:3063–70
114. Wang G, Zhang J, Hu X, Zhang L, Mao L, Jiang X, Liou AK, Leak RK, Gao Y, Chen J. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J Cereb Blood Flow Metab* 2013;**33**:1864–74
115. Tian Y, Zhu P, Liu S, Jin Z, Li D, Zhao H, Zhu X, Shu C, Yan D, Dong Z. IL-4-polarized BV2 microglia cells promote angiogenesis by secreting exosomes. *Adv Clin Exp Med* 2019;**28**:421–30
116. Liu X, Liu J, Zhao S, Zhang H, Cai W, Cai M, Ji X, Leak RK, Gao Y, Chen J, Hu X. Interleukin-4 is essential for microglia/macrophage M2 polarization and long-term recovery after cerebral ischemia. *Stroke* 2016;**47**:498–504
117. Tian DS, Li CY, Qin C, Murugan M, Wu LJ, Liu JL. Deficiency in the voltage-gated proton channel Hv1 increases M2 polarization of microglia and attenuates brain damage from photothrombotic ischemic stroke. *J Neurochem* 2016;**139**:96–105
118. Zhang H, Lu M, Zhang X, Kuai Y, Mei Y, Tan Q, Zhong K, Sun X, Tan W. Isosteviol sodium protects against ischemic stroke by modulating microglia/macrophage polarization via disruption of GAS5/miR-146a-5p sponge. *Sci Rep* 2019;**9**:12221
119. Nonnenmacher Y, Hiller K. Biochemistry of proinflammatory macrophage activation. *Cell Mol Life Sci* 2018;**75**:2093–109
120. Stunault MI, Bories G, Guinamard RR, Ivanov S. Metabolism plays a key role during macrophage activation. *Mediators Inflamm* 2018;**2018**:2426138
121. Sadiku P, Walmsley SR. Hypoxia and the regulation of myeloid cell metabolic imprinting: consequences for the inflammatory response. *EMBO Rep* 2019;**20**:e47388
122. Ghesquière B, Wong BW, Kuchnio A, Carmeliet P. Metabolism of stromal and immune cells in health and disease. *Nature* 2014;**511**:167–76
123. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* 2016;**213**:15–23
124. Langston PK, Shibata M, Horng T. Metabolism supports macrophage activation. *Front Immunol* 2017;**8**:61
125. Fukuzumi M, Shinomiya H, Shimizu Y, Ohishi K, Utsumi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. *Infect Immun* 1996;**64**:108–12
126. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, Wagner RA, Greaves DR, Murray PJ, Chawla A. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* 2006;**4**:13–24
127. Ivashkiv LB. The hypoxia-lactate axis tempers inflammation. *Nat Rev Immunol* 2020;**20**:85–6
128. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, Cline GW, Phillips AJ, Medzhitov R. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 2014;**513**:559–63
129. Chouchani ET, Pell VR, Gaude E, Aksentijević D, Sundier SY, Robb EL, Logan A, Nadtochiy SM, Ord ENJ, Smith AC, Eyassu F, Shirley R, Hu CH, Dare AJ, James AM, Rogatti S, Hartley RC, Eaton S, Costa ASH, Brookes PS, Davidson SM, Duchen MR, Saeb-Parsy K, Shattock MJ, Robinson AJ, Work LM, Frezza C, Krieg T, Murphy MP. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* 2014;**515**:431–5
130. Strelko CL, Lu W, Dufort FJ, Seyfried TN, Chiles TC, Rabinowitz JD, Roberts MF. Itaconic acid is a mammalian metabolite induced during macrophage activation. *J Am Chem Soc* 2011;**133**:16386–9
131. Ackermann WW, Potter VR. Enzyme inhibition in relation to chemotherapy. *Proc Soc Exp Biol Med* 1949;**72**:19
132. Nemeth B, Doczi J, Csete D, Kacso G, Ravasz D, Adams D, Kiss G, Nagy AM, Horvath G, Tretter L, Mocsai A, Csepanyi-Komi R, Iordanov I, Adam-Vizi V, Chinopoulos C. Abolition of mitochondrial substrate-level phosphorylation by itaconic acid produced by LPS-induced Irg1 expression in cells of murine macrophage lineage. *FASEB J* 2016;**30**:286–300
133. Lampropoulou V, Sergushichev A, Bambouskova M, Nair S, Vincent EE, Loginicheva E, Cervantes-Barragan L, Ma X, Huang SC, Griss T, Weinheimer CJ, Khader S, Randolph GJ, Pearce EJ, Jones RG, Diwan A, Diamond MS, Artyomov MN. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab* 2016;**24**:158–66
134. Cordes T, Lucas A, Divakaruni AS, Murphy AN, Cabrales P, Metallo CM. Itaconate modulates tricarboxylic acid and redox metabolism to mitigate reperfusion injury. *Mol Metab* 2020;**32**:122–35
135. Zhang H, Chen T, Ren J, Xia Y, Onuma A, Wang Y, He J, Wu J, Wang H, Hamad A, Shen C, Zhang J, Asara JM, Behbehani GK, Wen H, Deng M, Tsung A, Huang H. Pre-operative exercise therapy triggers anti-inflammatory trained immunity of Kupffer cells through metabolic reprogramming. *Nat Metab* 2021;**3**:843–58
136. Lauterbach MA, Hanke JE, Serefidou M, Mangan MSJ, Kolbe C-C, Hess T, et al. Toll-like receptor signaling rewires macrophage metabolism and promotes histone acetylation via ATP-citrate lyase. *Immunity* 2019;**51**:997–1011.e7
137. Meiser J, Kramer L, Sapcariu SC, Battello N, Ghelfi J, D'Herouel AF, Skupin A, Hiller K. Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. *J Biol Chem* 2016;**291**:3932–46
138. Speakman JR, Mitchell SE. Caloric restriction. *Mol Aspects Med* 2011;**32**:159–221
139. Castoldi A, Naffah de Souza C, Camara NO, Moraes-Vieira PM. The Macrophage Switch in Obesity Development. *Front Immunol* 2016;**6**:637
140. Gomez-Hernandez A, Beneit N, Diaz-Castroverde S, Escribano O. Differential role of adipose tissues in obesity and related metabolic and vascular complications. *Int J Endocrinol* 2016;**2016**:1216783
141. Luo W, Ai L, Wang B, Wang L, Gan Y, Liu C, Jensen J, Zhou Y. Eccentric exercise and dietary restriction inhibits M1 macrophage polarization activated by high-fat diet-induced obesity. *Life Sci* 2020;**243**:117246
142. Orillion A, Damayanti NP, Shen L, Adelaiye-Ogala R, Affronti H, Elbanna M, Chintala S, Ciesielski M, Fontana L, Kao C, Elzey BD, Ratliff TL, Nelson DE, Smiraglia D, Abrams SI, Pili R. Dietary protein restriction reprograms tumor-associated macrophages and enhances immunotherapy. *Clin Cancer Res* 2018;**24**:6383–95
143. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol* 2013;**191**:1486–95
144. Zhang N, Liu C, Jin L, Zhang R, Wang T, Wang Q, Chen J, Yang F, Siebert HC, Zheng X. Ketogenic diet elicits antitumor properties through inducing oxidative stress, inhibiting MMP-9 expression, and

rebalancing M1/M2 tumor-associated macrophage phenotype in a mouse model of colon cancer. *J Agric Food Chem* 2020;**68**:11182–96

145. Lin J, Huang Z, Liu J, Huang Z, Liu Y, Liu Q, Yang Z, Li R, Wu X, Shi Z, Zhu Q, Wu X. Neuroprotective effect of ketone metabolism on inhibiting inflammatory response by regulating macrophage polarization after acute cervical spinal cord injury in rats. *Front Neurosci* 2020;**14**:583611
146. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF Jr. Brain metabolism during fasting. *J Clin Invest* 1967; **46**:1589
147. Pan JW, Rothman TL, Behar KL, Stein DT, Hetherington HP. Human brain beta-hydroxybutyrate and lactate increase in fasting-induced ketosis. *J Cereb Blood Flow Metab* 2000;**20**:1502–7
148. Bernini A, Masoodi M, Solari D, Miroz JP, Carteron L, Christinat N, Morelli P, Beaumont M, Abed-Maillard S, Hartweg M, Foltzer F, Eckert P, Cuenoud B, Oddo M. Modulation of cerebral ketone metabolism following traumatic brain injury in humans. *J Cereb Blood Flow Metab* 2020;**40**:177–86
149. Prins M. Diet, ketones, and neurotrauma. *Epilepsia* 2008;49 Suppl **8**:111–3