



Immunometabolism of Phagocytes and Relationships to Cardiac Repair

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Specialty section:

This article was submitted to
Cardiovascular Biologics and
Regenerative Medicine,
a section of the journal
Frontiers in Cardiovascular Medicine

Received: 17 November 2018

Accepted: 22 March 2019

Published: 11 April 2019

Citation:

Zhang S, Bories G, Lantz C,
Emmons R, Becker A, Liu E,
Abecassis MM, Yvan-Charvet L and
Thorp EB (2019) Immunometabolism
of Phagocytes and Relationships to
Cardiac Repair.
Front. Cardiovasc. Med. 6:42.
doi: 10.3389/fcvm.2019.00042

Cardiovascular disease remains the leading cause of death worldwide. Myocardial ischemia is a major contributor to cardiovascular morbidity and mortality. In the case of acute myocardial infarction, subsequent cardiac repair relies upon the acute, and coordinated response to injury by innate myeloid phagocytes. This includes neutrophils, monocytes, macrophage subsets, and immature dendritic cells. Phagocytes function to remove necrotic cardiomyocytes, apoptotic inflammatory cells, and to remodel extracellular matrix. These innate immune cells also secrete cytokines and growth factors that promote tissue replacement through fibrosis and angiogenesis. Within the injured myocardium, macrophages polarize from pro-inflammatory to inflammation-resolving phenotypes. At the core of this functional plasticity is cellular metabolism, which has gained an appreciation for its integration with phagocyte function and remodeling of the transcriptional and epigenetic landscape. Immunometabolic rewiring is particularly relevant after ischemia and clinical reperfusion given the rapidly changing oxygen and metabolic milieu. Hypoxia reduces mitochondrial oxidative phosphorylation and leads to increased reliance on glycolysis, which can support biosynthesis of pro-inflammatory cytokines. Reoxygenation is permissive for shifts back to mitochondrial metabolism and fatty acid oxidation and this is ultimately linked to pro-reparative macrophage polarization. Improved understanding of mechanisms that regulate metabolic adaptations holds the potential to identify new metabolite targets and strategies to reduce cardiac damage through nutrient signaling.

Keywords: macrophage, neutrophil, phagocyte, immunometabolism, hypoxia, reperfusion, cardiac repair

INTRODUCTION

According to the American Heart Association, ~720,000 individuals in the United States alone will succumb to a first hospitalized acute myocardial infarction (AMI), and ~335,000 more will have a recurrent event (1). Improvements in timeliness and efficiency of clinical treatment have reduced mortality after first heart attack. Nevertheless, the incidence of heart failure, including post-MI heart failure, has seen recent escalations (2). The progression to heart failure is often related to the degree of ventricular damage after acute insult. Occlusion of coronary arteries restricts blood flow to the myocardium. This leads to reduced oxygen availability and metabolic substrates that are essential to sustain active cardiomyocyte metabolism. Oxygen restriction also

modulates the immune response to the injured heart. Further clinical percutaneous reperfusion and re-oxygenation may cause unintended tissue damage in the ischemic heart (3).

Acute Coronary Syndromes Have Long Been Associated With Inflammation (4, 5) and Metabolic Syndromes. In the majority of patients who succumb to sudden death after AMI, cardiac pathology typically reveals significant accumulation of polymorphonuclear and mononuclear phagocytes. In general, acute inflammation is followed by a resolution program that acts to dampen the amplitude of inflammation and orchestrate tissue repair. However, in many diseases of aging, this transition fails and leads to a persistence of chronic inflammation (6). Defects in inflammation resolution are often linked to a systemic metabolic imbalance (7). A similar phenomenon may occur after AMI, in which inefficient inflammation resolution due to hyperlipidemia (8) is linked to maladaptive myeloid response (9). In experimental animals, the ischemic heart recruits a diverse repertoire of innate and adaptive immune cells. This is triggered by chemokines and cytokines that are secreted by cardiac resident and recruited cells. Neutrophils enter and accumulate in the ischemic heart soon after AMI, and this is followed by recruitment of monocyte subsets. Two monocyte sources are of particular interest in this context. This includes monocytes generated from hematopoietic stem cells in the bone marrow, and mature monocytes released from the splenic reservoir (10). Independent of their site of origin, these monocytes differentiate into cardiac macrophages (11), which may either contribute to promote or resolve the inflammatory response (11). This occurs by the liberation of cytokines and growth factors that are recognized by parenchymal and stromal cells and therefore modulate the tissue response to injury (12).

Within the ischemic myocardium, reductions in both oxygen and nutrient supply are associated with acute cell death and necrosis (13). Prolonged ischemia impairs mitochondrial oxidative phosphorylation that is coupled to the synthesis of adenosine triphosphate (ATP) synthesis. In order to meet bioenergetic demands needs, cells must then rely on glycolysis, which in turn increases the concentration of lactate. Intracellular lactate accumulation lowers cytosolic pH whereas extracellular lactate is sensed by immune cells and can lead to their activation (14). Accumulation of metabolically active immune cells also depletes local oxygen and nutrient substrate availability. A consequence of reduced oxygen availability is the induction of hypoxia-inducible transcription factors (HIF), which act transcriptionally to promote glycolysis. Additional triggers, including liberation of damage associated molecular patterns (DAMP) in heart (15), activate immune cell toll like receptors, that are also tied to signaling that induce glycolytic polarization. This glycolytic switch has functional consequences. This includes facilitating the diversion of metabolites from Krebs cycle and the pentose phosphate pathway (PPP), to substrates that promote cellular proliferation during tissue repair. As the repair site matures, macrophages polarize to phenotypes associated with mitochondrial oxidative phosphorylation, as discussed below. Most studies of myocardial

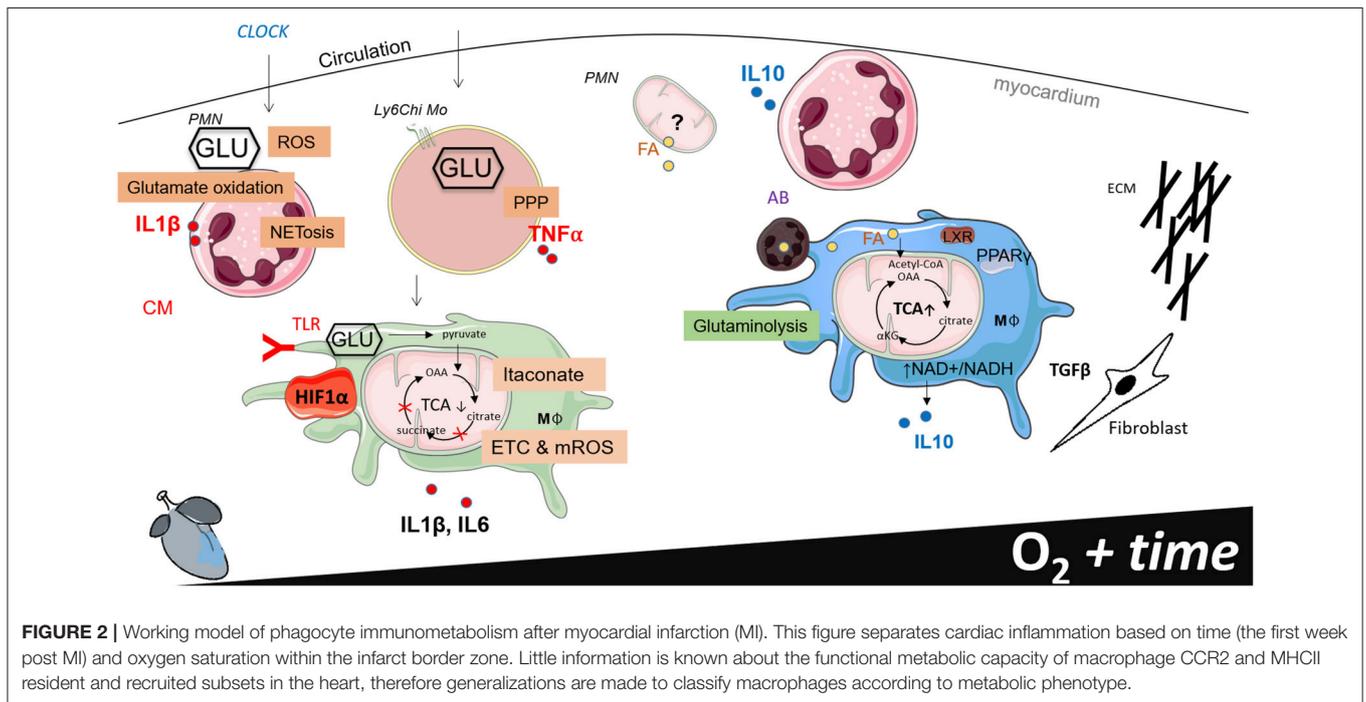
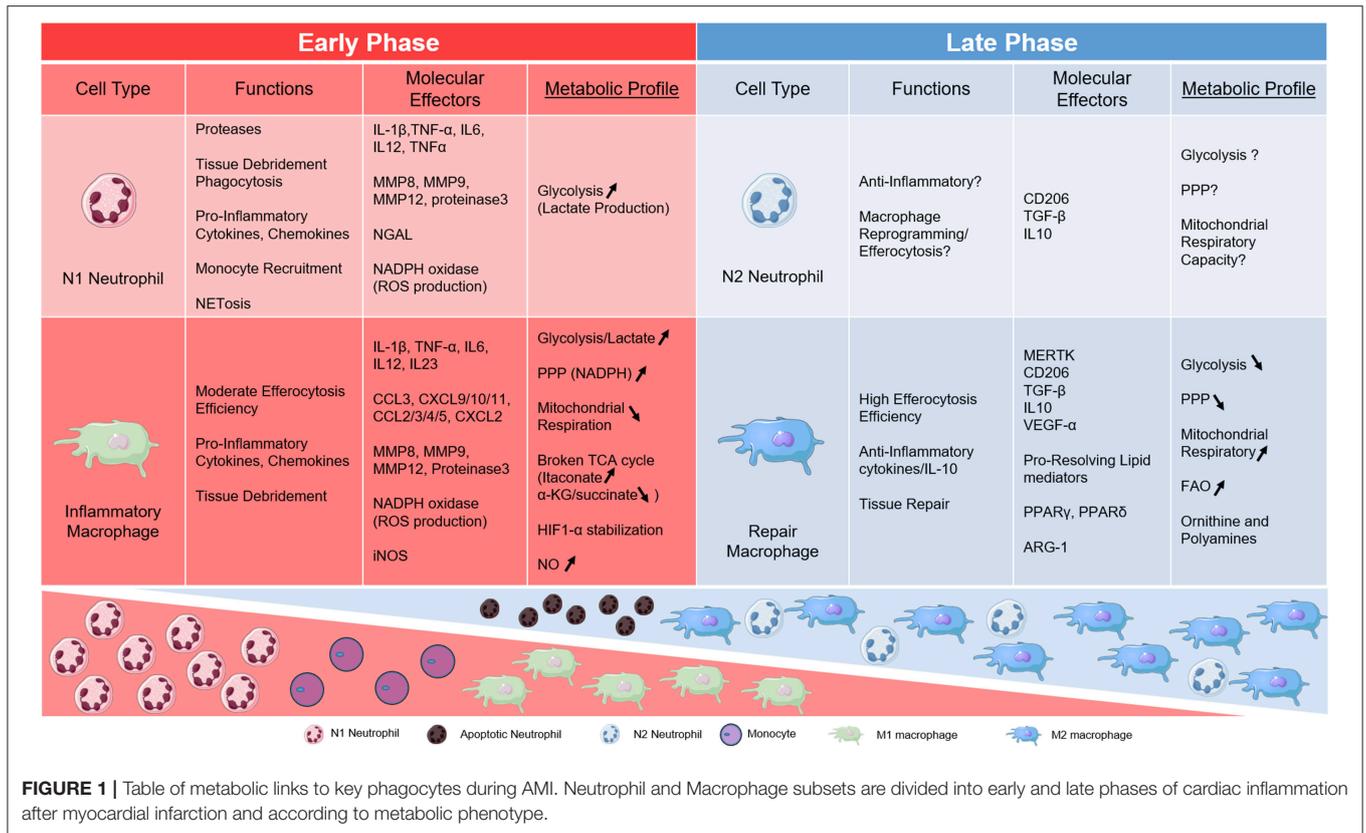
ischemic metabolism have focused on the myocyte. In contrast, discussed herein (**Figures 1, 2**) are advancements in the field of immunometabolism that hint at a significant role of phagocyte metabolism to cardiac repair.

NEUTROPHIL IMMUNOMETABOLIC LINKS TO HEART

As the predominant phagocyte in blood, the glycolytic-biased polymorpho-nuclear (PMN) neutrophil, is primed to rapidly respond to secreted alarms of tissue injury (16). PMNs have been referred to as the “wrecking crew” for their propensity to secrete proteases that facilitate wound breakdown that enables wound clearance. Thus, PMNs contribute to reparative functions after tissue injury, however they are also notoriously affiliated with causing maladaptive collateral tissue damage, particularly after clinical reperfusion. Patients in the highest tertile of circulating neutrophils track with heightened risk for MI (17). This bad reputation extends to after cardiac transplant, as pre-operative neutrophil phenotype is a biomarker for early allograft rejection (18). Elevated neutrophils are also prognostic for poor clinical outcome (19) and directly correlate with infarct size following percutaneous coronary intervention (20). Neutrophil depletion and inhibition of neutrophil-derived enzymes (21) have long been associated with experimental improvements with cardiac function (22). Harmful neutrophil effects may be amplified in the setting of metabolic syndromes (23). For example, neutrophils from hyperlipidemic patients exhibit elevated markers of superoxide release (24). Moreover, neutrophils from diabetic humans and experimental mice are more susceptible to cell death by NETosis (neutrophil extracellular traps), which impairs wound healing (25) and is linked to glucose metabolism. In contrast, neutrophil depletion may aggravate cardiac function after AMI, which is consistent with a conserved reparative function (26). The mechanism underlying this protective role may be linked to crosstalk with macrophages, which can polarize toward a reparative phenotype after PMN efferocytosis. This dichotomy between deleterious vs. protective roles of PMNs after AMI may be better understood by examining PMN function at distinct stages of AMI inflammation vs. resolution vs. reperfusion, as further discussed below.

Recent Reports Highlight our Evolving Understanding of Neutrophil Polarity (16)

For instance, neutrophils isolated from infarcted mouse hearts on the first days after coronary ligation expressed pro-inflammatory markers IL1 β and TNF α . By contrast, the neutrophil profile was more anti-inflammatory a few days later (27). In tumors, neutrophils have been classified into N1 and N2 polarization states (28), akin to the widely but overly simplistic M1/M2 macrophage classification. In this context, it is tempting to speculate that neutrophil polarization states may reflect unique metabolic rewiring, as illustrated in the M1/M2 dichotomy.



Neutrophil variation also manifests through diurnal variation. For example, immune regulation may be tied to a metabolic clock (29). It has been estimated that ~15% of all metabolites

may be under circadian control (30). This includes amino acid, lipid, and carbohydrate metabolic pathways (31). A molecular clock may integrate daily metabolic changes driven

by feeding-fasting and energy storage, to immune function. Indeed, the master inflammatory regulator NF κ B is required for maintenance of behavioral rhythm in mice (32). Among immune cells, neutrophils appear unique in their daily pattern of release, re-entry, and clearance from the bone marrow (33). In this context, neutrophil recruitment to sites of tissue injury is altered by time of day. In heart, elevations in chemokine-dependent recruitment of neutrophils occurred during non-sleep activity periods, relative to rest. During cardiac injury, heightened cardiac neutrophil infiltration was also observed during these activity periods (34). This was in parallel to systemic metabolic fluctuations in hepatic gluconeogenesis, elevated serum glucose (35), and leukocyte mobilization from the bone marrow (36). Nevertheless, it remains unclear if rhythmic neutrophil recruitment is also associated with neutrophil metabolic and inflammatory polarization. Although causal molecular clock mechanisms in neutrophils have not been characterized in detail, clock genes such as *Bmal1* exhibit significant daily variation in human neutrophils. Furthermore, human peripheral neutrophil pools display daily oscillation that correlates with time-dependent changes in superoxide production and phagocytosis (37). Circadian clock also controls the activity of the NAD⁺ dependent deacetylase *Sirtuin3*, which dampens mitochondrial respiration and oxidative enzyme activity (38). Interestingly, Sirtuin 3 deficiency aggravates experimental thrombosis and leads to increased neutrophil extracellular traps (NET). Taken together, intriguing links between circadian rhythm, neutrophil function, and metabolism are brought to light (39).

Recent Findings Implicate Mitochondrial Respiration Pathways in Neutrophil Maturation

For example, autophagy-dependent generation of fatty acids is important during neutrophil differentiation. Autophagy-defective neutrophil precursors have increased glycolytic activity but impaired mitochondrial respiration and fatty acid oxidation. This led to lipid droplet accumulation and diminished neutrophil development (40). These data suggest that autophagy is necessary for the release of free fatty acids from intracellular stores and within neutrophil precursor cells. That is, lipophagy may be required to provide mitochondrial fuel to support oxidative phosphorylation necessary for neutrophil maturation. On the other hand, mice lacking autophagy related 5 (*Atg5*), a gene essential for autophagosome formation, exhibited increased neutrophil proliferation and maturation (41). Furthermore, treatment of myeloid progenitors with ATP-synthase inhibitor oligomycin, reduced electron transport chain flow and neutrophil differentiation, highlighting the essential role of oxidative phosphorylation during neutrophil maturation (42). Finally, mitochondrial dysfunction has been associated with an impaired unfolded protein stress response and mitochondrial dysfunction, leading to reduced neutrophil differentiation (43, 44). The contribution of most of these pathways in heart are largely untested.

As Early as the 1960s, Observations Were Made in Guinea Pigs, Reporting That Mature Polymorphonuclear Leukocytes Are Glycolytic (45)

This is consistent with ultrastructural imaging, which often fails to identify significant numbers of mitochondria in mature neutrophils (46). Furthermore, mitochondrial respiration in neutrophils was found to be low (47). These observations have led to a hypothesis of high glycolytic reliance and a selective role of the mitochondria as a platform for apoptotic signaling (47, 48). Indeed, neutrophils rely greatly on glycolysis for their inflammatory functions (48). Glycolysis is increased in neutrophils during phagocytosis, and in the absence of glucose, the rate of ATP generation in neutrophils drops drastically. Furthermore, neutrophils from patients with chronic granulomatous disease have the same rate of glycolysis and ATP content as normal cells, consistent with resistance to defective respiration with glycolytic bias (49). After release, NETs become crosslinked to plasma fibrinogen, leading to thrombosis. This is a reason for the “no-reflow” phenomenon observed when artery circulation is restored. Thus, an experimental treatment approach employing DNase-based and thrombolytic agent combinations to treat ischemia-reperfusion injury has been shown to reduce NET formation and result in long-term benefit in heart function (50). NETs have also been identified in patients with ST-elevation myocardial infarction (STEMI) (51). Glycolysis and the glucose transporter (*Glut-1*) have also been implicated in NET formation (52). The pentose phosphate pathway (PPP) is also critical for NET release. Glucose diversion toward the PPP allows the production of nicotinamide adenine dinucleotide phosphate (NADPH), which in turn fuels NADPH oxidase to produce superoxide, and NETs release (53).

In Addition to Their Pro-Inflammatory Roles, Neutrophils and Their Metabolism Also Play a Role in Inflammation Resolution

For example, neutrophils metabolize the specialized pro-resolving lipid Resolvin E1 (RvE1), derived from omega-3 eicosapentaenoic acid, into inactive byproducts. This may be part of a coordinated return to homeostasis as RvE1 is a pro-resolving lipid mediator that is pro-phagocytic at low nM concentrations (54). Neutrophils at steady state also facilitate a diurnal IL-23 signaling axis to alert the need for additional myeloid cells (55). During inflammation, neutrophils recruit inflammatory monocytes and macrophages (56). Inflammatory neutrophils secrete chemoattractants such as heparin binding protein (HBP) (57) and LL-37 (58) that regulate monocyte and macrophage infiltration. In heart, neutrophils trigger macrophage polarization toward an anti-inflammatory and a reparative phenotype (26), whereas depleting neutrophils with monoclonal Ly-6g antibodies in mice inhibited Ly-6Chi monocyte release from splenic reservoirs. This in turn increased macrophage proliferation in the infarct, and increased cardiomyocyte death, fibrosis, and markers of heart failure. An

interesting question to answer is whether neutrophils may also instruct cardiac macrophage reprogramming through rewired cellular metabolism. Phagocytic uptake of dying neutrophils and apoptotic cells is known to induce an anti-inflammatory response. The phagocytic program is regulated by nuclear receptors including LXR (59), PPAR γ (60), and PPAR δ , which are known to be master-regulators of lipid and mitochondrial metabolism (61).

MACROPHAGE METABOLISM AND LINKS TO CARDIAC REPAIR

For Decades we Have Appreciated That Activated Macrophages Often Exhibit Features of Heightened Glycolysis and Warburg Metabolism (62)

This phenotype is characterized by augmented glycolysis, increased expression of glycolytic enzymes (63), and diminished oxygen consumption. Although a less efficient path for the generation of ATP, glycolysis does not require oxygen, which is rate limiting during myocardial hypoxia. Oxygen reductions stabilize macrophage HIFs (64) and accumulation of the HIF-1 α isoform is linked to glycolytic induction, including glucose transporter GLUT-1 (65), hexokinase (66), and 6-phosphofructokinase (67). Simultaneously, HIF-1 α may antagonize oxidative phosphorylation by shunting pyruvate away from the mitochondria through the action of PDK1 (68). Interestingly, HIFs also sense changes in metabolism from extracellular sources. For example, lactic acid produced by glycolytic tumor cells, induces a HIF-1 α -dependent polarization of tumor-associated macrophages (69). In macrophages, metabolic regulation by HIFs appears to be a property specific to the HIF-1 α isoform. HIF-2 α in contrast induces inflammatory cytokines, independent of significant changes in glycolysis (70). During myocardial infarction, *Hif-1 α* deficiency in myeloid cells decreased leukocyte recruitment inside the damaged tissue and improved cardiac function post MI (71). Glycolysis and HIFs are also triggered by the activation of macrophage toll like receptors (72). In heart, liberation of endogenous damage-associated molecular patterns (DAMPs) become ligands for TLR activation (73). TLR4-activation stimulates glycolysis and triggers pyruvate kinase-M2, in cooperation with HIF-1 α and to transactivate the expression of the *Il1 β* gene (74). As discussed further below, HIF-1 α is activated after TLR4 stimulation through the accumulation of TCA-derived succinate (75). Under aerobic conditions, AKT-mTOR signaling activates HIF-1 α to “train” immunity (76) through epigenetic remodeling. Trained immune cells are also characterized by a high ratio of nicotinamide adenine dinucleotide (NAD $^{+}$) relative to its reduced form NADH. NAD $^{+}$ has been implicated in key macrophage signaling events. For example, NAD $^{+}$ depletion can induce macrophage necroptosis while NAD $^{+}$ replenishment protects cell from necroptosis and alleviates cytotoxicity (77). In a therapeutic context, intraperitoneal injection of NAD precursor nicotinamide mononucleotide (NMN)

significantly increased NAD $^{+}$ in heart and protected from I/R injury (78).

The propensity for glycolytic activation in innate immune cells during inflammation is exploited in the clinic. For example, glucose analog 2-(18F)-fluoro-2-deoxy-D-glucose (FDG) concentrates in tissue with high glycolytic activity (79), including organs rich in inflammatory macrophages (80); this is readily detected by positron emission tomography. Granulocyte-macrophage colony-stimulating factor (GM-CSF), which attracts phagocytes to the heart (81), enhances macrophage glycolytic activity and 18F-FDG-uptake during inflammation *in vivo* (82), and after MI (83). More recent approaches have non-invasively imaged glycolytic cardiac inflammation with hyperpolarized magnetic resonance through employ of [1- 13 C] pyruvate and lactate (84). Development of similar clinical techniques to monitor the balance between immune cell glycolysis and oxidative phosphorylation may be useful in differentiating discreet stages of inflammation and inflammation resolution.

The PPP

The switch to glycolysis from oxidative phosphorylation is more efficient for the generation of biosynthetic intermediates that may fuel macrophages in the response to tissue injury. This is in part due to the pentose phosphate pathway (PPP), or hexose monophosphate shunt. Increased glycolytic utilization drives buildup of metabolites like glucose-6-phosphate, which flux into the PPP. This shunt is an alternative glucose-oxidizing pathway that is essential for the production of purines and pyrimidines for nucleic acid synthesis. The PPP also produces NADPH through Glucose-6-phosphate dehydrogenase (G6PD) and phosphogluconate dehydrogenase (PGD). NADPH is fed into NADPH oxidase and NOX2 on phagosomes (85, 86) to generate localized reactive oxygen species; this may also be useful for the breakdown of engulfed apoptotic bodies during transport to the lysosome. NADPH is additionally important during light-chain 3 associated phagocytosis (87). Thus, the PPP may serve as an important metabolic shunt in phagocytes during early phagocytic phases of MI. Interestingly, metabolite profiling of blood from patients undergoing planned MI, revealed a signature that included alterations in the PPP (88). Due to its dual role, the PPP is also essential to protect macrophages against oxidation by fueling with numerous NADPH-dependent antioxidative enzymes as glutathione-disulfide reductase (89). In the case of excess of circulating glucose, as in diabetic patients, G6PD, a rate-limiting enzyme of the PPP, may be reduced. This can elevate oxidative stress and cell death susceptibility (90). Hyperglycemia promotes myelopoiesis, increases circulating neutrophils, and impairs inflammation resolution in atherosclerosis (91). Elevated myelopoiesis is also a risk factor for impaired cardiac healing post MI (92). Moreover, hyperglycemia leads to increased advanced glycation end products and inflammatory macrophage RAGE signaling (93); this may be targeted by RAGE antagonists (93). The consequences of insulin resistance on macrophage immunometabolic function requires further study.

Glycolytic Pathways Also Feed Into the Mitochondria

Beyond their energetic function, mitochondria act as signaling organelles (94). This was prominently exemplified by the discovery that mitochondrial cytochrome c is released into the cytosol to activate cell death signaling (95). Mitochondria also release metabolites and reactive oxygen species (96) which activate cytosolic signaling. Signaling complexes may also form on outer mitochondrial membranes and form connections with the endoplasmic reticulum as mitochondria-associated membranes and transport lipids between organelles (97).

In the case of inflammation, toll like receptor signaling triggers re-localization of mitochondria to sites proximal to phagosomes. This subcellular trafficking augments the production of mitochondrial reactive oxygen species (ROS) (98).

ROS is also generated by the mitochondrial electron transport chain/ETC. The ETC is composed of four respiratory complexes. Complex I, III, and IV have been found to assemble as larger molecular super-complexes (99). Interestingly, TLR signaling and inflammasome NLRP3 activation reduce the formation of these super-complexes, in turn leading to increased CII activity. The adaptation of ETC complex assembly, including CII activity, is linked to macrophage activation (100). The ETC is also tightly coupled to the TCA. During inflammation, integrated metabolomics and transcriptomics analyses have revealed TCA cycle breaks. For example, one disruption occurs at isocitrate dehydrogenase (IDH) (101). This IDH break leads to the escalation of itaconate (102), which is highly produced by activated macrophages (103). Itaconate in activated macrophages inhibits dehydrogenase-mediated oxidation of succinate and is anti-inflammatory in culture as well as after myocardial ischemia-reperfusion injury (102). As introduced above, a second inflammation-induced TCA break may occur at succinate (101). Succinate is linked to macrophage activation through the activity of succinate dehydrogenase (104). Accumulation of succinate can be transported from the mitochondria to cytosol where in excess it impairs the activity of Prolyl hydroxylases (PHDs), which in turn leads to HIF-1 α stabilization and pro-inflammatory IL-1 β production (74, 104). Accumulation of succinate from the tricarboxylic acid cycle contributes to activation of the NLRP3 (NOD-like receptor family, pyrin domain containing) inflammasome (105) to activate IL-1 β . Succinate also contributes to mitochondrial ROS (mROS) formation. mROS exacerbates issue injury after ischemia-reperfusion (106). Accumulations in succinate may be reoxidized by succinate dehydrogenase upon reperfusion, driving extensive ROS generation by reverse electron transport at mitochondrial complex I (104). Decreasing ischemic succinate accumulation is sufficient to ameliorate ischemia reperfusion injury in murine models of heart attacks (107).

Accumulating evidence highlights the importance of mitochondrial dysfunction in inflammation and cardiovascular disease. In macrophages, mitochondrial dysfunction prevents repolarization of inflammatory macrophages (108), and the NLRP3 inflammasome senses mitochondrial dysfunction (105). Recent findings provide evidence for mitochondrial DNA damage and decreased activity of mitochondrial electron

transport complexes (109) and mitochondrial respiration (110) in coronary artery disease; restoring mitochondrial DNA copy number in these settings may be ameliorative (111). Mitochondrial dysfunction is characterized by uncoupling of the electron transport chain, reduced production of adenosine triphosphate, and elevated production of ROS. Excessive mROS may do damage to mitochondrial DNA. Mitochondrial DNA damage and dysfunction is also associated with oxidative stress post MI. For example, compared to control, the mtDNA-encoded gene transcripts of CI and CIII of the electron transport chain are decreased, along with the enzyme activity of complexes I, III, and IV after MI (112). Ultimately, dysfunctional mitochondria may be cleared or metabolized through mitophagy.

Mitochondria in Anti-inflammatory Processes

In comparison to initial links between glycolytic metabolism and pro-inflammatory macrophage activation, our understanding of metabolic integration with anti-inflammatory macrophage polarization has trailed behind, yet has made significant recent strides forward. During aerobic respiration, glycolytic pyruvate is shuttled to the mitochondria for entry into the enzyme-catalyzed reactions of the tricarboxylic acid cycle (113). In studies that employed 2-deoxy-D-glucose (2DG) to competitively inhibit the production of glucose-6-phosphate from glucose, glycolysis was implicated in generation of pyruvate for entry into the TCA cycle, as well as regulation of interleukin-4 dependent oxidative phosphorylation (114). In addition, glycolysis may contribute to alternative macrophage activation via an M-CSF-induced mTORC2-IR4 axis, acting in parallel with IL-4R α /Stat6 signaling (114). However, a recent study concluded that glycolysis is not required for alternative activation of macrophages. The differences between the studies may be explained by off-target effects of 2-DG on oxidative phosphorylation and the TCA cycle (115). Prior studies associated cell-intrinsic lipolysis and fatty acid oxidation to IL-4 induced macrophage polarization by etomoxir (ETO), an inhibitor of carnitine palmitoyl transferase (CPT1) (116). Subsequent studies in *Cpt1a-deficient* macrophages did not reveal contributions of LC-fatty acid oxidation during alternative macrophage polarization. Rather, etomoxir was found to have the potential to reduce the pool of Coenzyme A (CoA) during IL-4-triggered macrophage polarization (117). Furthermore, *Cpt-2-deficient* macrophages also did not show impaired macrophage polarization, raising further questions on the role of long chain fatty acid oxidation (LC-FAO) and macrophage polarization (118). Nevertheless, inhibition of mitochondrial oxidative phosphorylation has been shown to prevent repolarization from classically activated to alternatively activated macrophages. For example, inhibiting nitric oxide production in classically activated macrophages dampens declines in mitochondrial function and facilitates repolarization to the alternative state (108). IL4 may have potential post MI in promoting reparative phase cardiac macrophages (119).

Mitochondrial Metabolism and Epigenetic Modulation

Epigenetics play a key role in the establishment of the so called M1 and M2 program. α -KG produced by the Krebs cycle, or generated from glutaminolysis, is an important co-factor for numerous enzymes involved in epigenetic modification of DNA and histones (120). Among these enzymes, *Jmjd3*, an essential H3K27 demethylase, has been reported to promote M2 activation whereas its activity attenuates inflammation in classically activated macrophages. In M2 macrophages, α -KG accumulates whereas its abundance is decreased in M1 macrophages due to its broken Krebs cycle, as well as a higher α -KG dehydrogenase activity to the benefit of succinate accumulation (102). Interestingly, α -KG/succinate ratio can modulate *Jmjd3* activity (121). Thus, manipulation of this ratio may allow the control of macrophage polarization state. Another example of the epigenetic control over macrophage inflammatory status is given by TET2, a deoxyribonucleic acid (DNA) methyltransferase that is highly expressed in macrophages. TET2 restricts inflammatory gene activation through DNA methylation at 5-methylcytosine sites, whereas its deletion increases inflammation at basal levels and after LPS stimulation (122). Interestingly, in experimental mice harboring *Tet2* deletion, myeloid *Tet2* deficiency accelerated heart failure through the IL-1 β /NLRP3 inflammasome (123). Clonal hematopoiesis associated with *Tet2*-deficiency has been shown to accelerate atherosclerosis in experimental mice (124, 125), whereas whole-exome sequencing of carriers of clonal hematopoiesis of indeterminate potential (CHIP) patients indicated that mutations in TET2 are associated with heightened coronary heart disease risk (125).

Clearance and Catabolism of Dying Cardiomyocytes and Apoptotic Cells by Efferocytosis (126) Is Necessary for Cardiac Repair (127, 128)

Efferocytosis is distinct mechanistically and metabolically to the substrates acquired during microbial phagocytosis. The engulfment of dying cells has the capacity to double the phagocyte cellular content and provide new nutrient substrates for signaling reactions. Early studies connected the cellular metabolism of efferocytosis to generation of high energy phosphates which are necessary for actin-mediated engulfment of apoptotic bodies (45). Later studies elucidated roles for mitochondrial uncoupling proteins in regulating continued dying cell engulfment (129). Also, mitochondrial fission machinery (130) is necessary for multiple rounds of efferocytosis, which is likely critical to tissue injury that is characterized by heightened cell turnover. These later scenarios suggest conserved relationships and feedback mechanisms between mitochondrial metabolic sensing and uptake of dying cells. In the case of myocardial infarction and after ischemic reperfusion, dying cardiomyocytes are engulfed by recruited and resident cardiac macrophages (128, 131); this correlates directly with both heightened macrophage oxygen consumption and anti-inflammatory *Il10* cytokine expression. IL-10 is a hallmark cytokine produced in disparate settings of tissue injury. Within

the re-perfused myocardium, IL-10 is produced and linked to the regulation of extracellular matrix biosynthesis (132) and cardiac repair (133). IL10 is linked to inflammation resolution and may inhibit toll like receptor-associated glucose uptake through mTOR and induce macrophage polarization toward oxidative phosphorylation (134). Consistent with these relationships, macrophages fed apoptotic cells exhibit a transcriptional and metabolic signature polarized for fatty acid oxidation, and mitochondrial organelles can be found in close proximity to phagosomes. So how might mitochondrial metabolism be linked to anti-inflammatory cytokine production? One answer may be NAD. The citric acid cycle generates NADH reducing equivalents which are fed into the electron transport chain for oxidative phosphorylation (113). Efferocytosis increases NAD⁺, and NAD is sufficient to for efferocytic-induced IL10 through the activation of Sirtuin-mediated signaling (135). In comparison, glucose availability is linked to both lower cytosolic NADH:NAD⁺ ratios and reduced NF- κ B activation, and macrophage pro-inflammatory gene expression. This can be rescued by forced elevation of NADH, or reduced expression of the NADH-sensitive transcriptional co-repressor *CtBP* (136). Efferocytosis also triggers phagocyte polarization and metabolic parallels and distinctions may be made between efferocytic-induced polarization vs. cytokine-induced. For example, alternative polarization of macrophages by interleukin-4 (IL-4) has also been linked to the mobilization of intra- and extra-cellular free fatty acids (FFAs). However, unlike after IL-4, glycolytic requirements may be unnecessary for efferocytic-induced IL10.

An understanding of these basic mechanisms of efferocytic metabolism is important in the context that cardiovascular disease is associated with impaired phagocytosis (137, 138) and metabolic imbalance. For example, obesity is linked to defective efferocytosis in association with elevated levels of saturated fatty acids. This may be corrected by the action of omega-3 fatty acids (139). Impairments of cholesterol efflux from macrophages under conditions of excess lipid, as occurs during cardiovascular disease, may lead to macrophage death (140). Interestingly, lysosomal cholesterol hydrolysis couples efferocytosis to anti-inflammatory oxysterol production (141). Thus, cellular imbalances of metabolites during disease could in principle dysregulate the integrated metabolic response of macrophages to promote tissue repair.

Amino Acids

Amino acids are utilized for numerous anabolic reactions in macrophages and are sensed by mammalian target of the rapamycin (mTOR) (142). mTOR-inhibitors such as rapamycin block immune cell activation and can inhibit allograft rejection. mTOR is particularly sensitive to branched-chain amino acids/BCAAs (143). The BCAA isoleucine is required for cell proliferation in a mTORC1-dependent pathway. For example, experimental mice fed a BCAA-reduced diet exhibited decreased numbers of Foxp3⁺ T-regulatory cells (144). BCAA catabolism is initiated by branched-chain aminotransferase (BCAT) enzymes. BCATs transfer α -amino groups from BCAAs to α -KG and BCAT1 deficiency can lead to alpha-KG accumulation (145). Furthermore, selective inhibition of BCAT1

activity by the compound ERG240 leads to decreased oxygen consumption and glycolysis through the down regulation of IRG1 expression, which leads to decrease itaconate production in human macrophages. ERG240 has also been shown to reduce macrophage migration and inflammatory responses in immune-mediated inflammation (146). Further studies are warranted to test this pathway in disparate inflammatory settings of inflammation, including heart.

One of the earliest links between amino acids and phagocytes was the discovery that arginine is important to macrophage function. Classically activated macrophages metabolize L-arginine through inducible nitric oxide synthase (147) whereas alternatively activated macrophages metabolize L-arginine through arginase-1 (Arg1) (148). Macrophages that are stimulated with TLR ligands express nitric oxide synthase, which metabolizes arginine to nitric oxide and citrulline (149). Extracellular arginine supports the initial burst of NO, which is also required for control of infection (150). In comparison, alternatively activated macrophages express Arg1 that hydrolyzes arginine to ornithine and urea. In this setting, *Arg1-deficiency* in macrophages leads to increased pro-inflammatory macrophage polarization (151). In heart, L-arginine is associated with a higher risk of ischemic heart disease, and L-arginine administration post-infarction may be associated with a higher post-infarction mortality (152). Interestingly, oral administration of arginine improved wound healing and increased insulin-like growth factor-1 (IGF1) in post-surgery patients (153). Also, inhibition of Arg1 activity did not alter alternatively activated macrophage numbers but increased local inflammation and defects in matrix deposition (154).

Glutamine

may also be important in proliferating immune cells as an alternative input for the TCA cycle, or as a source of citrate for fatty acid synthesis. For example, glutamine has recently been implicated in T-cell metabolism (155). In phagocytes, glutamine metabolism provides support for macrophage activation. Multiple polarization states of macrophages rely on glutamine to mount desirable immune functions. For example, classically activated macrophages exhibit increased glucose and glutamine consumption and suppress oxidative metabolism (156). In IL-4 activated macrophages, the TCA cycle is intact and glutamine is metabolized into UDP-GlcNAc, which is important for glycosylation of lectin or mannose receptors for pathogen recognition. Glutamine deprivation reduces this macrophage polarization (101). Later studies reported that glutamine supports alternative macrophage activation through suppressing NF κ B-dependent classical macrophage activation. α KG generated through glutaminolysis is required for PHD-dependent proline hydroxylation of IKK β , thus suppressing NF- κ B downstream of LPS. *In vivo*, glutamine metabolism supports induction of endotoxin tolerance in an α KG dependent manner (121). Glutamine metabolism has also been shown to be increased in trained monocytes. For example, glutaminolysis led to replenishment of the TCA cycle and accumulation of fumarate. Fumarate induced monocyte epigenetic reprogramming by inhibiting KDM5

histone demethylases. Inhibiting glutaminolysis and cholesterol metabolism inhibited blocked trained immunity in monocytes (157). Moreover, glutamine deficiency reduced lipid-induced lysosome dysfunction, mTORC1 activity, and thereby increased autophagy and reduced cell death in macrophages (158). During ischemic heart disease, glutamine enhances recovery from acute ischemia in isolated rat hearts. Furthermore, post-ischemic reperfusion of isolated rat hearts with glutamine resulted in a full recovery of cardiac output (159).

Summary and Future Research

The field of immunometabolism arose to integrate cellular nutrient metabolism with innate and adaptive immune cell function. Largely absent are past preconceived notions that cellular nutrient processing is solely for energetic currency. Nutritional status has long been associated with optimal wound healing, yet early molecular interest grew in part from pioneering recognition of the association between excess metabolism and cell stress (160). During tissue injury, the integration of information between nutrient availability and anabolic needs leads cells to adapt their metabolism to ensure their functions.

In the translational and clinical arena, current interest of relationships between metabolism and inflammation is high. Compounds that were initially prioritized for metabolic benefit, also act through immunometabolic nodes. For example, the insulin sensitizer metformin (dimethylbiguanide) has effects beyond glucose control. Metformin stimulates the energy sensor AMP-activated protein kinase (AMPK), which is activated during nutrient deficiency (161). Induction of AMPK elevates the fatty acid transporter CPT1 and drives β -oxidation. Simultaneously, AMPK may antagonize inflammatory NF κ B gene expression and has been implicated in anti-inflammatory macrophages through an axis of SIRT1-PGC-1 α mitochondrial biogenesis (162). AMPK also inhibits the glycolytic-inhibitor mTOR (163), and mTOR-inhibitor rapamycin has been shown to attenuate myocardial damage after ischemia reperfusion (164). Separately, inhibitors of the sodium-glucose cotransporter SGLT2 may have promise in cardiovascular outcomes (165), yet their action on macrophages are unclear and caution must be taken given potential side effects of urinary tract infections. In comparison, drugs that directly target inflammatory cytokines, may also regulate cellular metabolism. For instance, IL-1 β is notable as introduced above for its sensitivity and responsiveness to metabolic alterations (166). Another case in point is the finding that elevated glucose levels after feeding induces macrophage IL-1 β secretion (167). In the case of myocardial infarction (MI), IL-1 β is markedly upregulated in infarcted myocardium (168) and in blood (169), paralleling peripheral cell blood counts. Preclinical studies have shown that anti-IL-1 β therapy reduced post MI hematopoiesis and leukocytosis, enhanced inflammation resolution in the infarct, and ameliorated post-MI heart failure (170). The landmark Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) inhibited interleukin 1 β (IL-1B) in survivors of myocardial infarction and reduced cardiovascular events (171, 172). However, IL-1 β exerts both detrimental and beneficial effects (173). Thus, further studies are warranted to optimize the full potential of

IL-1 β targeting in heart, as well as potential immunometabolic consequences (174). This is particularly the case in the face of recent negative clinical results after treatment with low dose methotrexate (175).

Because of the complicated integration and flux of numerous metabolic pathways, an informed future perspective will benefit from a coupled global network analysis of metabolomics and inflammatory signaling axes. As an example, integrated top-down transcriptional and metabolic omics approaches were key in revealing that pro-inflammatory macrophages exhibit a disrupted TCA cycle (101). These global and non-biased methods are necessary to completely appreciate the heterogeneity of a diverse phagocyte population. Insight into the metabolic activity of single cells holds the potential to uncover nuanced degrees of immunometabolic control, that we do not currently comprehend. In combination with genetic lineage tracing, fate mapping, and epigenetics signatures, we will also better understand how metabolism may regulate immune cell origins and their differentiation during inflammation (176). Surely, we have only scratched the surface of the spectrum of metabolites that are protagonists of signal transduction in immune cells and heart.

Finally, much of our understanding of immunometabolism has originated from studies in cell culture and experimental rodents. Therefore, it will be important to test the conservation of these findings in human cells and in the clinic. These are important considerations given that mice naturally exhibit a

basal metabolic rate per gram of body weight that is greater than that of humans. Advancements and accessibility of induced pluripotent stem cells that may be differentiated into immune cells (177) will facilitate human-mouse comparisons, as well as genetic associations. In experimental animals, multiple studies that have targeted inflammatory pathways have shown promise in enhancing cardiac repair, however, a disconnect exists between translating outcomes in preclinical models to improved patient outcomes (178). This is a challenge to understand experimental, species, and clinical distinctions between independent studies. It is also a challenge to dig deeper into fundamental mechanisms that regulate immunometabolic signaling in cardiovascular disease.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

ACKNOWLEDGMENTS

This work was supported by National Institute of Health grants HL122309, HL139812, and AI112522. Publication of this research was supported by the Sidney and Bess Eisenberg Memorial Fund.

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