Complement: a global immunometabolic regulator in amyotrophic lateral sclerosis

Martin W. Lo, John D. Lee*

Recently, McDonald et al. (2020) proposed that complement causes a metabolic switch to lipid consumption in amyotrophic lateral sclerosis (ALS), which raises the possibility of complement as a global immunometabolic regulator. In ALS, neuromuscular degeneration occurs concurrently with complement-mediated inflammation, glucose intolerance, and elevated lipid consumption. Moreover, complement is being increasingly recognized as a metabolic regulator that can induce insulin resistance and alter fuel source selection. Thus, the authors propose that chronic complement activation may be driving metabolic reprogramming in ALS, which has broad implications for the field of immunometabolism and would be the first mechanistic explanation of the "lipid switch" in ALS. Specifically, it suggests that complement can drive a global shift in resources during inflammation, in which glucose is redirected from peripheral tissues to immune cells to support their inflammatory actions. To illustrate this perspective, here we introduce immunometabolism and make a case for complement as a global immunometabolic regulator in ALS.

An introduction to immunometabolism: Immunometabolism refers to the ability of leukocytes to align their metabolic pathways with their effector functions (O'Neill et al., 2016). In all cells, these pathways transform fuel sources into the fundamental constituents of life (i.e., ATP, nucleotides, amino acids and reducing agents) and include glycolysis, the tricarboxylic acid (TCA) cycle, fatty acid oxidation and synthesis, the pentose phosphate shunt, and the amino acid pathways. These chemical reactions are organized into successive production lines whereby the outputs of one pathway feed into the next and are highly adaptable, in which their associated enzymes and transporters are modulated in response to various stimuli. In immunity, these metabolic pathways support both specific (e.g., cytokine production) and general immune processes (e.g., pro- and anti-inflammatory phenotypes) and thus, immunometabolism plays a fundamental role in enabling and shaping inflammation.

Glycolysis in immunity: Glycolysis is a fast and responsive means of energy production and is a metabolic hallmark of almost all pro-inflammatory leukocytes. It begins with the uptake of extracellular glucose, which is then converted in the cytosol into ATP and other biosynthetic precursors for fatty acid synthesis, the pentose phosphate shunt, and the serine amino acid pathway. Despite its inefficiencies (i.e., 1 glucose molecule yields 2 ATP molecules), glycolysis is the one pathway that is able to satisfy a cell's basic energy requirements and support high levels of anabolic activity. For this reason, it is favoured by proliferating cells and activated leukocytes, in which pro-growth signaling cascades (e.g., the mitogen-activated protein kinase pathway) and transcription factors (e.g., mechanistic target of rapamycin (mTOR)) upregulate various glycolytic transporters and enzymes. In immunity, glycolysis enables many pro-inflammatory processes including neutrophil degranulation and NETosis (Injarabian et al., 2019); macrophage cytokine production; dendritic cell (DC) antigen presentation; and lymphocyte effector function (O'Neill et al., 2016). In addition, neutrophils use glycolysis to support their basal metabolic function as they lack the mitochondria that is needed to support the TCA cycle (Injarabian et al., 2019). Thus, glycolysis is a key enabler of pro-inflammatory reactions.

The TCA cycle in immunity: By contrast, the TCA cycle is a slower but highly efficient energy pathway that supports the basal metabolic needs of most quiescent and non-proliferating cells. As the name suggests, this process is a cyclic series of reactions and occurs in the mitochondrial matrix where it acts as a nexus for many different metabolic pathways. For instance, glycolysis and fatty acid oxidation produce acetyl coenzyme A (acetyl-CoA), which undergoes aldol condensation with oxaloacetate to form citrate, whilst the amino acid glutamate can be directly converted into α-ketoglutarate (**Figure 1A**). With every pass, the TCA cycle produces 3 1,4-dihydronicotinamide adenine dinucleotide and 1 1,5-dihydroflavin adenine dinucleotide, which donate electrons to the electron transport chain for highly efficient ATP production via oxidative phosphorylation (OXPHOS) (i.e., 1 glucose molecule yields 36 ATP). In immunity, the TCA cycle supports most inactive leukocytes and promotes anti-inflammatory polarization in macrophages and T cells (**Figure 1A**). Indeed, M2 anti-inflammatory macrophages have an intact TCA cycle that allows them to produce UDP-N-acetyl-D-glucosamine, which is necessary for the glycosylation of M2-associated receptors (e.g., the mannose receptor). In contrast, M1 pro-inflammatory macrophages have TCA cycles that are broken in two places: (1) after citrate owing to a decrease in expression of isocitrate lyase and (2) after succinate due to an inhibition of succinate dehydrogenase. This fragmentation causes (1) the build-up of citrate, which promotes the production of fatty acids, biomembranes, nitric oxide, and eicosanoids, and (2) the accumulation of succinate, which stabilizes hypoxia-inducible factor 1α and sustains interleukin-1β (IL-1β) production (O'Neill et al., 2016). Moreover, the TCA cycle supports the actions of regulatory T cells and $CD8^+$ memory T cells, though the mechanisms that underlie this are incompletely understood (O'Neill et al., 2016). In this sense, this humble pathway is vital to basic cellular function and plays a key role in supporting anti-inflammatory processes.

Fatty acid oxidation in immunity: Feeding into the TCA cycle, fatty acid oxidation is a highly efficient energy producing pathway (i.e., 1 fatty acid molecule can yield > 100 ATP molecules) that is commonly used in anti-inflammatory leukocytes. In this process, fatty acids in the cytosol are converted into fatty acid acyl-CoA, which is then processed with respect to the length of the fatty acid's aliphatic tail. Short-chain fatty acids (< 6 tail carbons) are able to passively diffuse into the mitochondrial matrix, whilst medium- and long-chained fatty acids must be conjugated to carnitine via carnitine palmitoyltransferase 1A before being transported into the mitochondrial matrix for reconversion to fatty acid acyl-CoA. Here, fatty acid acyl-CoA from all fatty acids undergoes β-oxidation to yield large quantities of acetyl-CoA, 1,4-dihydronicotinamide adenine dinucleotide, and 1,5-dihydroflavin adenine dinucleotide, which are then able to enter the TCA cycle and drive OXPHOS. However, like in the degenerating tissues in ALS, chronic fatty acid oxidation can saturate the TCA cycle and cause adverse effects including ketoacidosis and oxidative stress. In immunity, fatty acid oxidation is primarily used by non-inflammatory leukocytes as an energy source. For example, M2 macrophages (as defined by activation with IL-4) are programmed by signal transducer and activator of transcription 6 and peroxisome proliferator-activated receptor γ coactivator-1β to rely on fatty acid oxidation, which works to inhibit inflammatory signals. Moreover, regulatory T cells and memory $CD8^+$ T cells express key fatty acid oxidation genes including carnitine palmitoyltransferase 1A, which suppresses effector T cell polarization and supports survival (O'Neill et al., 2016). Thus, this pathway works closely with the TCA cycle and supports both basal metabolic function and anti-inflammatory processes.

Fatty acid synthesis in immunity: As the reverse process, fatty acid synthesis converts a range of biosynthetic precursors to lipids and supports pro-inflammatory responses. To produce straight-chain fatty acids, citrate from the TCA cycle is exported out of the mitochondria to the cytosol where ATP citrate lyase converts it into acetyl-CoA and oxaloacetate. The former is then carboxylated by acetyl CoA carboxylase (ACC) to yield malonyl-CoA, which is then elongated in a dihydronicotinamide adenine dinucleotide phosphate-dependent manner by fatty acid synthase (FASN) until products such as palmitic acid are formed. Palmitic acid may then be converted into fatty acids of alternate chain lengths or become an unsaturated fatty acid. By contrast, branched-chain fatty acid synthesis requires specific amino acids such as valine and leucine as substrates for elongation. However, both fatty acid synthesis pathways are driven by mTOR signaling, which regulates many key enzymes including sterol regulatory element binding protein, FASN and ACC. In immunity, fatty acid synthesis is associated with various pro-inflammatory processes. For example, toll-like receptor stimulation upregulates fatty acid synthesis in DCs and allows them to activate $CD8^+$ T cells. T and B cells also use fatty acid synthesis to support their proliferation and differentiation. However, whilst fatty acid synthesis can support membrane biogenesis and the production of cholesterol-derived ligands for RORγt in T helper 17 cells, its precise role in immunity is incompletely understood (O'Neill et al., 2016). Nevertheless, in collaboration with glycolysis, fatty acid synthesis plays a key role in pro-inflammatory reactions.

The pentose phosphate shunt and amino acid pathways in immunity: By contrast, the pentose phosphate shunt and amino acid pathways have more specific roles in immunometabolism. Firstly, the pentose phosphate shunt converts various glycolytic intermediates into nucleotides and NADPH, which support cellular proliferation and reactive oxygen species (ROS) production respectively. In this pathway, two branches exist: (1) the oxidative branch, which is an irreversible series of linear reactions that generates 2 NADPH, and (2) the non-oxidative branch, which is a complex web of reversible reactions. These branches come together at ribulose-5-phosphate to facilitate downstream nucleotide synthesis. In immunity, the pentose phosphate shunt supports the NADPH oxidase-mediated production of anti-microbial ROS, DC activation, and M1-macrophage

function. Secondly, the amino acid pathways are a highly arborized network of reactions that interacts with almost all the aforementioned metabolic processes to produce the body's non-essential amino acids. Though their details are beyond the scope of this paper, suffice to say that the amino acid pathways support a variety of pro- and anti-inflammatory processes including T cell proliferation and cytokine and nitric oxide production. Thus, these biosynthetic pathways support highly specific processes in immunometabolism.

Complement-mediated immunometabolism: Complement-mediated immunometabolism is an emerging field with a small number of studies showing that C3a, C3b, and C5a shift the metabolic profiles of various leukocytes to support pro-inflammatory reactions. Indeed, C5aR1 signaling in neutrophils stimulates the sodium/hydrogen exchanger 1 transporter that promotes intracellular alkalinization and glycolysis (Denk et al., 2017) whilst CR3/4 signaling promotes macrophage cell cycling and proliferation (Hess and Kemper, 2016). In addition, autocrine CD46, C3aR and/or C5aR1 signaling are required for T cell homeostatic survival and effector function. In the former, inactive T cells turnover intracellular C3 via cathepsin L to support basal mTOR signaling and OXPHOS. By contrast, in the latter, activated T cells use CD46 signaling to drive the expression of late endosomal/lysosomal adaptor, mitogen-activated protein kinase, and MTOR activator 5, glucose transporter 1, and large neutral amino acid transporter, which promotes glycolysis, OXPHOS, and fatty acid synthesis. Similarly, autocrine C5aR1 signaling supports effector T cell function by increasing intracellular ROS and driving autocrine IL-1β signaling to upregulate glycolysis (Revu et al., 2018; West et al., 2018). Thus, these initial findings hint at complement's full immunometabolic potential and we greatly anticipate future developments in this field.

Complement as a global immunometabolic regulator in ALS: Elaborating on this, McDonald et al. (2020) proposed that complement may have synergistic effects on peripheral metabolism in ALS. Indeed, this condition is a rapidly progressive neurodegenerative disease that is associated with a biphasic complement-driven neuroinflammatory response. Initially, brain and spinal cord atrophy elicits a protective gliosis in which locally produced complement enables a range of processes. But as the burden of disease becomes unmanageable, danger signals are released in excess and cause a self-perpetuating cycle of inflammation and collateral damage. Eventually this affects the blood-brain barrier and blood-brain barrier breakdown then enables systemic complement activation and peripheral leukocyte invasion (Mantovani et al., 2014; Gustafson et al., 2017). In this later stage, the hypothesis from McDonald et al. (2020) suggests that complement may act as a global immunometabolic regulator, in which the anaphylatoxins cause a redistribution of fuel resources. Specifically, C3a and C5a may shunt glucose from muscles and motor neurons, which are affected in ALS, to leukocytes by indirectly driving lipid metabolism in the former (**Figure 1B**) and upregulating glycolysis in the latter (**Figure 1C**) (Wang et al., 2017). This then allows the anaphylatoxins to exert their various pro-inflammatory actions, which ultimately accelerates disease progression. Thus, McDonald et al. (2020) make the case that complement is one of the few if not only immune mediators that can marshal the entire body against a dangerous insult.

Concluding remarks: Complement-mediated immunometabolism is an emerging field with significant implications for chronic inflammatory diseases. Here, McDonald et al. (2020) suggest that complement can not only cause persistent inflammation and collateral damage but also provide a permissive metabolic environment with cachexic side effects. In this sense, complement has the potential to be a multi-faceted drug target for a range of non-communicable diseases and we look forward to future developments in this space.

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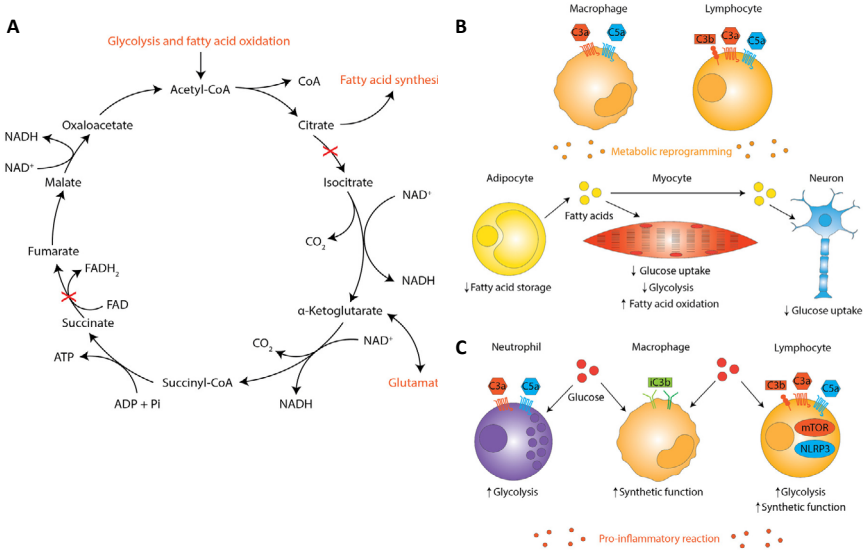

Figure 1 | **Schematic of TCA cycle and complement as a global immunometabolic regulator.**
(A) TCA cycle; precursors and products given at tails and heads of arrows respectively; input pathways given in orange; red crosses denote breaks in the cycle found in M1 macrophages. (B) C3a and C5a recruit and activate macrophages and lymphocytes that cause metabolic reprogramming and promote lipid metabolism in adipose tissue, muscle cells, and motor neurons; (C) C3a, C3b, and C5a cause metabolic reprogramming in neutrophils, macrophages, and lymphocytes that promote glycolysis and synthetic function that enables a pro-inflammatory reaction. Acetyl-CoA: Acetyl coenzyme A; ADP: adenosine diphosphate; ATP: adenosine triphosphate; CO_2: carbon dioxide; CoA: coenzyme A; FAD: flavin adenine dinucleotide; FADH2: 1,5-dihydroflavin adenine dinucleotide; mTOR: mammalian target of rapamycin; NAD: nicotinamide adenine dinucleotide; NADH: 1,4-dihydronicotinamide adenine dinucleotide; NLRP3: NLR family pyrin domain containing 3; Pi: phosphate; TCA: tricarboxylic acid.

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