

REVIEW ARTICLE

Complement's favourite organelle—Mitochondria?

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The complement system, well known for its central role in innate immunity, is currently emerging as an unexpected, cell-autonomous, orchestrator of normal cell physiology. Specifically, an intracellularly active complement system—the complosome—controls key pathways of normal cell metabolism during immune cell homeostasis and effector function. So far, we know little about the exact structure and localization of intracellular complement components within and among cells. A common scheme, however, is that they operate in crosstalk with other intracellular immune sensors, such as inflammasomes, and that they impact on the activity of key subcellular compartments. Among cell compartments, mitochondria appear to have built a particularly early and strong relationship with the complosome and extracellularly active complement—not surprising in view of the strong impact of the complosome on metabolism. In this review, we will hence summarize the current knowledge about the close complosome–mitochondria relationship and also discuss key questions surrounding this novel research area.

LINKED ARTICLES: This article is part of a themed issue on Canonical and non-canonical functions of the complement system in health and disease. To view the other articles in this section visit <http://onlinelibrary.wiley.com/doi/10.1111/bph.v178.14/issuetoc>

KEYWORDS

CD46, Complement, glycolysis, metabolism, mitochondria, OXPHOS

Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; AA, amino acid; ACC, acetyl CoA carboxylase; AIM2, absent in melanoma 2; AMPK, AMP-activated protein kinase; APC, antigen-presenting cell; ATP5D, ATP synthase subunit delta; BCL-XL, B-cell lymphoma extra large; C3aR, C3a receptor; C5aR, C5a receptor; CTL, cytotoxic T cells; CTRPs, C1q/TNF-related proteins; CTSL, cathepsin L; DAMP, danger-associated molecular pattern; DRP1/2, dynamin-related protein 1 or mitofusin 1 and 2; ER, endoplasmic reticulum; ERR α , oestrogen-related receptor- α ; ETC, electron transport chain; FAO, fatty acid oxidation; FAS, fatty acid synthesis; gC1qR, globular head C1q receptor; GLUT1, glucose transporter 1; HIF1 α , hypoxia-inducible factor 1 α ; ICAM-1, intercellular adhesion molecule 1; IP3R, inositol 1,4,5-triphosphate receptor; RyR, ryanodine receptor; MCU, mitochondrial calcium uniporter; IS, immunological synapse; LAIR1, leukocyte-associated immunoglobulin-like receptor 1; LAMTOR5, late endosomal/lysosomal adaptor, MAPK, and mTOR activator 5; LAT1, large neutral amino acid transporter; LFA-1, leukocyte adhesion molecule 1; LRR, leucine-rich repeat; MAC, membrane attack complex; MAMs, mitochondria-associated membranes; MASP, MBL-associated serine protease; MAVS, mitochondrial antiviral signalling; MBL, mannan-binding lectin; mtDNA, mitochondrial DNA; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; NFAT, nuclear factor of activated T cells; NLRP3, NOD-, LRR-, and pyrin domain-containing protein 3; NOD, nucleotide-binding oligomerization domain; OPA1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; RA, rheumatoid arthritis; RLRs, RIG-I-like receptors; SAM, S-adenosylmethionine; SLE, systemic lupus erythematosus; TCA, tricarboxylic acid; TCR, T-cell receptor; TF, transcription factor; TFAM, transcription factor A, mitochondrial; Th, T helper; TLRs, toll-like receptors; VDAC, voltage-dependent anion channel.

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1 | INTRODUCTION

The classic pattern recognition receptors (PRRs) of the innate immune system, such as the RIG-I like receptors (RLRs), the toll-like receptors (TLRs), the inflammasomes, and complement, are evolutionary old sensor systems that are critical to the host's defence against infections. They all detect molecular patterns derived from pathogens or from stressed, infected, dying, or otherwise injured host cells (pathogen- or danger-associated molecular patterns, PAMPs or DAMPs) and initiate—often employing extensive crosstalk with each other—catered cellular responses to remove the detected noxious targets (Guo, Callaway, & Ting, 2015; Heesterbeek, Angelier, Harrison, & Rooijackers, 2018; Liu, Olganier, & Lin, 2016; Odendall & Kagan, 2017; Vijay, 2018). PRR activation on innate and adaptive immune cells drives a whole battery of their respective protective effector responses, from migration of cells to the site of infection, the production of pro-inflammatory cytokines, induction of cytotoxic activity, and so forth. Although all of these activities require the engagement of several key cell metabolic pathways, traditionally, PRR-driven cell stimulation and cell metabolic reprogramming have been viewed as vital concurrent but not necessarily interdependent events. This perception has now changed: It is becoming increasingly clear that all PRRs directly impact on cell metabolism and, vice versa, that cell metabolites are key controllers of PRR activities (Haneklaus & O'Neill, 2015; Prochnicki & Latz, 2017)—thus, there is extensive cooperation between PRRs and metabolism during cell activation. One of the innate sensor systems, however, that was least expected to engage in such direct crosstalk with the cell metabolic machinery was the **complement system**. This was mostly due to the fact that complement was considered to be active exclusively in the extracellular space, while all other PRR systems operate intracellularly. The recent discovery of a cell-autonomous and intracellularly active complement system, the complosome (Liszewski et al., 2013), and the finding that the complosome regulates uniquely several central metabolic pathways (Hess & Kemper, 2016), however, places this ancient system now among the key orchestrators of cell physiology.

Our knowledge about the exact molecular pathways driven by these novel non-canonical functions of complement is currently limited. For example, it is unclear which subcellular compartments are directly impacted by intracellular complement and how. We know that in T cells, the complosome is required for the expression of nutrient transporters to allow for influx of “food” needed for T-cell activation (Kolev et al., 2015). Further, complement components are found in lysosomes and direct normal mammalian target of rapamycin complex 1 (mTORC1) lysosomal assembly and, in direct crosstalk with the NOD-, leucine-rich repeat (LRR)-, and pyrin domain-containing protein 3 (NLRP3) inflammasome, are needed for mitochondrial ROS production (Arbore et al., 2016). Lysosomes and mitochondria, on the other hand, are organelles that cooperate specifically during the regulation of metabolic activity of cells (Lawrence & Zoncu, 2019; Tiku, Tan, & Dikic, 2020). While the impact of complement on lysosomes is currently unexplored, it is acknowledged that extracellular and intracellular complement activity clearly affect mitochondrial

function—and this is becoming an exciting and buoying research area within the complement field. Here, we will summarize the current knowledge about the complosome's/complement's functional relationship particularly with mitochondria. We will also discuss briefly where we see this new complement–metabolism relationship “go” and what the most pressing questions surrounding the intracellular complement activities are.

2 | CELL-AUTONOMOUS INTRACELLULAR COMPLEMENT AND BASIC CELL METABOLISM

Complement is traditionally known as a liver-derived and serum-circulating PRR effector system. It is composed of over 50 fluid phase or cell-bound proteins and poses the first line of defence in the detection and removal of invading pathogens. Complement idles in the blood in a mostly inactive form and becomes activated in a cascade-like manner when one or more of the three main activation pathways, the classical, the lectin, or the alternative pathway, are engaged. This leads to the cleavage activation of the core complement components **C3** and **C5** into **C3a** and C3b, and **C5a** and C5b respectively (Arbore et al., 2016; Liszewski et al., 2013; Merle, Church, Fremeaux-Bacchi, & Roumenina, 2015). C3b is the host's principal opsonin and tags pathogens or other dangerous targets for removal by C3b receptor-expressing phagocytes. Deposition of C5b onto target cells seeds the activation of the terminal pathway and generation of the pore-forming membrane attack complex (MAC) and their lysis. The anaphylatoxins C3a and C5a engage their GPCRs, C3a receptor (**C3aR**) and C5a receptor 1 (**C5aR1**) or 2, on innate and adaptive immune cells to initiate their migration, activation, and effector function via induction of specific signalling events (Hajishengallis, Reis, Mastellos, Ricklin, & Lambris, 2017). Importantly, not all immune response-mediating complement needs to be sourced from the liver. For example, key immune cells such as antigen-presenting cells (APCs) and T cells can express and secrete C3 and C5, which are then activated in the extracellular space and engage activation fragment receptors in an autocrine fashion to induce APC antigen presentation and cytokine secretion and T-cell activation during the cognate APC–T-cell interaction (Lalli et al., 2008; Liu et al., 2005; Strainic et al., 2008). The critical role of systemic and local complement activity in instructing immune cells to remove pathogens is underpinned by the fact that deficiencies in key complement components lead to severe and recurrent infections (Morgan & Kavanagh, 2018; Ricklin, Hajishengallis, Yang, & Lambris, 2010). Furthermore, and similar to other PRR systems, complement also recognizes DAMPs displayed on the surface of stressed and dying cells, and perturbed complement DAMP sensing is therefore associated with a range of autoimmune diseases (Holers & Banda, 2018; Toubi & Vadasz, 2019; West, Kolev, & Kemper, 2018). Thus, it is widely accepted that complement is central to host (immune cell) homeostasis—it was unexpected, however, that complement achieves this often via directly controlling cell metabolism.

The intimate connection between complement and single-cell metabolism became apparent during studies that addressed the functional significance of cell-intrinsic intracellularly active complement, the complosome, which was initially discovered in human CD4⁺ T lymphocytes (Liszewski et al., 2013). Specifically, human T cells, in circulation, express low levels of C3 and C5 and store those (at minimum) in the endoplasmic reticulum (ER), endosomes, and lysosomes. These intracellular C3 and C5 stores are continuously activated on a basal level in circulating T cells: T-cell-intrinsic C3 is cleaved by the ancient protease cathepsin L (CTSL), while the specific protease or the enzyme complex (e.g., C5 convertases that activate extracellular C5) that cleaves intracellular C5 is currently not defined. The engagement of an intracellular C3aR expressed on lysosomes by such intrinsic intracellularly generated C3a sustains tonic mammalian target of rapamycin (mTOR) signalling, which the cell needs for homeostatic survival (Kolev et al., 2015). Leukocyte adhesion molecule 1 (LFA-1) engagement on T cells by endothelial cell-expressed intercellular adhesion molecule 1 (ICAM-1) induces increased C3 and CTSL expression in trans-migrating T cells (Kolev et al., 2020), while T-cell receptor (TCR) stimulation in conjunction with CD28 co-stimulation augments activation of

the cell-autonomous C3 pool (Kolev et al., 2020) (Figure 1). In addition, T-cell stimulation induces rapid C3a and C3b cell surface translocation where these C3 activation fragments engage surface-expressed C3aR and CD46, a receptor for C3b (Liszewski et al., 2013). Signalling events triggered by such autocrine engagement of CD46 induce the high levels of glycolysis and oxidative phosphorylation (OXPHOS) needed specifically for IFN- γ production and hence T helper cell 1 lineage induction (Liao, Lin, & Leonard, 2011; Liao, Lin, & Leonard, 2013; West et al., 2018). CD46 triggers also an increase in activation of the intracellular C5 storages into bioactive C5a and C5b. C5a, in turn, induces ROS generation within the cell, which leads to activation of the canonical NLRP3 inflammasome and subsequent secretion of mature IL-1 β , which sustains Th1 responses (Arbore et al., 2016). The complosome is also present and operative in human cytotoxic CD8⁺ T cells (CTLs), with autocrine C3b-driven CD46 stimulation supporting IFN- γ and granzyme B production via increasing fatty acid metabolism during CTL stimulation (Arbore et al., 2018).

Importantly, the complosome is not only an integral part of successful Th1 and CTL induction, this cell-autonomous system also participates in the contraction phase of T-cell responses. For example,

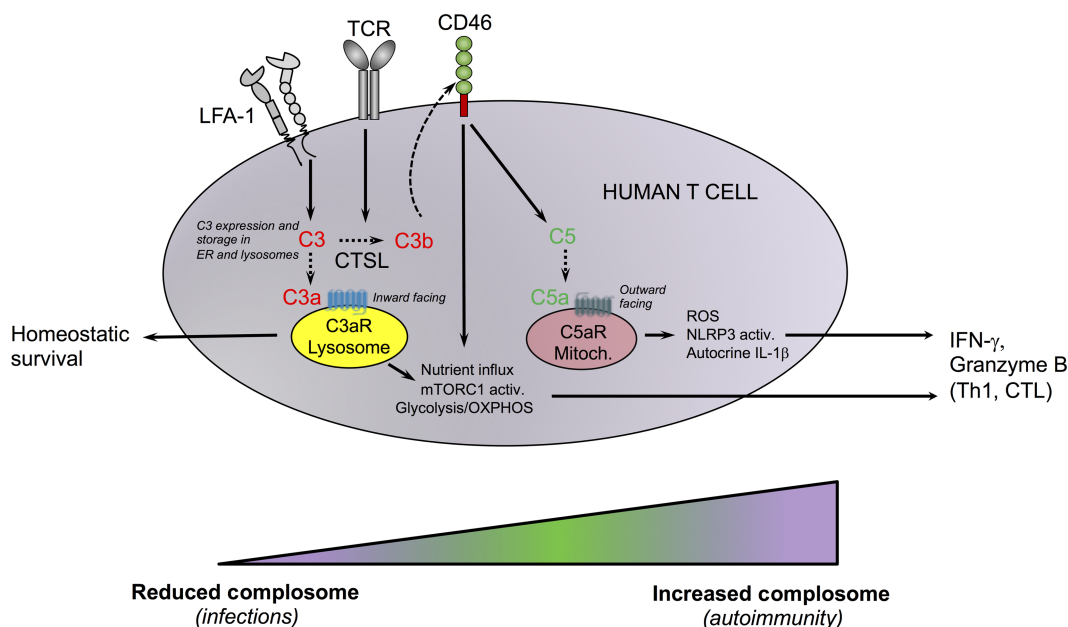


FIGURE 1 Cell-autonomous complement-induced metabolic events in human T cells. Summary schematic depiction of the key metabolic impacts of the cell-autonomous complosome in human T cells that underlie successful effector function induction in CD4⁺ (T helper type 1 [Th1] responses) and cytotoxic CD8⁺ T cells (CTLs). Circulating T cells contain storages of C3 within lysosomes and the endoplasmic reticulum (ER) that are continuously cleaved by cathepsin L (CTSL) to generate intracellular C3b and C3a. C3a within the lysosomes engages the lysosomal, inward facing, C3a receptor (C3aR) and sustains tonic mechanistic target of rapamycin complex 1 (mTORC1) activity required for homeostatic survival. Diapedesis of T cells induces leukocyte adhesion molecule 1 (LFA-1) engagement that significantly increases C3 gene transcription. Concurrent T-cell receptor (TCR) activation (and CD28 co-stimulation, not shown here) triggers rapid translocation of intracellular C3b to the cell surface and autocrine engagement of CD46. CD46 signalling mediates three key metabolic events: expression of glucose and amino acid transporters allowing nutrient influx, mTORC1 assembly at the lysosomes fostering glycolysis and oxidative phosphorylation (OXPHOS), and increased activation of intracellular C5 pools. Intracellularly generated C5a stimulates the mitochondrial C5a receptor (C5aR) resulting in ROS production and nucleotide-binding oligomerization domain-, leucine-rich repeat-, and pyrin domain-containing protein 3 (NLRP3) inflammasome activation. How C5 is activated within T cells and how C5a, contained in vesicles, activates the outward facing mitochondrial C5aR1 are currently unclear. Together, these events drive the induction of IFN- γ production and granzyme B expression denoting Th1 and CTL effector activity. Reduced or pathologically increased complosome activity contributes to recurrent infections and autoimmunity respectively

after successful Th1 induction, CD46-mediated signals, in cooperation with the IL-2 receptor, induce IL-10 coproduction and a (self)regulative contraction phase in these cells (Cardone et al., 2010; Liszewski & Kemper, 2019). CD46-mediated Th1 contraction involves the expression regulation of distinct CD46 isoforms, the induction of the cholesterol biosynthesis pathway, activation of the IL-10-driving transcription factor (TF) c-MAF, and the overall reduction of glycolysis and OXPHOS back to basal levels (Liao et al., 2011; Perucha et al., 2019). Th1 contraction is further supported by autocrine engagement of the alternative **C5aR2** by the des-arginized form of C5a, **C5a-desArg**, which represses the activating C5aR1 signal (Arbore et al., 2016). The centrality of the complosome to normal T-cell immunity is underpinned by the observations that reduced complosome activity is associated with recurrent infections, while pathologically augmented complosome activity contributes to Th1 hyperactivity in rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), scleroderma, and multiple sclerosis (Astier, Meiffren, Freeman, & Hafler, 2006; Cardone et al., 2010; Ellinghaus et al., 2017; Arbore et al., 2020).

Thus, a cell-autonomous and in part intracellularly active complement system exists in human T cells and is an integral component of T-cell effector function induction and contraction via the regulation of key cell metabolic pathways (Hess & Kemper, 2016; Kolev & Kemper, 2017; West & Kemper, 2019).

3 | CANONICAL COMPLEMENT VERSUS NON-CANONICAL COMPLOSOME—DRIVEN BY EVOLUTION?

With the finding that an intracellular complement system exists, a general picture emerges in which the localization of complement activity drives its function: Classic liver-derived complement is key to the detection and removal of pathogens, locally produced, extracellular complement directs immune cell activation, and the complosome regulates basic cellular processes (West, Kunz, & Kemper, 2020). We had previously suggested that such bifurcated development of complement activity makes sense if one argues that complement originated as an intracellular sensor system during evolution and became a secreted and “systemic” system when life evolved from single cell to multicell and then to multi-tissue/organ organisms (Arbore, Kemper, & Kolev, 2017; Kolev & Kemper, 2017).

Thus, complement may have begun as intrinsic regulator of intracellular physiological (nutrient) balance and cell survival early on and then branched out to directing cell-autonomous immunity via regulation of effector functions and finally evolved into the liver-provided guardian of the extracellular space as we know it today. This view is supported by the analysis of C3 structures throughout the animal kingdom that indicates that the oldest known C3 forms were bigger in size and contained additional domains that all have high homology to functional domains of metabolic enzymes (Kolev & Kemper, 2017). In addition, there is accumulating evidence that the C3 (and C5) protein forms generated by cells are distinct from those secreted by the liver

with regard to transcription initiation start point, structure, post-translational modifications, and in the way they are activated (King et al., 2019; Liszewski et al., 2013). Furthermore, it is becoming evident that the intracellular receptors for the complement activation fragments, such as the anaphylatoxin receptors, engage different signalling downstream pathways when stimulated compared with the cell surface activation of the same receptor (Arbore et al., 2016) and that intracellular C3 activation fragments and the cytoplasmic domains of CD46 are directly involved in the regulation of gene expression (Kolev et al., 2015; Kremlitzka et al., 2019). Together, these findings indicate that the complosome follows its own rules when it comes to the structure of its components, and their respective activation modes and functions (see below, final section). This is a concept that aligns well with what we know about the functional evolution of other ancient innate immune sensors and key effectors. For example, it is now broadly accepted that the TLRs and inflammasomes are also extensively involved in normal cell physiology. Furthermore, perturbations in those PRRs have strong associations with inflammatory and/or metabolic disease states (Konner & Bruning, 2011; Loftus & Finlay, 2016) but less so with recurrent infections, which lends further argument to the notion that PRRs may not have evolved primarily to protect against infection but to rather sense and rectify metabolic changes or imbalances (Coll, O'Neill, & Schroder, 2016). Similarly, several members of the IL-1 cytokine family, a pillar of the host protective immune response, such as IL-1 β and IL-33, emerged during evolution early and before their respective receptors and can directly act as TFs in the nucleus to instruct cell activities (De la Fuente, MacDonald, & Hermoso, 2015; Dinarello, 2007). In the same vein, the NLRP3 molecule, independent of inflammasome assembly, is a TF that drives the expression of the *Gata3* gene in mouse T cells and with this initiates the Th2 programme required for responses to helminth parasites (Bruchard et al., 2015).

Therefore, the recent discoveries about the non-canonical cell-autonomous and intracellular activities of complement are possibly less unexpected than initially thought but rather intuitive.

4 | GOING DEEPER INTO THE CELL—COMPLEMENT AS KEY MITOCHONDRIAL REGULATOR

How exactly intracellular complement components regulate T-cell metabolism is not clear. For example, we do not know yet which subcellular compartments, aside from the ER, endosomes, and lysosomes, contain C3 or C5 or can generate their activation fragments or which organelles express activation fragment receptors aside from lysosomes and the ER. Further, it remains to be explored which organelles beyond lysosomes are directly impacted by the complosome and how. Among the many subcellular compartments that could engage in a direct functional crosstalk with intracellular complement and/or be impacted by complement receptor cell surface signalling, mitochondria, however, are among the prime candidates.

4.1 | Mitochondria

Mitochondria are known as the “powerhouses of the cell” as they take in nutrients and break them down for the generation of the energy (ATP) that all cellular processes rely on. They have first been described by Siekevitz (1957) and are thought to originate from ancient α -proteobacteria, which invaded archaea-type hosts. Bacteria and host ultimately settled on a fruitful symbiotic relationship with the bacteria developing into mitochondria that supported the energetic demands that emergence of eukaryotic cells approximately 1.5 billion years ago required (Dyall, Brown, & Johnson, 2004). Mitochondria are structurally unique among the subcellular compartments and considered semi-autonomous because they bring their own specific DNA and mRNA translation machinery and can hence produce several of their basic structural building blocks in part independently from the host nuclear machinery. Mitochondria are composed of an outer and an inner membrane, which have different lipid structures and levels of membrane permeability. For example, the outer membrane is more porous and, while physically separating mitochondria from the cytoplasmic environment, allows relatively easy entry or exit of smaller molecules/ions (Pfanner, Warscheid, & Wiedemann, 2019). Larger molecules such as proteins that seek import or export into/from

mitochondria are handled by specific translocases that mediate transport of these molecules through the outer membrane (Kuhlbrandt, 2015). A major transporter system is the pore-forming voltage-dependent anion channel (VDAC), which transports Ca^{2+} , nucleotides, and metabolites between the cytosol and the mitochondrial intermembrane space. Importantly, the outer membrane also contains a number of enzymes that support key metabolic activities of these organelles, such as respiration and fatty acid oxidation (FAO) (Gellerich et al., 2000). The inner mitochondrial membrane poses a tighter gate and is compartmentalized into so-called cristae that increase the surface area of the inner membrane dramatically (Kuhlbrandt, 2015). Cristae are also equipped with membrane transporters that selectively allow specific ions/molecules to transverse. The inner membrane harbours the electron transport chain (ETC), a series of protein complexes that cooperatively pass along electrons in a series of redox reactions to generate ATP (Figure 2). The energy released by these reactions is captured as a proton gradient, which is utilized for the generation of ATP during chemiosmosis, and the combined activity of the ETC and chemiosmosis is generally called OXPHOS. The electrons and hydrogens needed for the ETC are produced in the mitochondrial matrix by the tricarboxylic acid cycle (TCA, or Krebs cycle or citric acid cycle) from pyruvate that is imported into

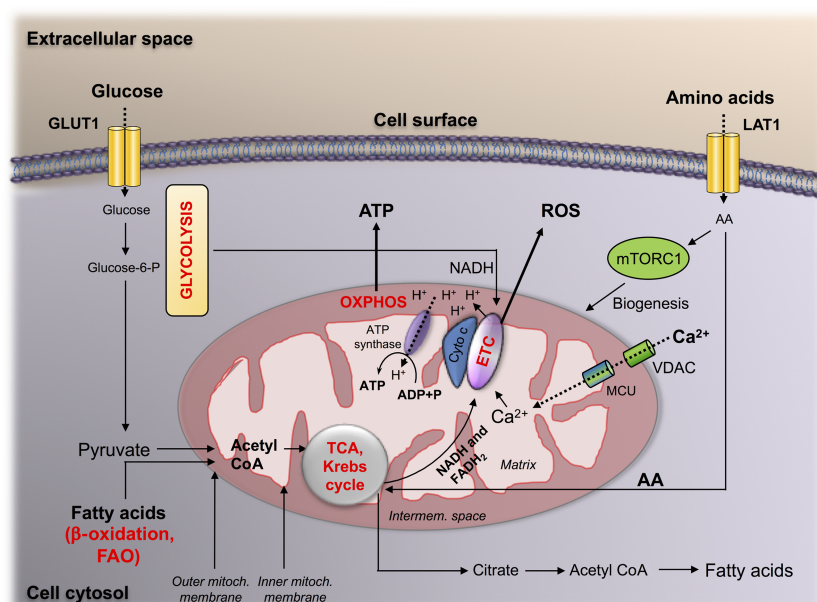


FIGURE 2 Key pathways driving mitochondrial ATP generation. A major task of mitochondria is to convert incoming nutrients into ATP, which provides the energy required for all cell physiological processes. The outer mitochondrial membrane controls import of proteins, ions, and molecules (e.g., via voltage-dependent anion channel [VDAC] or mitochondrial calcium uniporter [MCU]) into the intermembrane space. The inner mitochondrial membrane is organized into cristae and harbours the respiration electron transport chain (ETC), while tricarboxylic acid cycle (TCA) (also known as citric acid cycle or Krebs cycle) activity occurs in the matrix. Pyruvate generated by glycolysis in the cell's cytoplasm or amino acid (AA) taken up by the cell are shunted into the mitochondria's matrix and ultimately utilized by the TCA to generate electron donors (e.g., NADH), which are then stepwise oxidized by the ETC via exergonic redox reactions that couple electron transfer with the transfer of protons into the intermembrane space. This electrochemical proton gradient drives the synthesis of ATP via ATP synthases (collectively referred to as oxidative phosphorylation [OXPHOS]). The generation of ROS is also a by-product of ETC activity, while the TCA also generates the important cellular building blocks, fatty acids. This is a highly simplified schematic with focus on the pathways discussed in this review, and it omits many additional activities of mitochondria, such as generation of signalling-capable metabolites and control of cell death. Cyto c, cytochrome c; FADH₂, flavin adenine dinucleotide; FAO, fatty acid oxidation; GLUT1, glucose transporter 1; LAT1, large neutral amino acid transporter; mTORC1, mammalian target of rapamycin complex 1

the mitochondria as the last step of glycolysis in the cell's cytoplasm. Fatty acids and amino acids (AAs) can also fuel the TCA (Schell & Rutter, 2013) (Figure 2).

4.2 | Mitochondria and immune cell activity

A major part of studies that defined the central role of mitochondria in the regulation of immunity was/is performed using macrophages and T cells. This body of work showed that mitochondria not only deliver the energy that the cell needs to become activated and proliferate but that mitochondrial activity also triggers a range of additional events that control macrophage and T-cell polarization as well as T-cell memory responses (Angajala et al., 2018). For example, sensing of LPS or bacteria by macrophages induces a shift from mitochondrial ATP production to glycolysis-derived ATP and a pro-inflammatory M1 phenotype (Van den Bossche et al., 2016). The differentiation or switch of (M1) macrophages into a more resolution and tissue repair supporting phenotype, however, is driven by preferential engagement of OXPHOS and FAO (Vats et al., 2006). Similarly, naive T cells can be sustained mostly by OXPHOS, while activated T cells, after differentiation into T helper subsets, rely on higher glycolysis and mTORC1 activity compared with OXPHOS. CTLs, memory T cells, and regulatory T cells, on the other hand, engage FAO and display variable mTORC1 activity (Angajala et al., 2018). Thus, the exact balance of how T cells engage key metabolic pathways such as glycolysis, OXPHOS, and FAO (and others) determines their specific function, and perturbations in these intricate balances contribute to a range of T-cell-mediated disease states, including infections, autoimmunity, and cancer (Bantug, Galluzzi, Kroemer, & Hess, 2018; Gaber, Chen, Krauss, & Buttgerit, 2019; Patel & Powell, 2017). Moreover, mitochondrial (downstream) metabolites, such as acetyl CoA, S-adenosylmethionine (SAM), NAD⁺, and ROS, control the activity of enzymes that regulate gene accessibility, such as histone acetylases/deacetylases and histone and DNA methylases/demethylases (Gill & Levine, 2013; Kaminski et al., 2010; Kamiński et al., 2012; Lozoya et al., 2019). Conversely, mitochondria-driven epigenetic mechanisms regulate also the expression of metabolic genes, thereby further impacting on the cell's net-metabolic and behavioural activity (Mohammed, Ambrosini, Luscher, Paneni, & Costantino, 2020). Finally, the release of mitochondrial DNA (mtDNA) during (hyper) T-cell activation can function as an endogenous danger signal and induce the activation of DAMP systems including the NLRP3 and absent in melanoma 2 (AIM2) inflammasomes that collectively tip the balance between Th1 induction and T-cell death (Arbore et al., 2016; Li et al., 2019).

4.3 | Indirect effects of complement activity on mitochondria

Th1 cell differentiation requires a metabolic shift from low- to high-grade metabolism to generate the energy and cellular building blocks

allowing cell proliferation and effector activity. Upon TCR stimulation, large amounts of glucose and AAs flux into cells and key signalling events are induced, including calcium flux, PI3K-AKT-mTOR signalling, ERK and MAPK activation, together with the concomitant activation of NF-κB, c-MYC, and oestrogen-related receptor-α (ERRα) transcriptional networks. Mitochondrial biogenesis is greatly enhanced at this point to support the generation and utilization of metabolites (Mishra & Chan, 2016; Schrepfer & Scorrano, 2016; Westermann, 2012). The human-specific complement receptor CD46 is a critical node for triggering these events. TCR-induced autocrine engagement of cell surface CD46 directly induces the expression of genes encoding key nutrient transporters, such as SLC2A1 (glucose transporter 1 [GLUT1]) and SLC7A5 (large neutral amino acid transporter [LAT1]), thereby mediating glucose and AA influx required for successful T-cell activation (Kolev et al., 2015) (Figure 3a). Importantly, aside from allowing glucose influx, CD46 also directly augments the activity several glycolytic enzymes including GAPDH. This CD46-mediated increase in net glycolysis then leads to the production of increased pyruvate that is shunted into the mitochondria to support elevated OXPHOS observed during Th1 induction (Kolev et al., 2015). Moreover, the increased amounts of AA in conjunction with CD46-induced assembly of mTORC1 at the lysosomes via up-regulation of the scaffolding protein late endosomal/lysosomal adaptor, MAPK, and mTOR activator 5 (LAMTOR5) elevate the cell into a high mTORC1 activity state (Figure 3a). Activated mTORC1 not only further drives high glycolysis and pyruvate flux into mitochondria but also impacts directly on these organelles (Figure 3a). For example, mTORC1 regulates the expression of mitochondrial genes encoded in the nucleus such as ATP synthase subunit delta (ATP5D) or encoding TFs including transcription factor A, mitochondrial (TFAM) that are needed for the transcription of mtDNA (Morita et al., 2015). Further, mTORC1 activity is also required for subsequent translation of these mRNAs key to mitochondrial activity as it supports the production of the RNA translation machinery component eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) (Morita et al., 2013). Finally, mTORC1 can also directly interact with mitochondria via anti-apoptotic protein B-cell lymphoma extra large (BCL-XL) and the VDAC1 complex on the outer membrane of mitochondria. This interaction fosters sustained aerobic glycolysis and mitochondrial respiration over anaerobic glycolysis and regulates cell proliferation (Ramanathan & Schreiber, 2009). Although CD46 drives lysosomal mTORC1 assembly, it remains to be explored whether CD46 activation also induces such mTORC1-mitochondria interaction in T cells. Nonetheless, aligning with a key upstream role for CD46 in the indirect regulation of mitochondrial activity during T-cell stimulation, T cells from patients with CD46 deficiency fail to increase mTORC1 activation, glycolysis, and OXPHOS upon TCR engagement and cannot produce IFN-γ (Kolev et al., 2015). CD46 may also impact on the structure of mitochondria: Th1 effector lineage induction requires mitochondrial biogenesis and fusion to increase cellular energetic output. This process is controlled by dynamin-related protein 1 or mitofusin 1 and 2 (DRP1/2) and optic atrophy 1 (OPA1) proteins. OPA1 is positively controlled in several cells by NF-κB and/or ERK1/2

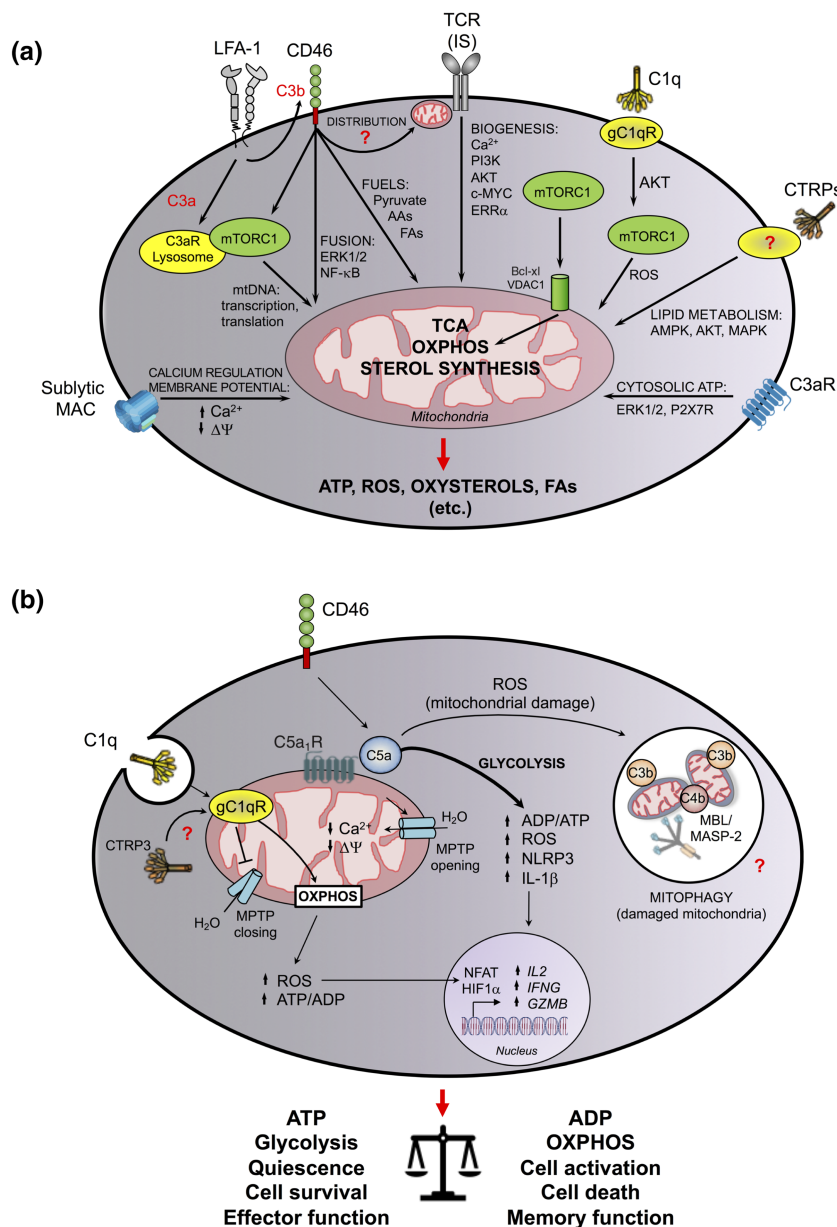


FIGURE 3 Indirect and direct effects of complement on mitochondria. Depicted are the currently known (a) indirect and (b) direct effects of cell-intrinsic complement activation (leukocyte adhesion molecule 1 [LFA-1], T-cell receptor [TCR], C3a receptor [C3aR], and CD46) events as well as complement-driven events triggered by extrinsically sourced complement summarized within one model cell. For example, under (a) are shown the main effects of surface complement receptor engagement on mitochondrial activity with the main mediator molecules or pathways depicted. These include the provision of nutrients (fuels) and the activation of signalling molecules/events (ERK1/2, AKT, Ca²⁺ flux, modulation of cytosolic ATP levels, etc.) and transcription factors (c-MYC, oestrogen-related receptor-α [ERRα], etc.) that ultimately affect mitochondrial DNA (mtDNA) transcription, general biogenesis, fusion and fission, and cellular distribution. Under (b) are depicted the currently known effects of complement receptor engagement directly on mitochondria, which include the of ROS generation via C5a1R (via a currently unknown mechanism) and the positive or negative control of oxidative phosphorylation (OXPHOS) through intracellular C1q. The net outcome of these complement-mitochondrial crosstalk(s) on ATP production and the balance of glycolysis and OXPHOS then ultimately dictate cellular behaviour and activity, including cytokine and granzyme production, survival versus death, and activation versus quiescence. Question marks denote either unknown receptors for many C1q/TNF-related proteins (CTRPs) (please note that it is unclear with what C1q-like portion CTRP3 interacts with putative receptors) or events, such as complosome-mediated mitophagy, or mitochondrial redistribution, which are likely to occur but need to be verified experimentally. 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1; AA, amino acid; AKT, PKB; AMPK, AMP-activated protein kinase; BCL-XL, B-cell lymphoma extra large; ETC, electron transport chain; FAs, fatty acids; gC1qR, globular head C1q receptor; HIF1α, hypoxia-inducible factor 1α; IS, immunological synapse; MAC, membrane attack complex; MASP-2, MBL-associated serine protease 2; MBL, mannan-binding lectin; MPTP, mitochondrial permeability transition pore; mTORC1, mammalian target of rapamycin complex 1; NFAT, nuclear factor of activated T cells; NLRP3, nucleotide-binding oligomerization domain-, leucine-rich repeat-, and pyrin domain-containing protein 3; P2X7R, P2X purinoreceptor 7; TCA, tricarboxylic acid; VDAC, voltage-dependent anion channel

activation, which together support mitochondrial fusion (Cook, Stuart, Gilley, & Sale, 2017; Albensi, 2019). Autocrine CD46 co-stimulatory signals are also required for normal NF- κ B and ERK1/2 activation during Th1 induction (Kolev et al., 2015). We further noticed that the mitochondria of CD46-deficient CD4⁺ T cells indeed displayed altered morphology when compared with healthy T cells (unpublished data); however, an unequivocal causal relationship between CD46, NF- κ B, ERK1/2 activation, and mitochondrial fission control in T cells remains to be established. CD46 does, however, clearly impact on the activity of mitochondria to produce oxysterols, such as **25-hydroxycholesterol**, which are commonly connected with a Th1/immune cell shutdown programme (Perucha et al., 2019). Mitochondria can also utilize fatty acids for ATP generation and, conversely, provide citrate needed for fatty acid synthesis (FAS) in the cytosol (Figure 2). Thus, the finding that CD46 increases specifically fatty acid synthase and fatty acid production for membrane growth and controls cholesterol flux in human T cells during TCR activation (Arbore et al., 2018) indicates that the complosome regulates metabolic nodes both upstream, within, and downstream of mitochondria.

Another complement component with strong metabolic ties and particularly with mitochondria is C1q. C1q belongs to the PRRs of the complement system and consists of six globular heads that recognize antigen-bound IgM and IgG and a collagen-like stalk region (Ghebrehiwet, Hosszu, Valentino, & Peerschke, 2012) and is the canonical inducer of the classic complement pathway. C1q came to broader fame when it was discovered as being central to the detection and removal of apoptotic cells with deficiencies in C1q noted as the major underlying reason for SLE (Botto & Walport, 2002). It has since been defined as a central modulator of general immune cell activity with the globular head C1q receptor (gC1qR) mediating many of the C1q effects, although the collagen-like stalk region can also engage certain receptors (Ghebrehiwet, Geisbrecht, Xu, Savitt, & Peerschke, 2019). C1q induces mitochondria-driven ROS production and oxidative stress in a mouse model of neonatal hypoxic brain injury (Ten et al., 2010). Furthermore, binding of C1q to gC1qR on cell surfaces or other complexes such as leukocyte-associated immunoglobulin-like receptor 1 (LAIR1) often induces AKT activation (Jingushi et al., 2019), which is central in the induction of downstream mTOR activation and cell proliferation (Figure 3a). Because of its strong impact on cell proliferation, C1q is currently intensively studied as a critical player in several cancers and in the process of aging, “conditions” that are both defined by significant cell metabolic deviations (Cho, 2019; Mangogna et al., 2019). Importantly, a highly conserved family of 15 secreted, hormone-like, molecules that are hybrids between C1q and the key growth factor tumour necrosis factor (TNF) (C1q/TNF-related proteins [CTRP]) exists. The vast majority of CTRPs affect cell activity via impact on their metabolism and particularly lipid metabolism (reviewed in Seldin, Tan, & Wong, 2014) (Figure 3a). With few exceptions (Klonisch et al., 2017), the receptors for CTRP1 to CTRP15 have not yet been identified, but a common scheme among them is that they activate specifically the classic “metabolic nodes,” 5' AMP-activated protein kinase (AMPK), AKT, and p44/42 MAPK signalling cascades. For example, CTRP1, CTRP3,

and CTRP9 activate MAPKs and AKT and trigger mTOR activity, and CTRP2, CTRP5, CTRP6, CTRP9, and CTRP13 augment FAO via activation of AMPK and/or acetyl CoA carboxylase (ACC) in several cell types (Seldin et al., 2014). The biology of CTRPs is currently a hot topic, and the existence of these C1q–TNF hybrid proteins is intriguing as TNF itself is a master regulator of a broad range of metabolic pathways in almost all cell subpopulations. We therefore expect to hear a lot more of these exciting molecules, particularly on the dissection of the combined functions of CTRPs versus those of “stand-alone” C1q and TNF.

The complement MAC, the final product of the terminal complement pathway, is usually perceived as a prime killing machine as it lyses pathogens and target cells with high efficiency (Morgan, Boyd, & Bubeck, 2017). However, it has long been acknowledged in the field that sublytic MAC insertion into host membranes can be non-lethal and triggers signalling events that most often support a pro-inflammatory cell phenotype (Fishelson & Kirschfink, 2019; Sala-Newby, Taylor, Badminton, Rembold, & Campbell, 1998). For example, sublytic MAC attack of human lung epithelial cells significantly increased cytosolic Ca²⁺ concentration via its release from the ER in an inositol 1,4,5-triphosphate receptor (IP3R)- and ryanodine receptor (RyR)-dependent fashion. The cytosolic Ca²⁺ increase is accompanied by Ca²⁺ influx and accumulation via the mitochondrial calcium uniporter (MCU) into the mitochondrial matrix, a subsequent significant drop in mitochondrial transmembrane potential, and “controlled” mitochondrial damage and cytochrome c release (Triantafilou, Hughes, Triantafilou, & Morgan, 2013) (Figure 3a). As the latter is an intrinsic DAMP for inflammasome activation, sublytic MAC insertion indeed induces NLRP3 activation and IL-1 β release and also apoptotic pathways. The amounts of sublytic MAC inserted into host membranes may hence determine if the cell survives its pro-inflammatory contribution to the host response.

Mitochondrial output of ATP into the cytosol is a key determinant of the cytosolic ATP/ADP ratio, with a balance in favour of ATP inhibiting glycolysis in resting cells (Maldonado & Lemasters, 2014). Activated immune cells display the bioenergetics of a Warburg phenotype with suppressed mitochondrial activity and hence a lower ATP/ADP ratio that supports glycolysis. Cell surface C3aR activation on human monocytes induces the rapid release of cytosolic ATP into the extracellular space via ERK1/2-mediated induction of an as of yet undefined ATP channel during stimulation with LPS (Asgari et al., 2013) (Figure 3a). Extracellular ATP triggers the **P2X7** purinergic receptor on monocytes in an autocrine fashion to activate the NLRP3 inflammasome and to support pro-inflammatory IL-1 β production by monocytes (Asgari et al., 2013). Albeit not assessed in this study, the C3aR-mediated cytosolic drop in ATP likely also augments glycolysis, which is needed for the transcription of the *IL1B* gene in these cells as a second complement-mediated mechanism to support monocyte effector activity (Moon et al., 2015).

In sum, cell-autonomous C3 and CD46 activity emerges as key orchestrator of the upstream events (nutrient influx, glycolytic enzyme activity, and signalling cascades) that dictate aerobic versus non-aerobic respiration in T cells, while extrinsic complement-driven signals

impact on mitochondrial function of immune cells by regulating lipid anabolism versus catabolism and intracellular Ca^{2+} and ATP amounts.

4.4 | Direct effects of complosome activity on mitochondria

The stimulation of cell surface-expressed C5aR1 on neutrophils induces their activation including respiratory burst and ROS production (Schreiber et al., 2009), and the C3aR can induce similar events in eosinophils (Elsner et al., 1994), suggesting that cell surface anaphylatoxin receptors are indirect regulators of mitochondrial activity. However, there is strong indication that the C5aR1 has also a direct effect on mitochondria. We recently demonstrated that intracellular C5aR1-driven ROS production is also important in endowing human Th1 cells with optimal capacity to produce IFN- γ . Importantly, resting human CD4⁺ T cells only expressed the C5aR1 intracellularly, and C5aR1-mediated ROS production required the intracellular engagement of this receptor by intrinsic C5a generation (Arbore et al., 2016). Of note, this study did not pin down the exact source of ROS and the organelle on which C5aR1 is expressed in T cells. We now, however, have gathered evidence that mitochondria in human immune cells express the C5aR1 on the outer membrane (mtC5aR1) with the C5a-binding site facing into the cytosol and that mtC5aR1 engagement induces ROS production by directly affecting the ETC activity and thus cytosolic ATP/ADP balance (manuscript under review) (Figure 3b). C5aR1-driven ROS production induces the canonical NLRP3 inflammasome and autocrine IL-1 β production by T cells, which supports sustained Th1 activity (Arbore et al., 2016). Interestingly, intrinsic IL-1 β production during normal protective Th1 induction does not induce pyroptosis (Arbore et al., 2016). In Th1 cells from patients with RA, on the other hand, mitochondria undergo bioenergetic failure and leak mtDNA, which induce NLRP3- and AIM2-generated IL-1 β and IL-18 as well as T-cell pyroptosis and general tissue inflammation (Li et al., 2019). The authors did not evaluate potential changes in intracellular C5aR1 activity and/or ROS generation in their model, and it may be interesting to test whether mtC5aR1 indeed contributes to Th1 hyperactivity in RA. ROS also modulates the expression of several key TFs including hypoxia-inducible factor 1 α (HIF1 α), and nuclear factor of activated T cells (NFAT), which are needed to induce the second Th1 signature cytokine, IL-2, as well as proliferation (Sena et al., 2013; Tatla, Woodhead, Foreman, & Chain, 1999). Excessive ROS activity, however, just like pathologically increased intrinsic IL-1 β production, can damage proteins and DNA and induce cell death (Liu et al., 2018). Therefore, intracellular C5 activation and C5aR1 engagement will likely need to be tightly regulated to sustain mitochondrial function supporting immune cell effector activity but to concurrently restrain overt cell damage and death (Figure 3b).

C1q was traditionally considered an extracellular DAMP. However, C1q is expressed by many cells and often intracellularly retained (Ghebrehiwet et al., 2012; Thielens, Tedesco, Bohls, Gaboriaud, & Tenner, 2017). Further, the gC1qR is located on/in mitochondria

(Dedio, Jannen-Dechent, Bachmann, & Muller-Esterl, 1998). Indeed, intracellular C1q can directly augment mitochondrial ROS production by cortical neurons in a model of neonatal hypoxic-ischaemic brain injury (Ten et al., 2010) (Figure 3b). Also, cell-autonomous production and intracellular activity of the C1q-TNF hybrid CTRP3 drives OXPHOS-supported protein expression and mitochondrial ROS production within smooth muscle cells (Feng et al., 2016), and gC1qR engagement on mitochondria inhibits the mitochondrial permeability transition pore and protects against oxidative stress-induced death and regulates ATP generation (McGee & Baines, 2011; McGee, Douglas, Liang, Hyder, & Baines, 2011) (Figure 3b). A recent elegant study by Marina Botto and colleagues also dissected why C1q, but not C3 deficiency, is associated with SLE. The group found that intracellular C1q restrains the adaptive response to self-antigens by modulating CTL metabolism. Specifically, CD8⁺ T cells can internalize C1q, which then engages the gC1qR on mitochondria to control glycolysis versus respiration-derived ATP balance and via this the magnitude of CTL activity as well as the tipping point between generation of short-lived effector cells and long-lived memory CTLs (Ling et al., 2018).

Overall, there is now increasing evidence that a broad range of intracellular complement components participate in the regulation of cell metabolism via direct impact on mitochondria.

4.5 | Mitochondria positioning and clearance by intracellular complement

Mitochondria developed successfully because probacteria were accepted and not killed or expelled by their evolutionary early hosts. Indeed, isolated mitochondria do not evoke significant complement activation or destruction per se by it (Dyall et al., 2004). Intuitively, this rings well with the presence of a complosome as intracellular complement attack of organelles could have detrimental effects on the cell. However, we would like to make the case that “moderate” or controlled complement opsonization of mitochondria or other cellular subcompartments may actually serve a beneficial purpose. Mannan-binding lectin (MBL), L-ficolin, and M-ficolin can recognize purified mitochondria and colocalize with mitochondria in cells (Brinkmann et al., 2013). Furthermore, MBL in complex with MBL-associated serine protease 2 (MASP-2) can then seed activation of the lectin pathway and deposit C4b onto purified mitochondria when the components are present in the reaction mixture. Interestingly, when the authors challenged mice with the injection of mitochondria into the liver, they noted that C3 was consumed, however, in the absence of any signs of inflammation. The authors suggest that the lectin pathway may direct sterile immune handling of mitochondria released during cell death. Although the study places this new role for complement in safe mitochondrial clearance outside cells, it is entirely feasible that this mechanism occurs also intracellularly: The dynamic process of fragmentation or fusion of mitochondria during cell activation as well as strong mtC5aR1-driven ROS production will likely generate damaged mitochondria. Intracellular tagging of such compromised mitochondria with complement could induce

mitophagy, thus their safe removal, and sustain continuous cellular integrity (Buck et al., 2016) (Figure 3b). Of note, other key complement-mediated events such as changes in Ca^{2+} influx/intracellular pools, a stark drop in cytosolic ATP, and membrane depolarization (see above) are also known triggers for the cell mitophagy programme (Hamacher-Brady & Brady, 2016).

Cell-autonomous complement may impact on not only the morphology and integrity of mitochondria but also their distribution within cells. LFA-1 engagement during immune cell diapedesis or the formation of the immunological synapse (IS) during the cognate APC–T-cell interaction induces cell-intrinsic C3 gene transcription and C3 protein expression (Kolev et al., 2020). This, in turn, allows for autocrine CD46 engagement and metabolic reprogramming underlying successful Th1 induction. The IS is composed of clusters of TCR surrounded by adhesion molecules. Formation of the IS requires extensive cytoskeletal rearrangements and is metabolically demanding to the cell (Quintana, Kummerow, Junker, Becherer, & Hoth, 2009). Interestingly, LFA-1 triggers the rapid recruitment of mitochondria to the IS where they benefit from the fast Ca^{2+} influx into the cell during stimulation and hence can provide the targeted heightened energy needed to build and sustain the IS (Quintana et al., 2009). CD46 indeed has been shown to induce cytoskeletal rearrangements in T cells (Ludford-Menting et al., 2011) and could hence easily contribute to such mitochondrial redistribution downstream of LFA-1 activity (Figure 3b).

Thus, the complosome likely contributes to pro-inflammatory (mitochondrial activation and distribution) and resolution/homeostasis (removal of damaged mitochondria/organelles) events surrounding mitochondria in the cell and, with this, actually mirrors the functions of extracellular complement.

5 | WHERE TO GO FROM HERE?

There is now ample evidence that intracellular complement activity is central to the regulation of normal cell metabolic activity. Although our knowledge about the new complosome-metabolism axis has been mostly carved so far in T cells, the complosome can be observed in almost all cells analysed (Liszewski et al., 2013). In fact, it is the defining feature of immune cells in tissue (Kolev et al., 2020), and it is therefore feasible to view it as a broad regulator of key metabolic nodes and with this of memory development, tissue residency, wound repair, and so forth. Moreover, intracellular complement is also operative in non-immune cells as cell-intrinsic C3 induces autophagy and protects pancreatic beta cells and lung epithelial cells from stress-induced cell death (King et al., 2019; Kulkarni et al., 2019), and intracellular C3 regulates Paneth cell turnover during repair of intestinal epithelial cells after injury (Zhang et al., 2018). Thus, we suggest that the regulation of cell metabolism by the complosome is an integral part of general normal cell activity and hence of broad significance.

However, this new field is currently defined rather by more questions than answers, and addressing even the most pressing will not be trivial. For example, we argue that the complosome is of vital significance to cell integrity and function, yet patients with C3 deficiency

exist and “only” suffer from recurrent infections during childhood. At this moment, we cannot deliver satisfactory and conclusive reasons for these observations but only provide some potential explanations. So far, all patients with serum C3 deficiency that have been assessed for intracellular C3, C3b, or C3a presence in T cells produce normal amounts of C3 and C3 activation fragments on the intracellular level (Jimenez-Reinoso et al., 2018; Liszewski et al., 2013). We recently showed that intracellular C3 is not only critical to normal Th1 induction but also required for optimal CTL activity and monocyte IL-1 β production (Arbore et al., 2018; Kolev et al., 2020); thus, it is feasible that the functional intracellular C3 activation components in these patients may be sufficient to drive (protective immune) cell activity beyond their early childhood. Another possibility is that the C4-derived activation fragment C4b, which also binds CD46 strongly, may instead foster CD46 engagement in these patients or that other proteins/pathways compensate for lack of C3 activity within cells. In fact, the ability of cells to quickly adapt to their metabolic needs by engaging compensatory pathways is notorious in the metabolism field and poses a hurdle in the interpretation of experiments that employ animals/cells with engineered alterations of metabolic genes (Ma et al., 2019). To define the exact functional consequences of the C3 gene mutations leading to selective reduction of normal C3 secretion in humans and possibly identifying patients with absent intracellular C3 production will be important in better understanding the function and significance of intracellular C3.

The usage of (full) C3-deficient mice will unfortunately not be a suitable model to address these questions as intracellular C3 in murine T cells is not processed solely by CTSL (Liszewski et al., 2013). Further, mice lack the expression of CD46 on somatic cells (Tsujimura et al., 1998) and with this the key C3b receptor driving many of the metabolic and cell activating pathways in humans. An “alternative” C3b receptor in mice that initiates “CD46-similar” events has so far not been discovered. Also puzzling to us, mice with gene deletions in C3, C5, C3ar1, or C5ar1 seem healthy if not challenged. However, none of these animals have been systematically assessed for whole-body or single-cell metabolic changes over time, and such experiments may be needed to unearth deviations in their metabolic machinery.

We currently know very little about the exact structures and localizations of intracellular complement components, their activation modes, and the pathways that regulate the complosome. How C3 and C5 and activation fragment receptors are distributed within the cell and subcellular compartments (King et al., 2019) and how they meet their ligands are also currently a “black box.” In some instances, this can be envisioned relatively easily and in some not. For example, the C3aR is expressed in T-cell lysosomes, and these organelles also contain C3a (Liszewski et al., 2013). The C3a binding site of the C3aR faces with high probability inwards into the lysosome in line with the majority of lysosomal GPCRs that are transported via controlled trafficking through the endosomal–lysosomal system into this organelle (Marchese, Paing, Temple, & Trejo, 2008). Thus, engagement of the C3aR at this location can readily occur. Subsequent productive C3aR signalling is also supported as mature lysosomes contain lipid rafts and the signalling portion of lysosomal GPCRs is exposed to cytosolic

proteins that include G proteins and all important downstream signal transduction components, such as ERK1/2 (Bagshaw, Mahuran, & Callahan, 2005). Furthermore, lysosomes are becoming now recognized as central cell signalling hubs (Ballabio & Bonifacino, 2020), and the specialized protein environment close to the lysosomal membrane (which is distinct to that of the inner leaf of the surface cell plasma membrane) could also explain as to why the C3aR evokes distinct signals when engaged at the cell surface versus an organelle (Gan et al., 2019). The situation is more complex with regard to the mitochondrial C5aR1: This receptor faces outwards, and we have not observed C5a “free” in the cytosols but only located within lysosomes and other not yet defined vesicular structures (Arbore et al., 2016). Thus, it is not clear how the mitochondrial C5aR1 is engaged by intracellular C5a—and defining the underlying mechanism is currently a research subject in our laboratory.

Probing intracellular complement receptor signalling, however, is not straightforward. As discussed, the intrinsic complosome components differ in structure and activation modes from the classic liver-derived components and may hence not be recognized by antibodies raised against the latter; indeed, the β -chain of C3 within T cells is not recognized by an antibody that readily detects the β -chain of hepatic C3 (Liszewski et al., 2013). In consequence, intracellular complement components may not respond to known activation inhibitors. Thus, our currently available research tools are probably not adequate to comprehensively study the complosome in deep detail. Moreover, the dissection of cell surface versus intracellular receptor signalling will be difficult as knock-down of respective receptors or usage of mice with receptor gene deletions will ablate both their surface and intracellular activities. So far, we have made use of cell-permeable versus cell-non-permeable inhibitors, but these reagents may have leaky or off-target activities. The signals directing the intracellular locations of GPCRs (specifically opposed to those that direct them to the cell surface) are in most cases not defined. There is accumulating evidence that suggests that the C-terminal portions of GPCRs harbour structures or domains for post-translational modifications that can be detected and interpreted by the cell sorting machinery (Marchese et al., 2008). Thus, exploring these for targeted intracellular ablation of anaphylatoxin receptors may be a way forward—but it will be a tedious journey.

Also, findings in mouse models may not always be transferable to human cells (West et al., 2020). This is the case not only for CD46, as discussed above, but also for the anaphylatoxin receptors, which are often expressed on different cells and in different subcellular locations between mouse and human immune cells (Laumonier, Karsten, & Kohl, 2017; Liszewski & Kemper, 2019). Moreover, it is now also widely accepted that complement receptors play distinct roles on different cells and that they often also function in a temporal fashion—for example, the C3aR (similar to human CD46) contributes to the initiation of immune responses and also to their contraction phase (Coulthard & Woodruff, 2015). This means that, ideally, future research should employ animals with cell-specific and inducible (e.g., *floxed* gene loci sensitive to tamoxifen exposure) ablations of complement components. In addition, data obtained using mice need to be probed for transferability to

humans—possibly through a better integration of data derived from systematic screens of large human-based data sets for disease gene discovery and sensitive whole-metabolome/proteome analyses of cells from (newly) selected patient groups.

Finally, we have not even scratched the surface of the intracellular networks that the complosome may engage in. One of such networks would be that of the communication occurring between subcellular compartments. For example, C3 is present in lysosomes and in the ER (Liszewski et al., 2013), and both of these compartments interact directly with mitochondria via mitochondria-associated membranes (MAMs) and via this heavily impact on ETC and TCA activities. Furthermore, lysosomes and mitochondria most often act in concert to direct cell metabolism, and the cytoskeleton (which is controlled by the complosome) is a key orchestrator of organelle positioning and movement within cells. The autophagosome that is also impacted by the complosome will also likely add complexity to the functional communication of this subcompartmental network. A second major network that the complosome engages with are other intracellular PRRs. CD46 induces the NLRP3 inflammasome via mitochondrial ROS (Arbore et al., 2016), and C3 activation fragments shunted into cells via C3b-opsonised viruses support mitochondrial antiviral signalling (MAVS) during viral infections (Tam, Bidgood, McEwan, & James, 2014) (Figure 3b). We speculate that direct communication with intracellular TLRs and the RIG-I system occurs also in most cells. Detangling these new functional and highly complex crosstalks will be challenging (see above) and a long walk. We suggest, however, that this will be rewarding, particularly with regard to a better understanding of human diseases: The heavy involvement of intracellular complement in cell metabolism suggests that many metabolic diseases that have traditionally not been connected to complement perturbations may indeed have an as of yet undiscovered complement dimension.

Nothing behind me, everything ahead of me, as is ever
so on the road. (Jack Kerouac, 1922–1969, *The Road*)

5.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org> and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander, Christopoulos, et al., 2019; Alexander, Fabbro, et al., 2019a, b; Alexander, Kelly, et al., 2019a, b).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

- Albensi, B. C. (2019). What is nuclear factor kappa B (NF- κ B) doing in and to the mitochondrion? *Frontiers in Cell and Development Biology*, 7, 154.
- Alexander, S. P. H., Christopoulos, A., Davenport, A. P., Kelly, E., Mathie, A., Peters, J. A., ... Collaborators, C. (2019). The Concise Guide to PHARMACOLOGY 2019/20: G protein-coupled receptors. *British Journal of Pharmacology*, 176(Suppl 1), S21–S141.
- Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., ... Collaborators, C. (2019a). The Concise Guide to PHARMACOLOGY 2019/20: Catalytic receptors. *British Journal of Pharmacology*, 176(Suppl 1), S247–S296.
- Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., ... Collaborators, C. (2019b). The Concise Guide to PHARMACOLOGY 2019/20: Enzymes. *British Journal of Pharmacology*, 176(Suppl 1), S297–S396.
- Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., ... Collaborators, C. (2019a). The Concise Guide to PHARMACOLOGY 2019/20: Introduction and other protein targets. *British Journal of Pharmacology*, 176(Suppl 1), S1–S20.
- Alexander, S. P. H., Kelly, E., Mathie, A., Peters, J. A., Veale, E. L., Armstrong, J. F., ... Collaborators, C. (2019b). The Concise Guide to PHARMACOLOGY 2019/20: Transporters. *British Journal of Pharmacology*, 176(Suppl 1), S397–S493.
- Angajala, A., Lim, S., Phillips, J. B., Kim, J. H., Yates, C., You, Z., & Tan, M. (2018). Diverse roles of mitochondria in immune responses: Novel insights into immuno-metabolism. *Frontiers in Immunology*, 9, 1605.
- Arbore, G., Kemper, C., & Kolev, M. (2017). Intracellular complement—the complosome—in immune cell regulation. *Molecular Immunology*, 89, 2–9.
- Arbore, G., Ong, V. H., Costantini, B., Denton, C. P., Abraham, D., Placais, L., ... Hourcade, D. E. (2020). *Deep phenotyping detects a pathological CD4⁺ T-cell complosome signature in systemic sclerosis*. *Cell Mol Immunol*.
- Arbore, G., West, E. E., Rahman, J., Le Fricc, G., Niyonzima, N., Pirooznia, M., ... Kemper, C. (2018). Complement receptor CD46 co-stimulates optimal human CD8⁺ T cell effector function via fatty acid metabolism. *Nature Communications*, 9(1), 4186. <https://doi.org/10.1038/s41467-018-06706-z>
- Arbore, G., West, E. E., Spolski, R., Robertson, A. A., Klos, A., Rheinheimer, C., ... Kemper, C. (2016). T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4⁺ T cells. *Science*, 352(6292), aad1210.
- Asgari, E., Le Fricc, G., Yamamoto, H., Perucha, E., Sacks, S. S., Kohl, J., ... Kemper, C. (2013). C3a modulates IL-1 β secretion in human monocytes by regulating ATP efflux and subsequent NLRP3 inflammasome activation. *Blood*, 122(20), 3473–3481. <https://doi.org/10.1182/blood-2013-05-502229>
- Astier, A. L., Meiffren, G., Freeman, S., & Hafler, D. A. (2006). Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis. *The Journal of Clinical Investigation*, 116(12), 3252–3257. <https://doi.org/10.1172/JCI29251>
- Bagshaw, R. D., Mahuran, D. J., & Callahan, J. W. (2005). A proteomic analysis of lysosomal integral membrane proteins reveals the diverse composition of the organelle. *Molecular & Cellular Proteomics*, 4(2), 133–143. <https://doi.org/10.1074/mcp.M400128-MCP200>
- Ballabio, A., & Bonifacino, J. S. (2020). Lysosomes as dynamic regulators of cell and organismal homeostasis. *Nature Reviews. Molecular Cell Biology*, 21(2), 101–118. <https://doi.org/10.1038/s41580-019-0185-4>
- Bantug, G. R., Galluzzi, L., Kroemer, G., & Hess, C. (2018). The spectrum of T cell metabolism in health and disease. *Nature Reviews. Immunology*, 18(1), 19–34. <https://doi.org/10.1038/nri.2017.99>
- Botto, M., & Walport, M. J. (2002). C1q, autoimmunity and apoptosis. *Immunobiology*, 205(4–5), 395–406. <https://doi.org/10.1078/0171-2985-00141>
- Brinkmann, C. R., Jensen, L., Dagnaes-Hansen, F., Holm, I. E., Endo, Y., Fujita, T., ... Degn, S. E. (2013). Mitochondria and the lectin pathway of complement. *The Journal of Biological Chemistry*, 288(12), 8016–8027. <https://doi.org/10.1074/jbc.M112.430249>
- Bruchard, M., Rebe, C., Derangere, V., Togbe, D., Ryffel, B., Boidot, R., ... Ghiringhelli, F. (2015). The receptor NLRP3 is a transcriptional regulator of T_H2 differentiation. *Nature Immunology*, 16(8), 859–870. <https://doi.org/10.1038/ni.3202>
- Buck, M. D., O'Sullivan, D., Klein Geltink, R. I., Curtis, J. D., Chang, C. H., Sanin, D. E., ... Pearce, E. L. (2016). Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell*, 166(1), 63–76. <https://doi.org/10.1016/j.cell.2016.05.035>
- Cardone, J., Le Fricc, G., Vantourout, P., Roberts, A., Fuchs, A., Jackson, I., ... Kemper, C. (2010). Complement regulator CD46 temporally regulates cytokine production by conventional and unconventional T cells. *Nature Immunology*, 11(9), 862–871. <https://doi.org/10.1038/ni.1917>
- Cho, K. (2019). Emerging roles of complement protein C1q in neurodegeneration. *Aging and Disease*, 10(3), 652–663. <https://doi.org/10.14336/AD.2019.0118>
- Coll, R. C., O'Neill, L., & Schroder, K. (2016). Questions and controversies in innate immune research: What is the physiological role of NLRP3? *Cell Death Discov*, 2, 16019.
- Cook, S. J., Stuart, K., Gilley, R., & Sale, M. J. (2017). Control of cell death and mitochondrial fission by ERK1/2 MAP kinase signalling. *The FEBS Journal*, 284(24), 4177–4195. <https://doi.org/10.1111/febs.14122>
- Coulthard, L. G., & Woodruff, T. M. (2015). Is the complement activation product C3a a proinflammatory molecule? Re-evaluating the evidence and the myth. *Journal of Immunology (Baltimore, Md. : 1950)*, 194(8), 3542–3548.
- De la Fuente, M., MacDonald, T. T., & Hermoso, M. A. (2015). The IL-33/ST2 axis: Role in health and disease. *Cytokine & Growth Factor Reviews*, 26(6), 615–623. <https://doi.org/10.1016/j.cytogfr.2015.07.017>
- Dedio, J., Jahnhen-Dechent, W., Bachmann, M., & Muller-Esterl, W. (1998). The multiligand-binding protein gC1qR, putative C1q receptor, is a mitochondrial protein. *Journal of Immunology*, 160(7), 3534–3542.
- Dinarello, C. A. (2007). Historical insights into cytokines. *European Journal of Immunology*, 37(Suppl 1), S34–S45.
- Dyall, S. D., Brown, M. T., & Johnson, P. J. (2004). Ancient invasions: From endosymbionts to organelles. *Science*, 304(5668), 253–257.
- Ellinghaus, U., Cortini, A., Pinder, C. L., Le Fricc, G., Kemper, C., & Vyse, T. J. (2017). Dysregulated CD46 shedding interferes with Th1-contraction in systemic lupus erythematosus. *European Journal of Immunology*, 47(7), 1200–1210. <https://doi.org/10.1002/eji.201646822>
- Elsner, J., Oppermann, M., Czech, W., Dobos, G., Schopf, E., Norgauer, J., & Kapp, A. (1994). C3a activates reactive oxygen radical species production and intracellular calcium transients in human eosinophils. *European Journal of Immunology*, 24(3), 518–522. <https://doi.org/10.1002/eji.1830240304>
- Feng, H., Wang, J. Y., Zheng, M., Zhang, C. L., An, Y. M., Li, L., & Wu, L. L. (2016). CTRP3 promotes energy production by inducing mitochondrial ROS and up-expression of PGC-1 α in vascular smooth muscle cells. *Experimental Cell Research*, 341(2), 177–186. <https://doi.org/10.1016/j.yexcr.2016.02.001>

- Fishelson, Z., & Kirschfink, M. (2019). Complement C5b-9 and cancer: Mechanisms of cell damage, cancer counteractions, and approaches for intervention. *Frontiers in Immunology*, 10, 752.
- Gaber, T., Chen, Y., Krauss, P. L., & Buttgerit, F. (2019). Metabolism of T lymphocytes in health and disease. *International Review of Cell and Molecular Biology*, 342, 95–148. <https://doi.org/10.1016/bs.ircmb.2018.06.002>
- Gan, L., Seki, A., Shen, K., Iyer, H., Han, K., Hayer, A., ... Meyer, T. (2019). The lysosomal GPCR-like protein GPR137B regulates Rag and mTORC1 localization and activity. *Nature Cell Biology*, 21(5), 614–626. <https://doi.org/10.1038/s41556-019-0321-6>
- Gellerich, F. N., Trumbeckaite, S., Opalka, J. R., Seppet, E., Rasmussen, H. N., Neuhoﬀ, C., & Zier, S. (2000). Function of the mitochondrial outer membrane as a diffusion barrier in health and diseases. *Biochemical Society Transactions*, 28(2), 164–169. <https://doi.org/10.1042/bst0280164>
- Ghebrehiwet, B., Geisbrecht, B. V., Xu, X., Savitt, A. G., & Peerschke, E. I. B. (2019). The C1q receptors: Focus on gC1qR/p33 (C1qBP, p32, HABP-1)¹. *Seminars in Immunology*, 45, 101338.
- Ghebrehiwet, B., Hosszu, K. K., Valentino, A., & Peerschke, E. I. (2012). The C1q family of proteins: Insights into the emerging non-traditional functions. *Frontiers in Immunology*, 3.
- Gill, T., & Levine, A. D. (2013). Mitochondria-derived hydrogen peroxide selectively enhances T cell receptor-initiated signal transduction. *The Journal of Biological Chemistry*, 288(36), 26246–26255. <https://doi.org/10.1074/jbc.M113.476895>
- Guo, H., Callaway, J. B., & Ting, J. P. (2015). Inflammasomes: Mechanism of action, role in disease, and therapeutics. *Nature Medicine*, 21(7), 677–687. <https://doi.org/10.1038/nm.3893>
- Hajishengallis, G., Reis, E. S., Mastellos, D. C., Ricklin, D., & Lambris, J. D. (2017). Novel mechanisms and functions of complement. *Nature Immunology*, 18(12), 1288–1298. <https://doi.org/10.1038/ni.3858>
- Hamacher-Brady, A., & Brady, N. R. (2016). Mitophagy programs: Mechanisms and physiological implications of mitochondrial targeting by autophagy. *Cellular and Molecular Life Sciences*, 73(4), 775–795. <https://doi.org/10.1007/s00018-015-2087-8>
- Haneklaus, M., & O'Neill, L. A. (2015). NLRP3 at the interface of metabolism and inflammation. *Immunological Reviews*, 265(1), 53–62. <https://doi.org/10.1111/imr.12285>
- Heesterbeek, D. A. C., Angelier, M. L., Harrison, R. A., & Rooijackers, S. H. M. (2018). Complement and bacterial infections: From molecular mechanisms to therapeutic applications. *Journal of Innate Immunity*, 10(5–6), 455–464. <https://doi.org/10.1159/000491439>
- Hess, C., & Kemper, C. (2016). Complement-mediated regulation of metabolism and basic cellular processes. *Immunity*, 45(2), 240–254. <https://doi.org/10.1016/j.immuni.2016.08.003>
- Holers, V. M., & Banda, N. K. (2018). Complement in the initiation and evolution of rheumatoid arthritis. *Frontiers in Immunology*, 9, 1057.
- Jimenez-Reinoso, A., Marin, A. V., Subias, M., Lopez-Lera, A., Roman-Ortiz, E., Payne, K., ... Regueiro, J. R. (2018). Human plasma C3 is essential for the development of memory B, but not T, lymphocytes. *The Journal of Allergy and Clinical Immunology*, 141(3), 1151–1154e1114. <https://doi.org/10.1016/j.jaci.2017.09.037>
- Jingushi, K., Uemura, M., Nakano, K., Hayashi, Y., Wang, C., Ishizuya, Y., ... Nonomura, N. (2019). Leukocyte-associated immunoglobulin-like receptor 1 promotes tumorigenesis in RCC. *Oncology Reports*, 41(2), 1293–1303. <https://doi.org/10.3892/or.2018.6875>
- Kamiński, M. M., Sauer, S. W., Kamiński, M., Opp, S., Ruppert, T., Grigaravičius, P., ... Gülöw, K. (2012). T cell activation is driven by an ADP-dependent glucokinase linking enhanced glycolysis with mitochondrial reactive oxygen species generation. *Cell Reports*, 2(5), 1300–1315. <https://doi.org/10.1016/j.celrep.2012.10.009>
- Kaminski, M. M., Sauer, S. W., Klemke, C. D., Süß, D., Okun, J. G., Krammer, P. H., & Gülöw, K. (2010). Mitochondrial reactive oxygen species control T cell activation by regulating IL-2 and IL-4 expression: Mechanism of ciprofloxacin-mediated immunosuppression. *Journal of Immunology*, 184(9), 4827–4841.
- King, B. C., Kulak, K., Krus, U., Rosberg, R., Golec, E., Wozniak, K., ... Blom, A. M. (2019). Complement component C3 is highly expressed in human pancreatic islets and prevents β cell death via ATG16L1 interaction and autophagy regulation. *Cell Metabolism*, 29(1), 202–210e206. <https://doi.org/10.1016/j.cmet.2018.09.009>
- Klonisch, T., Glogowska, A., Thanasupawat, T., Burg, M., Krcek, J., Pitz, M., ... Hombach-Klonisch, S. (2017). Structural commonality of C1q TNF-related proteins and their potential to activate relaxin/insulin-like family peptide receptor 1 signalling pathways in cancer cells. *British Journal of Pharmacology*, 174(10), 1025–1033. <https://doi.org/10.1111/bph.13559>
- Kolev, M., Dimeloe, S., Le Fric, G., Navarini, A., Arbore, G., Povoleri, G. A., ... Kemper, C. (2015). Complement regulates nutrient influx and metabolic reprogramming during Th1 cell responses. *Immunity*, 42(6), 1033–1047. <https://doi.org/10.1016/j.immuni.2015.05.024>
- Kolev, M., & Kemper, C. (2017). Keeping it all going—Complement meets metabolism. *Frontiers in Immunology*, 8, 1.
- Kolev, M., West, E. E., Kunz, N., Chauss, D., Moseman, E. A., Rahman, J., ... Kemper, C. (2020). Diapedesis-induced integrin signaling via LFA-1 facilitates tissue immunity by inducing intrinsic complement C3 expression in immune cells. *Immunity*, 52(3), 513–527e518. <https://doi.org/10.1016/j.immuni.2020.02.006>
- Konner, A. C., & Bruning, J. C. (2011). Toll-like receptors: Linking inflammation to metabolism. *Trends in Endocrinology and Metabolism*, 22(1), 16–23. <https://doi.org/10.1016/j.tem.2010.08.007>
- Kremlitzka, M., Nowacka, A. A., Mohlin, F. C., Bompada, P., De Marinis, Y., & Blom, A. M. (2019). Interaction of serum-derived and internalized C3 with DNA in human B cells—A potential involvement in regulation of gene transcription. *Frontiers in Immunology*, 10, 493.
- Kuhlbrandt, W. (2015). Structure and function of mitochondrial membrane protein complexes. *BMC Biology*, 13, 89.
- Kulkarni, H. S., Elvington, M. L., Perng, Y. C., Liszewski, M. K., Byers, D. E., Farkouh, C., ... Atkinson, J. P. (2019). Intracellular C3 protects human airway epithelial cells from stress-associated cell death. *American Journal of Respiratory Cell and Molecular Biology*, 60(2), 144–157. <https://doi.org/10.1165/rcmb.2017-0405OC>
- Lalli, P. N., Stranic, M. G., Yang, M., Lin, F., Medof, M. E., & Heeger, P. S. (2008). Locally produced C5a binds to T cell-expressed C5aR to enhance effector T-cell expansion by limiting antigen-induced apoptosis. *Blood*, 112(5), 1759–1766. <https://doi.org/10.1182/blood-2008-04-151068>
- Laumonnier, Y., Karsten, C. M., & Kohl, J. (2017). Novel insights into the expression pattern of anaphylatoxin receptors in mice and men. *Molecular Immunology*, 89, 44–58. <https://doi.org/10.1016/j.molimm.2017.05.019>
- Lawrence, R. E., & Zoncu, R. (2019). The lysosome as a cellular centre for signalling, metabolism and quality control. *Nature Cell Biology*, 21(2), 133–142. <https://doi.org/10.1038/s41556-018-0244-7>
- Li, Y., Shen, Y., Jin, K., Wen, Z., Cao, W., Wu, B., ... Weyand, C. M. (2019). The DNA repair nuclease MRE11A functions as a mitochondrial protector and prevents T cell pyroptosis and tissue inflammation. *Cell Metabolism*, 30(3), 477–492e476. <https://doi.org/10.1016/j.cmet.2019.06.016>
- Liao, W., Lin, J. X., & Leonard, W. J. (2011). IL-2 family cytokines: New insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. *Current Opinion in Immunology*, 23(5), 598–604. <https://doi.org/10.1016/j.coi.2011.08.003>
- Liao, W., Lin, J. X., & Leonard, W. J. (2013). Interleukin-2 at the crossroads of effector responses, tolerance, and immunotherapy. *Immunity*, 38(1), 13–25. <https://doi.org/10.1016/j.immuni.2013.01.004>
- Ling, G. S., Crawford, G., Buang, N., Bartok, I., Tian, K., Thielens, N. M., ... Botto, M. (2018). C1q restrains autoimmunity and viral infection by

- regulating CD8⁺ T cell metabolism. *Science*, 360(6388), 558–563. <https://doi.org/10.1126/science.aao4555>
- Liszewski, M. K., & Kemper, C. (2019). Complement in motion: The evolution of CD46 from a complement regulator to an orchestrator of normal cell physiology. *Journal of Immunology*, 203(1), 3–5.
- Liszewski, M. K., Kolev, M., Le Friec, G., Leung, M., Bertram, P. G., Fara, A. F., ... Kemper, C. (2013). Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. *Immunity*, 39(6), 1143–1157.
- Liu, J., Miwa, T., Hilliard, B., Chen, Y., Lambris, J. D., Wells, A. D., & Song, W. C. (2005). The complement inhibitory protein DAF (CD55) suppresses T cell immunity in vivo. *The Journal of Experimental Medicine*, 201(4), 567–577. <https://doi.org/10.1084/jem.20040863>
- Liu, Y., Olganier, D., & Lin, R. (2016). Host and viral modulation of RIG-I-mediated antiviral immunity. *Frontiers in Immunology*, 7, 662.
- Liu, Z., Ren, Z., Zhang, J., Chuang, C. C., Kandaswamy, E., Zhou, T., & Zuo, L. (2018). Role of ROS and nutritional antioxidants in human diseases. *Frontiers in Physiology*, 9, 477.
- Loftus, R. M., & Finlay, D. K. (2016). Immunometabolism: Cellular metabolism turns immune regulator. *The Journal of Biological Chemistry*, 291(1), 1–10. <https://doi.org/10.1074/jbc.R115.693903>
- Lozoya, O. A., Wang, T., Grenet, D., Wolfgang, T. C., Sobhany, M., Ganini da Silva, D., ... Santos, J. H. (2019). Mitochondrial acetyl-CoA reversibly regulates locus-specific histone acetylation and gene expression. *Life Sci Alliance*, 2(1).
- Ludford-Menting, M. J., Crimeen-Irwin, B., Oliaro, J., Pasam, A., Williamson, D., Pedersen, N., ... Russell, S. M. (2011). The reorientation of T-cell polarity and inhibition of immunological synapse formation by CD46 involves its recruitment to lipid rafts. *Journal of Lipids*, 2011, 521863.
- Ma, E. H., Verway, M. J., Johnson, R. M., Roy, D. G., Steadman, M., Hayes, S., ... Jones, R. G. (2019). Metabolic profiling using stable isotope tracing reveals distinct patterns of glucose utilization by physiologically activated CD8⁺ T cells. *Immunity*, 51(5), 856–870. <https://doi.org/10.1016/j.immuni.2019.09.003>
- Maldonado, E. N., & Lemasters, J. J. (2014). ATP/ADP ratio, the missed connection between mitochondria and the Warburg effect. *Mitochondrion*, 19(Pt A), 78–84.
- Mangogna, A., Agostinis, C., Bonazza, D., Belmonte, B., Zacchi, P., Zito, G., ... Bulla, R. (2019). Is the complement protein C1q a pro- or anti-tumorigenic factor? Bioinformatics analysis involving human carcinomas. *Frontiers in Immunology*, 10, 865.
- Marchese, A., Paing, M. M., Temple, B. R., & Trejo, J. (2008). G protein-coupled receptor sorting to endosomes and lysosomes. *Annual Review of Pharmacology and Toxicology*, 48, 601–629. <https://doi.org/10.1146/annurev.pharmtox.48.113006.094646>
- McGee, A. M., & Baines, C. P. (2011). Complement 1q-binding protein inhibits the mitochondrial permeability transition pore and protects against oxidative stress-induced death. *The Biochemical Journal*, 433(1), 119–125. <https://doi.org/10.1042/BJ20101431>
- McGee, A. M., Douglas, D. L., Liang, Y., Hyder, S. M., & Baines, C. P. (2011). The mitochondrial protein C1qbp promotes cell proliferation, migration and resistance to cell death. *Cell Cycle*, 10(23), 4119–4127. <https://doi.org/10.4161/cc.10.23.18287>
- Merle, N. S., Church, S. E., Fremaux-Bacchi, V., & Roumenina, L. T. (2015). Complement system part I—Molecular mechanisms of activation and regulation. *Frontiers in Immunology*, 6.
- Mishra, P., & Chan, D. C. (2016). Metabolic regulation of mitochondrial dynamics. *The Journal of Cell Biology*, 212(4), 379–387. <https://doi.org/10.1083/jcb.201511036>
- Mohammed, S. A., Ambrosini, S., Luscher, T., Paneni, F., & Costantino, S. (2020). Epigenetic control of mitochondrial function in the vasculature. *Front Cardiovasc Med*, 7, 28.
- Moon, J. S., Hisata, S., Park, M. A., DeNicola, G. M., Ryter, S. W., Nakahira, K., & Choi, A. M. K. (2015). mTORC1-induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. *Cell Reports*, 12(1), 102–115. <https://doi.org/10.1016/j.celrep.2015.05.046>
- Morgan, B. P., Boyd, C., & Bubeck, D. (2017). Molecular cell biology of complement membrane attack. *Seminars in Cell & Developmental Biology*, 72, 124–132. <https://doi.org/10.1016/j.semcd.2017.06.009>
- Morgan, B. P., & Kavanagh, D. (2018). Introduction to complement in health and disease: Novel aspects and insights. *Seminars in Immunopathology*, 40(1), 1–2. <https://doi.org/10.1007/s00281-017-0664-7>
- Morita, M., Gravel, S. P., Chénard, V., Sikström, K., Zheng, L., Alain, T., ... Sonenberg, N. (2013). mTORC1 controls mitochondrial activity and biogenesis through 4E-BP-dependent translational regulation. *Cell Metabolism*, 18(5), 698–711. <https://doi.org/10.1016/j.cmet.2013.10.001>
- Morita, M., Gravel, S. P., Hulea, L., Larsson, O., Pollak, M., St-Pierre, J., & Topisirovic, I. (2015). mTOR coordinates protein synthesis, mitochondrial activity and proliferation. *Cell Cycle*, 14(4), 473–480. <https://doi.org/10.4161/15384101.2014.991572>
- Odendall, C., & Kagan, J. C. (2017). Activation and pathogenic manipulation of the sensors of the innate immune system. *Microbes and Infection*, 19(4–5), 229–237. <https://doi.org/10.1016/j.micinf.2017.01.003>
- Patel, C. H., & Powell, J. D. (2017). Targeting T cell metabolism to regulate T cell activation, differentiation and function in disease. *Current Opinion in Immunology*, 46, 82–88. <https://doi.org/10.1016/j.coi.2017.04.006>
- Perucha, E., Melchiodi, R., Bibby, J. A., Wu, W., Frederiksen, K. S., Roberts, C. A., ... Cope, A. P. (2019). The cholesterol biosynthesis pathway regulates IL-10 expression in human Th1 cells. *Nature Communications*, 10(1), 498. <https://doi.org/10.1038/s41467-019-08332-9>
- Pfanner, N., Warscheid, B., & Wiedemann, N. (2019). Mitochondrial proteins: From biogenesis to functional networks. *Nature Reviews. Molecular Cell Biology*, 20(5), 267–284. <https://doi.org/10.1038/s41580-018-0092-0>
- Prochnicki, T., & Latz, E. (2017). Inflammasomes on the crossroads of innate immune recognition and metabolic control. *Cell Metabolism*, 26(1), 71–93. <https://doi.org/10.1016/j.cmet.2017.06.018>
- Quintana, A., Kummerow, C., Junker, C., Becherer, U., & Hoth, M. (2009). Morphological changes of T cells following formation of the immunological synapse modulate intracellular calcium signals. *Cell Calcium*, 45(2), 109–122. <https://doi.org/10.1016/j.ceca.2008.07.003>
- Ramanathan, A., & Schreiber, S. L. (2009). Direct control of mitochondrial function by mTOR. *Proceedings of the National Academy of Sciences of the United States of America*, 106(52), 22229–22232. <https://doi.org/10.1073/pnas.0912074106>
- Ricklin, D., Hajishengallis, G., Yang, K., & Lambris, J. D. (2010). Complement: A key system for immune surveillance and homeostasis. *Nature Immunology*, 11(9), 785–797. <https://doi.org/10.1038/ni.1923>
- Sala-Newby, G. B., Taylor, K. M., Badminton, M. N., Rembold, C. M., & Campbell, A. K. (1998). Imaging bioluminescent indicators shows Ca²⁺ and ATP permeability thresholds in live cells attacked by complement. *Immunity*, 9(3), 601–609. <https://doi.org/10.1046/j.1365-2567.1998.00004.x>
- Schell, J. C., & Rutter, J. (2013). The long and winding road to the mitochondrial pyruvate carrier. *Cancer & Metabolism*, 1(1), 6. <https://doi.org/10.1186/2049-3002-1-6>
- Schreiber, A., Xiao, H., Jennette, J. C., Schneider, W., Luft, F. C., & Kettritz, R. (2009). C5a receptor mediates neutrophil activation and ANCA-induced glomerulonephritis. *Journal of the American Society of Nephrology*, 20(2), 289–298. <https://doi.org/10.1681/ASN.2008050497>
- Schrepfer, E., & Scorrano, L. (2016). Mitofusins, from mitochondria to metabolism. *Molecular Cell*, 61(5), 683–694. <https://doi.org/10.1016/j.molcel.2016.02.022>

- Seldin, M. M., Tan, S. Y., & Wong, G. W. (2014). Metabolic function of the CTRP family of hormones. *Reviews in Endocrine & Metabolic Disorders*, 15(2), 111–123. <https://doi.org/10.1007/s11154-013-9255-7>
- Sena, L. A., Li, S., Jairaman, A., Prakriya, M., Ezponda, T., Hildeman, D. A., ... Chandel, N. S. (2013). Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity*, 38(2), 225–236.
- Siekevitz, P. (1957). Powerhouse of the cell. *Scientific American*, 197, 131–144.
- Strainic, M. G., Liu, J., Huang, D., An, F., Lalli, P. N., Muqim, N., ... Medof, M. E. (2008). Locally produced complement fragments C5a and C3a provide both costimulatory and survival signals to naive CD4⁺ T cells. *Immunity*, 28(3), 425–435. <https://doi.org/10.1016/j.immuni.2008.02.001>
- Tam, J. C., Bidgood, S. R., McEwan, W. A., & James, L. C. (2014). Intracellular sensing of complement C3 activates cell autonomous immunity. *Science*, 345(6201), 1256070. <https://doi.org/10.1126/science.1256070>
- Tatla, S., Woodhead, V., Foreman, J. C., & Chain, B. M. (1999). The role of reactive oxygen species in triggering proliferation and IL-2 secretion in T cells. *Free Radical Biology & Medicine*, 26(1–2), 14–24.
- Ten, V. S., Yao, J., Ratner, V., Sosunov, S., Fraser, D. A., Botto, M., ... Starkov, A. A. (2010). Complement component C1q mediates mitochondria-driven oxidative stress in neonatal hypoxic–ischemic brain injury. *The Journal of Neuroscience*, 30(6), 2077–2087. <https://doi.org/10.1523/JNEUROSCI.5249-09.2010>
- Thielens, N. M., Tedesco, F., Bohlsion, S. S., Gaboriaud, C., & Tenner, A. J. (2017). C1q: A fresh look upon an old molecule. *Molecular Immunology*, 89, 73–83. <https://doi.org/10.1016/j.molimm.2017.05.025>
- Tiku, V., Tan, M. W., & Dikic, I. (2020). Mitochondrial functions in infection and immunity. *Trends in Cell Biology*, 30(4), 263–275. <https://doi.org/10.1016/j.tcb.2020.01.006>
- Toubi, E., & Vadasz, Z. (2019). Innate immune-responses and their role in driving autoimmunity. *Autoimmunity Reviews*, 18(3), 306–311. <https://doi.org/10.1016/j.autrev.2018.10.005>
- Triantafilou, K., Hughes, T. R., Triantafilou, M., & Morgan, B. P. (2013). The complement membrane attack complex triggers intracellular Ca²⁺ fluxes leading to NLRP3 inflammasome activation. *Journal of Cell Science*, 126(Pt 13), 2903–2913.
- Tsujimura, A., Shida, K., Kitamura, M., Nomura, M., Takeda, J., Tanaka, H., ... Seya, T. (1998). Molecular cloning of a murine homologue of membrane cofactor protein (CD46): Preferential expression in testicular germ cells. *The Biochemical Journal*, 330(Pt 1), 163–168.
- Van den Bossche, J., Baardman, J., Otto, N. A., van der Velden, S., Neele, A. E., van den Berg, S. M., ... de Winther, M. P. (2016). Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Reports*, 17(3), 684–696. <https://doi.org/10.1016/j.celrep.2016.09.008>
- Vats, D., Mukundan, L., Odegaard, J. I., Zhang, L., Smith, K. L., Morel, C. R., ... Chawla, A. (2006). Oxidative metabolism and PGC-1 β attenuate macrophage-mediated inflammation. *Cell Metabolism*, 4(1), 13–24.
- Vijay, K. (2018). Toll-like receptors in immunity and inflammatory diseases: Past, present, and future. *International Immunopharmacology*, 59, 391–412. <https://doi.org/10.1016/j.intimp.2018.03.002>
- West, E. E., & Kemper, C. (2019). Complement and T cell metabolism: Food for thought. *Immunometabolism, 1T Cell Metabolic Reprogramming*, e190006.
- West, E. E., Kolev, M., & Kemper, C. (2018). Complement and the regulation of T cell responses. *Annual Review of Immunology*, 36, 309–338. <https://doi.org/10.1146/annurev-immunol-042617-053245>
- West, E. E., Kunz, N., & Kemper, C. (2020). Complement and human T cell metabolism: Location, location, location. *Immunological Reviews*, 295, 68–81. <https://doi.org/10.1111/imr.12852>
- Westermann, B. (2012). Bioenergetic role of mitochondrial fusion and fission. *Biochimica et Biophysica Acta*, 1817(10), 1833–1838. <https://doi.org/10.1016/j.bbabi.2012.02.033>
- Zhang, J., Ye, J., Ren, Y., Zuo, J., Dai, W., He, Y., ... Yuan, Y. (2018). Intracellular activation of complement C3 in Paneth cells improves repair of intestinal epithelia during acute injury. *Immunotherapy*, 10(15), 1325–1336. <https://doi.org/10.2217/imt-2018-0122>

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