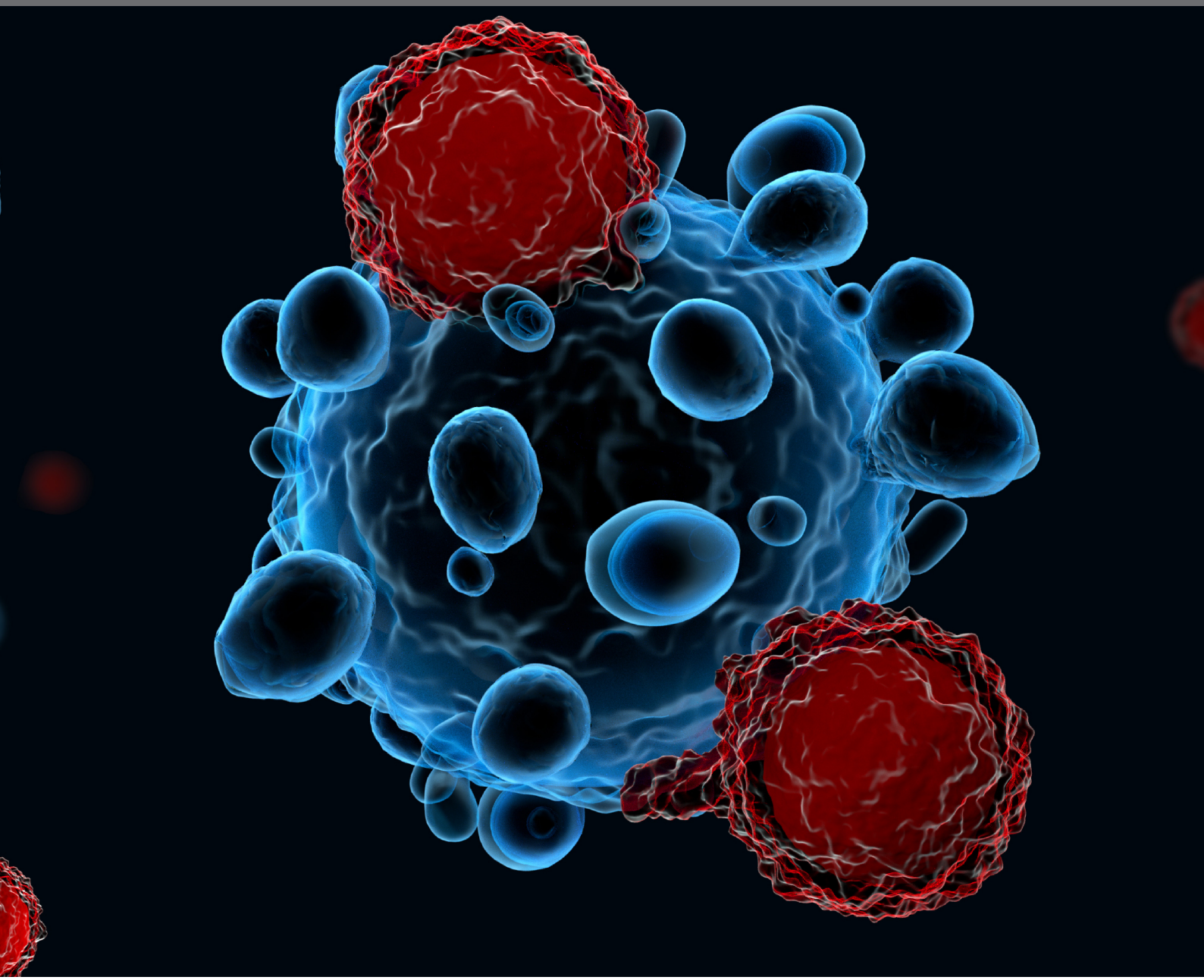
METABOLISM AND IMMUNE TOLERANCE

EDITED BY: Duncan Howie and Claudio Mauro
PUBLISHED IN: Frontiers in Immunology



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ISSN 1664-8714
ISBN 978-2-88945-725-0
DOI 10.3389/978-2-88945-725-0

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METABOLISM AND IMMUNE TOLERANCE

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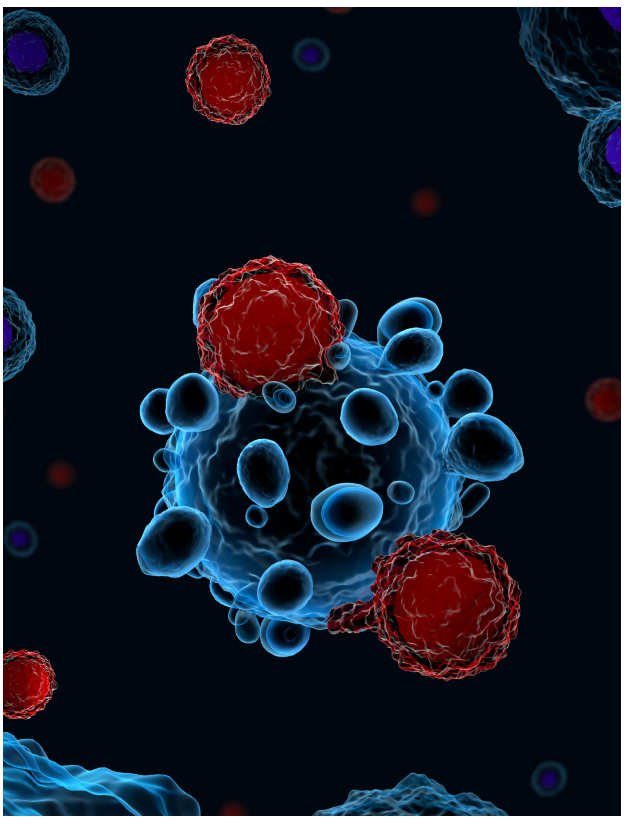

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Historically the study of the immune system and metabolism have been two very separate fields. In recent years, a growing literature has emerged illustrating how the multiple processes of cellular metabolism are intricately linked to several aspects of immune function and development. This Research Topic covers recent progress in the field now known as "Immunometabolism" and the role of metabolism in immune tolerance. Immune tolerance is operationally defined as a state where a host's immune system is balanced such that although self-reactive lymphocytes are present, they are kept in check by immune regulation. Perturbations to this homeostasis may result in self-reactive lymphocytes gaining the upper hand and mediating auto-immune disease. Maintenance of immune tolerance involves a large cast of different cell types including effector T cells, regulatory T cells, B cells, stromal cells, dendritic cells and macrophages.

Intracellular pathways and individual enzymes of metabolism have been shown to be harnessed by cells of both the adaptive and innate immune system to allow particular immune functions to be achieved. Examples include metabolic enzymes serving 'moonlighting' functions in mRNA translation, gene splicing, and kinase activation. Other examples include the requirement for de novo fatty acid synthesis for differentiation into Th17 effectors and CD8 memory T cells or products of the TCA cycle promoting pro-inflammatory cytokine production. Likewise, the availability of extracellular metabolic substrates has a large impact on the maintenance of local immune tolerance. For example, there are different requirements for glucose, glutamine and fatty acids for effector versus regulatory T cell development. Also tolerogenic dendritic cells mediate lowering of extracellular essential amino acids by their enhanced catabolism, promoting the induction of regulatory T cells. The purpose of this Research Topic is to provide an update on the current understanding of the multiple roles for metabolism in regulating the immune system.

Citation: Howie, D., Mauro, C., eds. (2019). Metabolism and Immune Tolerance. Lausanne: Frontiers Media. doi: 10.3389/978-2-88945-725-0

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frontiers in Immunology

EDITORIAL
published: 15 November 2018
doi: 10.3389/fimmu.2018.02678

Editorial: Metabolism and Immune Tolerance

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Keywords: immune tolerance, metabolism, regulatory T cell, transplantation, macrophage

Editorial on the Research Topic

Metabolism and Immune Tolerance

During the past decade amongst the immunology community there has been a renaissance of interest in cellular metabolism as it relates to immune functions. Much of the work has focused on the role of metabolism in facilitating or controlling immune cell differentiation and determination of effector mechanisms. Certain fundamental relationships between metabolic state and the differentiation status of innate and lymphoid cells have been worked out, using for the most part, reductionist mouse models. One major challenge ahead will be to understand to what extent the coordination of metabolism between the multiple cell types of an immune response; lymphoid, stromal, endothelial, epithelial impacts on immunity, and immune tolerance. Our aim in assembling this Research Topic is to highlight the current understanding of cellular metabolism as it relates to immune tolerance in a variety of settings.

The collection starts with a review from Degauque et al. on the role of the inflammatory metabolic microenvironment in allogeneic transplantation. This review describes the functions of metabolites such as lactate, acetate, adenosine, and extracellular ATP on cellular and humoral immunity, and the interplay of immunosuppression and metabolism in allogeneic tolerance. Alwarawrah et al. then give an overview of the effect of nutritional status on immunity, with a focus on T cells in human and mouse. Their review highlights the effects of over and under-nutrition on the metabolism and function of the immune system in protective immunity to viruses and bacteria as well as autoimmunity. Potential therapeutic targets of glycolysis, amino acid metabolism, and mitochondrial metabolism are discussed. This is followed by primary research from Jhun et al. who address the problem of how to predict which patients who have received allogeneic liver transplants will develop tolerance over time following tapering of immunosuppressive drugs. They present data measuring circulating levels of T cell markers for Treg, Th17, Th1, and CD8 cells and correlate changes in their relative abundance with propensity to develop tolerance. The role of metabolism in the fate and function of macrophages is discussed in a comprehensive review from Diskin and Palsson-McDermott. They focus on the role of metabolic pathways, substrates, and metabolites on the programming of inflammatory or anti-inflammatory macrophages in infection and resolution of inflammation. Nguyen et al. focus on the roles of metabolism in T cells following allogeneic hematopoietic cell transplantation therapy for hematological malignancies. One undesirable consequence of this procedure is graft vs. host disease (GVHD). This review describes the potential metabolic pathways that could be targeted for therapy of GVHD and the impact of current immunosuppressive drugs on these pathways. Wawman et al. then give a thorough overview of the current state of knowledge in the metabolism of T cells in the hepatic microenvironment, a hypoxic location exposed to a rich abundance of nutrients and metabolites. They give an in-depth appraisal of the prospects for metabolic intervention for therapy in autoimmune and allo-transplant settings. The role of amino acid sensing by GCN2 and its

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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *08 October 2018*
Accepted: *30 October 2018*
Published: *15 November 2018*

Citation:
Howie D and Mauro C (2018) Editorial: Metabolism and Immune Tolerance. Front. Immunol. 9:2678. doi: 10.3389/fimmu.2018.02678

relevance to immune regulation in metabolic and autoimmune disease is then reviewed by Battu et al. The relationship between TCR signal strength and underlying metabolic shift in human antigen-specific T cell clones is addressed in primary research by Jones et al. They demonstrate in their elegant study, using T cell clones and altered peptide ligands, that the signal strength between TCR and pHLA on antigen presenting cells governs the glycolytic shift in T cells. This observation may have therapeutic applications such as optimizing vaccination strategies. Finally, Tang and Mauro review the similarities in metabolic reprogramming between immune and endothelial cells. Immune cells and endothelial cells are intimately related in the physical space especially during diapedesis. The metabolic changes in inflammation and homeostasis in endothelial cells is important as it has huge implications for disease states such as atherosclerosis and cancer. In their review they put a spotlight on the roles of nitric oxide, hypoxia inducible factor, and adenosine monophosphate activated kinase in metabolic reprogramming of these cells.

We hope that this collection of primary research and review articles will prove useful to investigators interested in the current state-of-the-art in research into immune tolerance and cellular metabolism. We would also like to thank the many authors who generously contributed to this collection and to the Frontiers staff for their assistance.

AUTHOR CONTRIBUTIONS

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

ACKNOWLEDGMENTS

This work was supported by an ERC Advanced Grant 339402 Physician aided reconstitution of the Immune system, Principle Investigator Herman Waldmann, and by the British Heart Foundation (fellowship FS/12/38/29640 and project grant PG/15/105/31906), the Arthritis Research UK (clinical research fellowship 21386), and the Fondazione Cariplo (project grant 2015-0552) to CM and members of his team.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 25 June 2018
doi: 10.3389/fimmu.2018.01465

Regulation of the Immune Response by the Inflammatory Metabolic Microenvironment in the Context of Allotransplantation

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Antigen challenge induced by allotransplantation results in the activation of T and B cells, followed by their differentiation and proliferation to mount an effective immune response. Metabolic fitness has been shown to be crucial for supporting the major shift from quiescent to active immune cells and for tuning the immune response. Metabolic reprogramming includes regulation of the balance between glycolysis and mitochondrial respiration processes. Recent research has shed new light on the functions served by the end products of metabolism such as lactate, acetate, and ATP. At enhanced local concentrations, these metabolites have complex effects in which they not only induce T and B cell responses, cell mobility, and cytokine secretion but also favor the resolution of inflammation by promoting regulatory functions. Such mechanisms are instrumental in the context of the immune response in transplantation, not only to protect the graft and/or eliminate cells targeting it but also to maintain cell homeostasis *per se*. Metabolic adaptation thus plays an instrumental role on the outcome of the cellular and humoral responses. This, of course, raises the possibility of drugs that would interfere in these metabolic pathways to control the immune response but also highlights the risk that some drugs may perturb this metabolism and cell homeostasis and be deleterious for graft outcome. This review focuses on how metabolic alterations of the local immune microenvironment regulate the immune response and the impact of metabolic manipulation in allotransplantation.

Keywords: allotransplantation, immunometabolism, T lymphocytes, lactic acid, acetate, B cells, adenosine triphosphatases, inflammation

OPEN ACCESS

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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *10 April 2018*
Accepted: *12 June 2018*
Published: *25 June 2018*

Citation:
Degauque N, Brosseau C and Brouard S (2018) Regulation of the Immune Response by the Inflammatory Metabolic Microenvironment in the Context of Allotransplantation. Front. Immunol. 9:1465. doi: 10.3389/fimmu.2018.01465

INTRODUCTION

The research devoted to immunometabolism over the last decade has highlighted the cross talk between immune networks and metabolic pathways (1) to adjust the energetic machinery of lymphocyte and to fulfill the needs of an effective immune response. Whereas rapid replication of naïve and memory T and B cells results from the integration of antigen-driven stimuli, costimulatory molecules, and cytokine pathways, the effector immune response also results in the differentiation of T and B lymphocytes. B cells will ultimately differentiate into antibody (Ab)-producing plasma cells, whereas the cytokine secretion of various CD4 T helper cells (T_h1, T_h2, T_h17, and T_h22) will orchestrate the immune response. The combined functions of cytokine secretion and cytotoxic

action of CD8 T cells will elicit the elimination of target cells and protect the body from intracellular pathogens and tumors. A major contribution of immunometabolism research has been to highlight how metabolic fitness is crucial to support this shift from quiescent to active immune cells and how the survival of such long-term naïve and memory lymphocytes is fine-tuned by their metabolism. It is thus not surprising that transplant immunologists exhibit a growing interest in the field of immunometabolism. This is particularly true in fields of transplantation where the current suppressive drugs impact both immunity and metabolism. Understanding how the immune system adapts to the chronic stimulation triggered by allogeneic transplant and how metabolism interacts with treatment will likely improve the standard of care for patients and increase the survival of graft recipients. This review focuses on how metabolic alterations of the local immune microenvironment regulate the immune response and what implications these effects hold for allotransplantation.

CROSS TALK BETWEEN METABOLISM AND T CELL-BASED IMMUNITY

The ability of T cells to adapt their metabolic status in response to change in the external microenvironment has attracted increasing interest in recent years. The integration of metabolic signals has a profound impact on specific immune cell responses. Quiescent and activated T cells rely on different metabolic pathways to sustain their energetic needs. Oxidative phosphorylation (OXPHOS) is used mainly in the quiescent state by naïve or memory T cells. By contrast, the proliferation and effector functions of activated T cells upregulate their glycolytic flux to support the biosynthesis of proteins, nucleic acids, and lipids.

BALANCE OF GLUCOSE AND OXPHOS METABOLISM IN T CELL RESPONSE

A hallmark of T cell activation is the switch from OXPHOS to aerobic glycolysis. After uptake by the glucose transporter (GLUT), glucose molecules are converted into glucose-6-phosphate and will later (i) fuel the pentose phosphate pathway (PPP) to provide ribose-5-phosphate, a precursor for nucleotide synthesis; (ii) generate reducing equivalents (NADPH) for fatty acid synthesis; and (iii) fuel the tricarboxylic acid (TCA) cycle through the uptake of pyruvate by mitochondria. Glycolysis yields lactate as an end product and results in the net production of two ATP per glucose.

The metabolic conversion of T cell metabolism from OXPHOS to aerobic glycolysis is a pre-requisite for most but not all T cell function. Indeed, the activation of naïve CD4 T cells can occur when aerobic glycolysis is inhibited, rather than in the context of OXPHOS inhibition (2). The ATP synthase inhibitor oligomycin is sufficient to prevent the activation and proliferation of naïve CD4 T cells. Mitochondrial ATP from OXPHOS and not aerobic glycolysis is sufficient to support T cell proliferation as naïve CD4 T cells proliferate when activated in medium supplemented only with galactose. Upon activation, T cells can use either aerobic glycolysis or OXPHOS to support their proliferation, as shown by the proliferation of activated CD4 T cells despite the administration of oligomycin 2 days after activation. Nevertheless, aerobic glycolysis is needed to support cytokine production, as T cells cultured in galactose-supplemented medium have severe defects in IFN-γ and IL-2 production. The defective cytokine production is likely due to a block in translation rather than transcription as the expression of IFN-γ and IL-2 transcripts or the protein expression of transcription factor T-bet are similar in glucose- and galactose-cultured cells. Collectively, these data demonstrate that cytokine mRNA translation is regulated by aerobic glycolysis.

The glycolytic enzyme GAPDH not only has metabolic functions (3) but also acts as an mRNA-binding protein that regulates mRNA translation (4). GAPDH is able to bind to adenylate/uridylate-rich elements in the 3′ UTRs of IFN-γ and IL-2 mRNAs (5). CD4 T cells cultured in galactose-supplemented medium show a 10-fold increase in GAPDH-associated IFN-γ transcripts compared with cells cultured in glucose-supplemented medium (2). Therefore, aerobic glycolysis promotes effector cytokine production by distracting GAPDH from binding to cytokine transcripts. For instance, in a mouse model of infection with *Listeria monocytogenes*, the CD4 T cells expressing the highest amounts of GAPDH secrete low amounts of IFN-γ (2). Activated T cells increase also the expression of lactate dehydrogenase A (LDHA) to support aerobic glycolysis by relieving the burden on mitochondria that burn acetyl-CoA to generate ATP (6) and by regenerating NAD^+ consumed by GAPDH during glycolysis. For instance, LDHA-deficient CD4 T cells exhibit a 30% decrease in glucose consumption compared with WT cells (6). LDHA deficiency protects mice from lethal autoinflammatory disease induced by stable expression of IFN-γ in Yeti/Yeti mice (6, 7). LDHA helps maintain high concentrations of acetyl-CoA that can be readily used as a substrate for histone acetyltransferases for epigenetic regulation of target genes including IFN-γ. Thereby, aerobic glycolysis promotes effector T cell differentiation through an epigenetic mechanism.

In addition to its key role in eliciting T cell activation, glycolysis has been shown to control the inductive and suppressive functions of human regulatory T cells by modulating the expression of *FOXP3* splicing variants containing exon 2 (8). Suboptimal stimulation of T_{conv} cells leads to the generation of highly suppressive human iT_{reg} cells. These cells are characterized by a high-glycolytic rate and constitute the metabolically active fraction of T_{conv} cells. Inhibition of glycolysis by 2-DG (2-deoxy-D-glucose) blunts the IL-2–IL-2R–STAT5 signaling pathway and consequently limits the generation of human iT_{reg} cells and their suppressive functions (8). By contrast, the inhibition of fatty acid oxidation (FAO) by etomoxir (Etx) has the opposite effect and enhances the generation of iT_{reg} cells (8). It is interesting to observe that T_{conv} cells from patients with RRMS or T1D displayed an impairment of glycolysis despite showing no defect regarding proliferation after CD3/CD28 stimulation. Moreover, iT_{reg} cells generated from the T_{conv} cells of RRMS patients have diminished suppressive function compared with similar cells from age- and gender-matched healthy control individuals. The reduced suppressive function of iT_{reg} cells from RRMS patients is associated with lower expression of CTLA-4, PD-1, Foxp3-E2, and CD71 (8).

Collectively, these reports highlight the complexity of the regulation of T cell immune response by aerobic glycolysis; this metabolic pathway is critical to mount an efficient T cell response, as well as to generate and sustain the suppressive function of regulatory T cells by regulating the expression of Foxp3-E2, which is necessary for the suppressive function of human iT_{reg} cells.

LIPID METABOLISM AND ITS IMPACT ON T CELL RESPONSE

Lipids are key structural components of the cell membrane, and T cells double their lipid content in preparation for each round of cell division. In addition to their key structural properties, lipids are also used to generate energy through the process of β-oxidation. Interestingly, lymph nodes are surrounded by adipose tissue, and thereby facilitate the access of the immune system to lipid sources (9). *In vivo* LPS injection results in rapid but transient spontaneous lipolysis in the adipocytes surrounding the popliteal lymph nodes, whereas the response of adipocytes in other anatomical locations is limited (10). Similarly, there is a change in adipose tissue distribution, with fat depots surrounding lymphoid tissue in patients with chronic diseases such as Crohn's disease and in long-term treated HIV patients (11).

Enhanced mitochondrial FAO constitutes one of the hallmarks of metabolic reprogramming required for the generation of memory CD8 T cells (12). It has been shown that, upon activation, memory CD8 T cells do not increase their uptake of external long-chain fatty acids but rather synthesize fatty acids *de novo* to support FAO (13). The lysosomal hydrolase lysosomal acid lipase is more highly expressed in *in vitro*-induced memory CD8 T cells than in effector CD8 cells and supports the generation of free fatty acid (FA) and cholesterol in the lysosomes (13). Fatty acid metabolism also impacts the differentiation of CD4 T cells. Inhibition of fatty acid synthesis by selective inhibition of acetyl-CoA carboxylase 1 (ACC1) favors the generation of human and mouse regulatory $Foxp3^+$ T_{reg} cells and restrains the formation of pro-inflammatory T_h17 cells (14). Whereas T_{reg} cells take up exogenous fatty acids to produce phospholipids for the cell membrane, T_h17 cells rely on ACC1-mediated *de novo* fatty acid synthesis. As a consequence, provision of soraphen A, a specific inhibitor of ACC, attenuates the *in vivo* development of EAE by shifting the T_h17/T_{reg} balance toward a pro-tolerogenic profile. These data indicate that targeting fatty acid synthesis may be an option for immunomodulation.

LACTATE, MORE THAN A WASTE PRODUCT OF CELLULAR METABOLISM

For decades, lactate has been considered a waste product of cellular metabolism. Production of lactate occurs as a consequence of high-glycolytic flux in dividing cells or under hypoxic conditions. Two forms of lactate are present, either at higher pH as the ion salt (i.e., sodium lactate) or at low pH as the acid in its undissociated form (i.e., lactic acid). Thus, the negatively charged biologically active form (i.e., sodium lactate) represents the main form under physiological conditions (pH 7.2). The organic molecules that fuel mitochondrial metabolism *in vivo* are not fully understood, and it has been demonstrated recently that lactate fuels mitochondria in both normal and cancerous tissue (15).

Aerobic glycolysis was initially proposed by Otto Warburg in the 1920s after the observation of a high amount of lactate production by tumor cells *ex vivo*. This original observation led to the assumption that mitochondrial metabolism has a minor role in the production of macromolecules. Infusion of ^{13}C-glucose or ^{13}C-lactate tracer into patients with lung cancer has demonstrated this assumption to be wrong. Indeed, enhanced glycolysis is associated with fueling of the TCA cycle by lactate (15). The in and out transport of lactate is mediated by the lactate dehydrogenases LDHA and LDHB and by the proton-coupled monocarboxylate transporters (MCTs 1–4), members of the solute carrier 16a family of 12-transmembrane-domain, proton-linked monocarboxylic acid symporters (SLC16A1/7/8/3). MCTs bidirectionally cotransport H^+ ions and lactate anions, depending on their respective concentration gradients. Continuous lactic acid efflux is inhibited by an excess of external lactic acid, and thereby hampers T cell metabolism. Recently, Hui et al. demonstrated that lactate is, by contrast, the predominant fuel for mitochondrial metabolism (15). Lactate shows a 2.5-fold higher circulatory turnover flux than glucose in fasted mice, although glucose was previously considered the predominant circulating carbon source. Indeed, lactate has the highest circulatory turnover flux of any metabolite, exceeding that of glucose by 2.5-fold in fasting mice and 1.1-fold in fed mice (15). Thus, in all tissues except the brain, circulating lactate is the main contributor to tissue TCA metabolism. The Cori cycle postulates that lactate is produced upon anaerobic glycolysis in the muscle and later converted to glucose in the liver before being metabolized back to lactate after its return to the muscles. The potential for lactate to transfer carbon between tissues has already been shown (16, 17), leading to the concept that glycolysis and OXPHOS are tightly linked pathways and opposing processes, as the product of glycolysis provides the substrate for OXPHOS (16). The use of a ^{13}C tracker recently provided evidence to support this concept by demonstrating that glucose feeds TCA metabolism mainly through circulating lactate (15).

Regulation of T Cell Motility and Cytokine Secretion by Lactate

Glycolysis results in the production of pyruvate, which is reduced to lactate by LDHA/B, a process coupled with the conversion of NAD^+ to NADH. Lactate secretion maintains the intracellular pH by eliminating protons through the MCTs and therefore allows the persistence of the glycolysis rate. Increasing the amount of lactate in the external milieu inhibits glucose consumption by reversing the flux of lactate and can thereby inhibit T cell function. It has been shown, for instance, that lactate tightly regulates the motility and migration of CD4 and CD8 T cells (18). Lactic acid but not sodium lactate inhibits CD8 T cell migration (18). The progressive acidification of the medium induced by the provision of lactic acid does not account for the reduction of cellular motility of CD8 T cells. By contrast, sodium lactate and not lactic acid inhibits the migration of CD4 T cells by interfering with glycolysis and favors the production of the pro-inflammatory cytokine IL-17 but not IFN-γ. The modulation of T cell functions depends on the expression of

specific lactate transporters, namely, the lactic acid transporter Slc16a1 on CD8 T cells and the sodium lactate transporter Slc5a12 on CD4 T cells. Selective blockade of Slc16a1 and Slc5a12 reverses the blockade of transmigration of CD8 and CD4 T cells, respectively. Moreover, the selective blockade of Slc5a12 not only prevents T cell migration to the inflammatory site but also blocks the secretion of IL-17. Sodium lactate induces a decrease in extracellular acidification rate (ECAR) and in glucose uptake, and thereby blunts the glycolytic flux of CD4 T cells. Finally, a decrease in chemotaxis, as measured by an *in vitro* assay or *in vivo* in a model of T cell recruitment to the peritoneum, is observed after the direct or indirect inhibition of glycolysis with the glucose analog 2-DG or the mTOR inhibitor rapamycin. Therefore, after their migration into inflammatory sites such as the synovial joints of RA patients, T cells sense the local concentration of lactate and become trapped at the site. Despite the reduced CTL function of CD8 T cells, a chronic local inflammatory environment is sustained through the increased production of pro-inflammatory cytokines. The differential expression of lactate transporter by CD4 and CD8 T cells raises the question of how the nature of the inflammatory exudate (i.e., more lactic acid versus sodium lactate) regulates the differential distribution of T cells.

A key feature of inflammatory sites and tumors is the enhanced glycolysis resulting in the accumulation of lactic acid. Sodium lactate does not impact the proliferation of CTL CD8 induced by antigen-specific or mitogenic stimulation, whereas lactic acid can suppress their proliferation (19). Similarly, the production of IL-2 and IFN-γ by CTL is abolished by lactic acid at a concentration of 20 mM, whereas sodium lactate or acidification alone has no impact. The inhibition of CTL function by lactic acid is reversible by the removal of lactic acid. Lactate serum concentrations and tumor burden in cancer patients are positively correlated, and lactic acid but not the mere acidification of the environment inhibits key functions of CTL. The inhibitory effect of lactic acid on cytotoxic activity is also observed in NK cells and associated with reduced expression of perforin and granzyme (20).

In short, lactate acts as a complex immunomodulatory molecule to control T cell effector functions during inflammation and to favor the retention of activated CD4 T cells (**Figure 1**).

IMPACT OF SYSTEMIC METABOLIC ALTERATIONS ON IMMUNE CELL FUNCTION

Sensor molecules able to detect danger are necessary to mobilize an efficient immune response. Acetate and extracellular ATP (eATP) are two such molecules, and their metabolic pathways are involved in the regulation of inflammation.

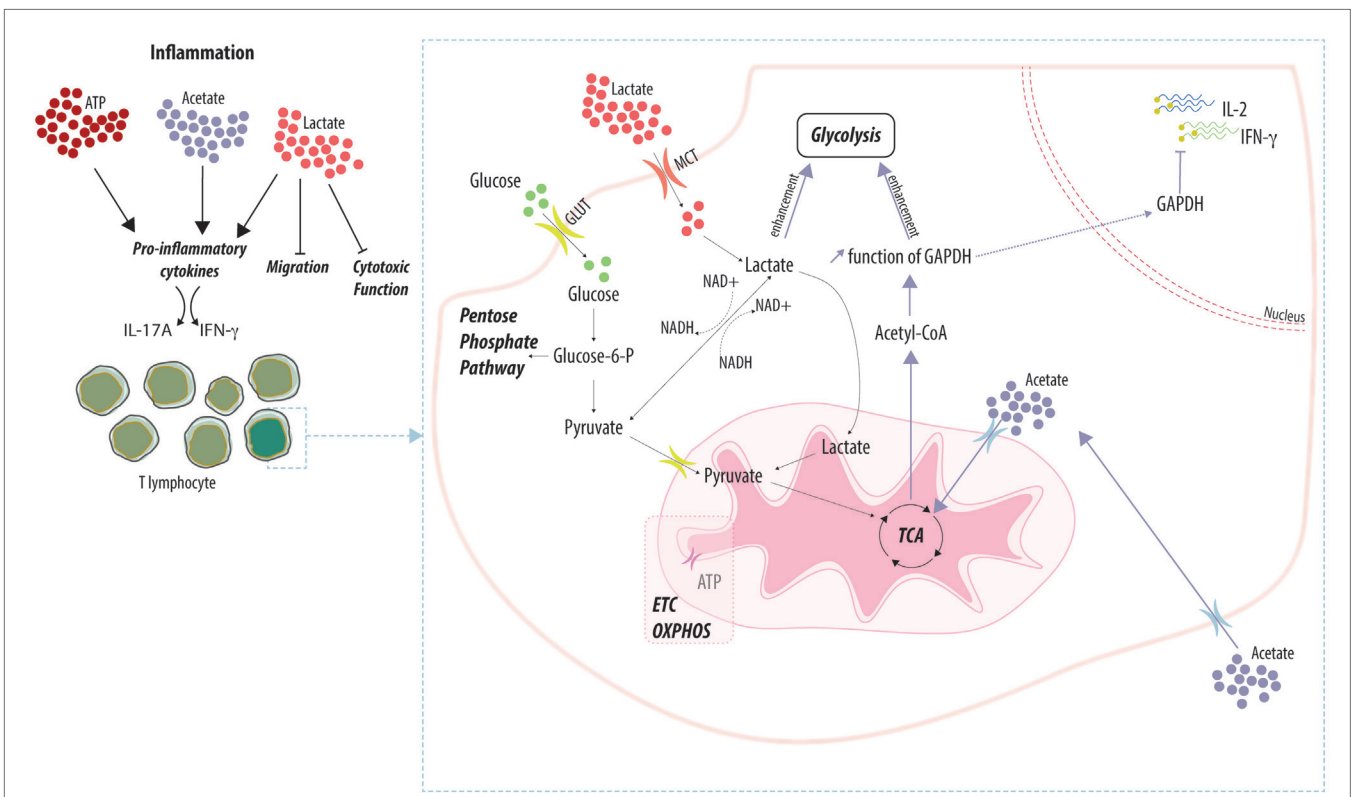

FIGURE 1 | Adaptation of T cell function to the local metabolic environment. Local concentrations of metabolic by-products (e.g., ATP, acetate, and lactate) differentially impact the function of T lymphocytes. Accumulation of metabolites favors the effector function by enhancing the secretion of pro-inflammatory cytokines while favoring the retention of T cells within the inflamed tissue. Lactate and acetate are actively imported into T cells and fuel the main metabolic pathways. Enhancement of the glycolytic rate prevents the inhibitory effect of the glycolytic enzyme GAPDH on the translation of pro-inflammatory cytokines.

Regulation of T Cell Response by Acetate Levels

It has become evident that lymphocyte T cells influence homeostasis and integrate environmental signs of danger (21). For instance, a systemic bacterial infection induces within hours an increase in acetate serum levels, and high acetate concentrations are needed to mount an optimal memory CD8 T cell response *in vitro* and *in vivo* (22). The primary production site of acetate released in the circulation is the liver (23). Hydrolysis of acetyl-CoA and release of acetate into the extracellular space can occur in other tissues under catabolic and metabolic stress conditions such as starvation or diabetes (24). Under physiological and normoxic conditions, the major source of cytosolic acetyl-CoA derives from the production of mitochondrial acetyl-CoA during glycolysis or β-oxidation (25). Mitochondrial acetyl-CoA is metabolized within the TCA to yield NADH, the main substrate for ATP synthesis *via* OXPHOS (26). Acetyl-CoA is also a central metabolite intermediate for lipid synthesis, and acetylation (provision of acetyl groups to the N-terminal residue of a protein) is one of the major post-translational protein modifications regulating the stability, localization, and function of proteins (25). The cellular function of metabolic enzymes is tightly regulated at the post-translational level through their acetylation (27). The concentration of acetate in the sera of mice infected with *L. monocytogenes*, *Salmonella typhimurium*, or *Escherichia coli* rises transiently within few hours after systemic infection (4–48 h) (22). Provision of acetate during the memory induction phase of *in vitro* differentiation of memory OT-I T cells results in increased and rapid secretion of IFN-γ upon rechallenge. Acetate has no impact on the expression of phenotypic markers or chemokine receptors. Hallmarks of memory T cells include the ability to switch rapidly to glycolysis to support IFN-γ production (28) and an increase in spare respiratory capacity (SRC) (29). Acetate exposure increases the glycolytic reserve of memory CD8 T cells by twofold in addition to increase the SRC (22). The increase in IFN-γ production upon acetate exposure is reversible, as the secretion of IFN-γ returns to the basal level when acetate is removed from previously acetate-exposed memory OT-I cells.

Therefore, a systemic increase of acetate during an inflammatory response is integrated by the immune system to favor the generation of efficient memory CD8 T cells, another new example of connection between the modulation of the immune response and systemic metabolism (**Figure 1**). The alteration of the tissue microenvironment by regulating the level of acetate will dictate the fate of T cells and is likely to represent an attractive way to control allo- and autoimmune response.

eATP: A Key Factor in Inflammation and Immune Responses

Intracellular adenosine triphosphate is well known as the energy source driving cell survival, proliferation, and metabolic function (30). However, under tissue stress such as hypoxia, apoptosis, necrosis, or inflammation, ATP can be released from cells into the extracellular environment (31–33). Two mechanisms are involved in the release of ATP into the extracellular space: (i) passive ATP release by necrotic cells by loss of cell membrane integrity and (ii) active ATP release through transporters/channels or exocytosis (34). This ATP release through transporters mainly involves connexin and pannexin channels and gap junction proteins (35). Exocytotic release of ATP into the extracellular environment has been reported in many cell types, such as neuronal cells (36), platelets (37), lymphocytes (38), mast cells (39), and endothelial cells (40). eATP then acts as a signaling molecule inducing anti- or pro-inflammatory responses depending on its binding to metabotropic P2Y purinergic or ionotropic P2X receptors, respectively, or depending on its concentration (41).

Consider a danger-associated molecular pattern and part of a group of molecules called "alarmins" (42), eATP is involved in recognition of intracellular pathogens and can mobilize an efficient innate immune response (43, 44). This occurs by the secretion of cytokines (45), recruitment of innate immune cells (such as macrophages, neutrophils, eosinophils, and mast cells) (46, 47), and production of nitric oxide (NO) and reactive oxygen species (ROS) (48). eATP also has an influence on specific immune response, since eATP activates T cells that express P2X7 by amplifying T cell receptor (TCR)-induced activation (49), inhibits the differentiation and function of Treg cells (50), and induces the differentiation of Th17 cells (51).

However, high concentrations of ATP and chronic stimulation of the P2X7 receptor also induce T cell apoptosis (52). Interestingly, the P2X7 receptor is expressed on B lymphocytes, and it mediates either cell death or proliferation (53). Wiley et al. showed that in patients with chronic lymphocytic leukemia, apoptosis of lymphocytes was observed upon the activation of the P2X7 receptor. By contrast, with very low eATP concentrations or chronic exposure, the receptor has an anti-apoptotic effect, resulting in an increase in B cell numbers (54). ATP also exerts anti-inflammatory effects *via* P2Y receptors by diminishing the Th1 cell-stimulatory capacity of DCs, by inhibiting lymphocyte effector functions, and by attenuating production by macrophages of pro-inflammatory cytokine (32).

Adenosine Production by ATP Degradation: Involvement in the Regulation of Immune Responses

To resolve the inflammatory response or to avoid ATP-induced pathological effects, eATP can be dephosphorylated into extracellular adenosine diphosphate and adenosine monophosphate by ectonucleoside triphosphate diphosphohydrolase 1 (or CD39). AMP can be further dephosphorylated to adenosine by 5′-ectonucleotidase (or CD73) (55). Indeed, uncontrolled or chronic inflammation resulting in cell and tissue damage may result from an overactivation of the immune system. Co-regulation between ATP and adenosine is due to purinergic receptors (P1 and P2 receptors for adenosine and ATP, respectively) that are ubiquitously co-expressed. The magnitude of purinergic signaling is controlled by the extracellular nucleotide concentrations that are regulated by the ectoenzymes CD39 and CD73. This purinergic feedback system is confirmed by several studies using ectoenzyme knockout models. Interestingly, $CD39^{-/-}$ and $CD73^{-/-}$ mice are prone during inflammatory conditions to tissue injury such

as acute lung injury or intestinal inflammation, highlighting the role of adenosine in terminating the inflammatory response (56, 57). Moreover, increasing the level of adenosine *in vivo* by administration of various adenosine kinase inhibitors down-regulates inflammation in different animal models of acute and chronic inflammation (32, 58, 59).

In general, the effects of adenosine on inflammation are opposite to those of ATP, and adenosine acts as an immunoregulatory signal through adenosine 2A receptor binding that inhibits and modulates adaptive and innate immune response functions (60, 61). Adenosine inhibits adhesion to endothelial cells, reduces superoxide anion production by neutrophils, and decreases the secretion of pro-inflammatory cytokines (62, 63) or facilitates the secretion of anti-inflammatory cytokines such as IL-10 (64). Opposite to the action of eATP, adenosine inhibits T cell responses following A2A receptor stimulation, inhibits T cell differentiation and proliferation by blocking TCR signaling and by decreasing IL-2 production (65, 66), reduces cytokine release, induces Treg cells, and inhibits Th17 cells (67, 68).

ATP and adenosine thus appear to be crucial endogenous signaling molecules in inflammation and immunity (**Figure 2**). They may have dual effects on inflammatory responses, depending on the concentration, the receptor used, and the duration of exposure. Therefore, the treatment of immune-related diseases such as graft dysfunction in transplantation may benefit from the control of purinergic signaling molecules.

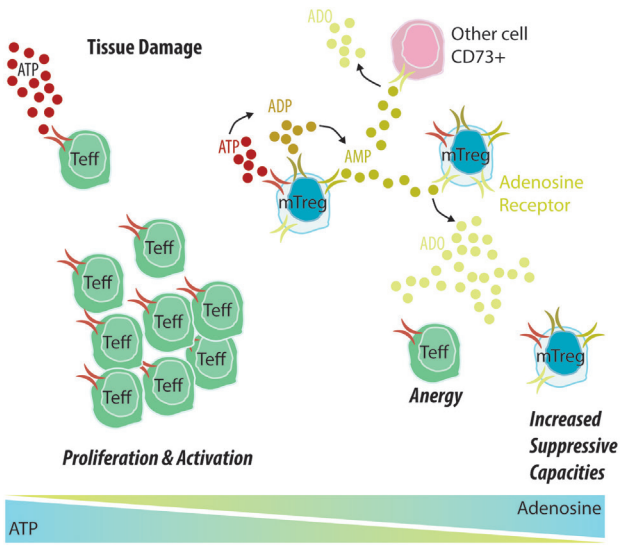

FIGURE 2 | Memory Treg (mTreg) cells induce the degradation of ATP into adenosine, promoting a tolerant environment. (1) An allograft undergoes constant attacks from the immune system, causing tissue damage that leads to the release of ATP into the extracellular environment. (2) In the context of allograft dysfunction, ATP will bind to CD39 expressed by Teff cells, leading to their activation and proliferation and potentiating the inflammatory environment. (3) In tolerant patients, ATP will mostly bind to CD39 expressed by mTreg cells and be further degraded into ADP and AMP by CD39 and into adenosine by CD73. An adenosine-rich environment will promote Teff anergy and increase the suppressive capacity of mTreg cells.

METABOLIC REGULATION OF THE IMMUNE HUMORAL RESPONSE

Whereas T cell immunometabolism has been a very intense field of investigation over the past few years, the characterization of B cell metabolism is still in its infancy. As is the case with T lymphocytes, the energetic needs of B cells are highly variable, with a necessary transition from the quiescent state to the rapid proliferation phase upon antigen encounter. Naïve and memory B cells as well as long-lived plasma cells persist in the body for years. Upon activation, B cells increase their uptake of glucose (69, 70), and Ab production requires an efficient glycolysis (70). Indeed, provision of the pyruvate dehydrogenase kinase inhibitor dichloroacetate inhibits the glycolysis and suppresses *in vitro* and *in vivo* B cell proliferation and Ab secretion. Mice with B cells deficient in Glut1 exhibit reduced peripheral B cell numbers and total IgM levels in the steady state, and antigen-specific IgM and IgG production fails to increase upon the immunization of $Glut1^{fl/fl}$CD19-Cre mice with NP-OVA (70). Oxidative metabolism or anabolism relies on glucose and glutamine uptake. The relative contributions of glucose and glutamine uptake to ATP generation or to supporting the synthesis of cellular constituents in the different B cell subsets are still unknown, and the use of metabolic trackers will be useful to track the *ex vivo* and *in vivo* fate of carbon donors. Activation of naïve B cells results from a complex integration of signals through BCR, CD40, IL-4R, and TLR (71–73) and causes the engagement of the PI3K/Akt/mTOR pathway. After BCR stimulation, glycolysis is regulated by the PI(3)K signaling pathway (71). IL-4 triggers Glut1 expression, glucose uptake, and glycolysis in splenic B cells (73). BCR activates protein kinase Cβ, which promotes an increase in glycolytic flux (74). The low oxygen tension within the GC light zone sensed by HIF inhibits B cell proliferation, increases their death, and impairs Ab class switching by limiting the expression of activation-induced cytosine deaminase (75).

Transition From the Quiescent State to Proliferation Requires Metabolic Reprogramming of B Cells

Glycogen synthase kinase 3 (Gsk3) is a metabolic sensor that promotes the survival of naïve circulating B cells. The kinase Gsk3 is ubiquitously expressed in its constitutively active form in nutrient-deprived and resting cells, but its expression rapidly decreases upon phosphorylation after growth factor stimulation (76). Two isoforms (α and β) of Gsk3 exist, and they have highly similar substrate specificities. The proliferation of B cells within the germinal center is associated with the inactivation of Gsk 3β (77). In antigen-driven responses, Gsk3 (α and β) is selectively required for the regulation of mitochondrial biogenesis, glycolysis, B cell size, and production of ROS, in a manner mediated by the costimulatory receptor CD40 and IL-4 (78).

Given the high plasticity of B cells and the continuum of B cell states from immature B cell to plasma cells, the quest to characterize the regulation and the impact of the different metabolic pathways will be challenging but will constitute a fascinating research area.

METABOLIC REGULATION OF MACROPHAGES

Macrophages are key elements of the innate immune response. Located throughout the body, macrophages maintain tissue homeostasis and behave as immune sentinels. By sensing locally tissue damage and inflammation, macrophages can rapidly modify their functional phenotype to facilitate the elimination of the pathogens and to favor tissue repair. The integration of stimuli from the local environment results in the differentiation of pro-inflammatory macrophages (M1 macrophages) in the presence of pathogen-associated molecular patterns such as LPS (79, 80). M1 macrophages are characterized by their secretion of pro-inflammatory cytokines and antimicrobial properties. By contrast, M2 macrophages differentiate in the presence of IL-4 and IL-13 and are involved in tissue repair and immunoregulatory functions (79, 80). The rewiring of metabolic pathways within macrophages in response to environmental stimuli is a key process for macrophage effector function. The polarizing signals activates canonical signaling pathways known to regulate metabolic processes (1), including the activation of Akt, mTORC1, mTORC2, and AMPK. The differentiation into M1 macrophages relies on aerobic glycolysis as shown by the defect in bacterial killing and myeloid cell infiltration when the metabolic switch to glycolysis is impaired upon HIF-1α deletion (81). M1 polarization is also associated with a defect in mitochondrial function and TCA cycle (82), the latter being truncated at the level of isocitrate dehydrogenase and succinate dehydrogenase (83). The shunt into the TCA participates to the production of itaconate, an important antimicrobial agent inhibiting the bacterial growth such as *Mycobacterium tuberculosis* and *Salmonella enterica* (84). M2 macrophages differs from M1 macrophages not only at their functional level but also at the metabolic processes, oxidative TCA cycle associated with OXPHOS being the major provider of ATP in M2 macrophages. FAO and glutamine metabolism fuels the oxidative TCA cycle in M2 macrophages (83, 85). M2 polarization activates glutamine catabolism and UDP-GlcNAc associated modules (83). Chemokine *Ccl22* production and defect in M2 polarization are observed upon glutamine deprivation (83). Finally, arginine metabolism is strikingly different in M1 and M2 macrophages (86). Production of antimicrobial agent NO is catalyzed by iNOS by converting L-arginine to L-citrulline. By contrast, expression of arginase (ARG-1) by M2 macrophages favors the catabolism of arginine to L-ornithine and urea. This arginase activity limits the production of NO, provides L-ornithine, precursor for the production of L-proline, and favors wound repair function of M2 macrophages through the synthesis of collagen. The alteration of metabolic pathways has been thus proposed as a promising strategy to repolarize macrophages. Macrophages are highly plastic cells that continuously adapt their function to their local environment. Given that most of the investigation on macrophages are performed with isolated cultured cells and singular stimulus, much work remains to be done to understand the adaptation of macrophages to complex *in vivo* environment with multiple stimuli simultaneously.

MANIPULATING THE METABOLIC PATHWAY IN TRANSPLANTATION

Given that metabolic reprogramming is an essential step to elicit an effector function, it is not surprising that interference with metabolic pathways has been attempted to control the immune response in various preclinical models. Successful attempts to prevent the development of auto- and alloimmune responses have been reported in animal models including tumor vaccination (87), hematopoietic stem cell transplantation (88–91), lupus (92, 93), EAE (94), and heart and skin transplantation (95). Metabolism being a key process shared by all cells within a given individual, one could wonder how to specifically target the metabolism of immune effector cells while preserving low toxicity. Hypothesis-driven experiments in this area rely on the shift to high-metabolic profiles in effector cells. Alloreactive T cells are deleted using a small-molecule inhibitor of the mitochondrial F_1F_0 adenosine triphosphate (F_1F_0-ATPase) while preserving hematopoietic engraftment and lymphocyte reconstitution in various models of bone marrow transplantation (91). This selective inhibition is based on increased superoxide production, decreased amounts of antioxidants, and hyperpolarization of the mitochondrial membrane potential of alloreactive T cells (91). During the course of GVHD, alloreactive T cells use FAs to support their *in vivo* activation, whereas T cells activated by cellular immunization do not (89). Pharmacological blockade of FAO has thus been shown to prevent GVHD in different models *via* induction of alloreactive T cell apoptosis while sparing the survival of T cells during normal immune reconstitution (89). The antidiabetic drug metformin has been used to target alloreactive T cells in GVHD (88, 90, 91), organ transplantation (95), and lupus (92). Disease progression and a CD4 T cell-skewed response in lupus-prone mice are reverted and controlled by combining 2-DG and metformin (92). Despite the treatment of lupus-prone mice with 2-DG and metformin for 3 months, the authors do not report any physiological side effects of such a long-term treatment. The absence of toxicity to normal tissues may seem surprising. A therapeutic window could exist for drugs that target metabolic treatment, as also demonstrated by the wide use of metformin to treat type 2 diabetes.

Examples of control of alloimmune response in models of organ transplantation are sparse. Combinatory treatment with 2-DG, DON, and metformin prevents or delays graft rejection in fully mismatched heart or skin allograft transplantation models (95). A better characterization of the metabolic requirement of allogeneic T cells during the course of allogeneic response is needed to design innovative treatments that could target immune cells that escape from standard immunosuppressive regimens. An increase of effector memory re-expressing CD45RA CD8 T cells (TEMRA CD8) is associated with an increased risk of kidney dysfunction in kidney transplant (KT) recipients (96). We have recently demonstrated that IL-15 activate TEMRA CD8 cells from KT recipients, despite immunosuppressive therapies, and promote endothelial inflammation as shown by the upregulation of CX3CL1 in human umbilical vein endothelial cells in an IFN-γ- and TNF-α-dependent manner (97). TEMRA CD8 cells exhibit an active metabolic state characterized by a large pool

of pre-formed ATP and high expression of genes involved in glycolysis and the PPP. TEMRA CD8 adapts their metabolism to stimulation by increasing their ECAR and oxygen consumption rate, demonstrating thereby their metabolic fitness. Finally, interfering with the processes of glycolysis and glutaminolysis in TEMRA CD8 cells from KT patients efficiently prevents the endothelial inflammation (97).

In the field of solid organ transplantation, eATP may be particularly important, since it participates in the fine modulation of the immune response, acting as a danger signal that will induce a pro-inflammatory environment. Inflammation is dampened upon ATP degradation, under the controlled expression of CD39 and CD73 (32). In particular, the expression of CD39 by APC or regulatory T cells appears to be of crucial importance in their immunomodulatory functions (98, 99). Moreover, CD39 expression is a fundamental determinant of human Treg function and stability (100), and recent studies have shown that CD39 expression is restricted to the most stable and suppressive subset of CD4 Treg cells (101), the human memory Treg (mTreg) cells, and show immune suppression through the production of adenosine (102, 103).

In the particular state of tolerance in kidney transplantation, we reported an increased proportion of mTreg cells in tolerant patients and not in patients with stable graft functions (104). Interestingly, mTreg cells from patients with stable graft function were unable to degrade eATP, whereas this ability was preserved in mTreg cells in tolerant patients (105). This lack of degradation capacity was not due to immunosuppressive treatments. Finally, a reduced mTreg and mTeff cells expressing CD39 was observed in patients with acute cellular rejection, and mTreg cells in transplant patients with stable graft function displayed more potent suppressive capacity than those of non-immunosuppressed controls (106). Thus, the authors of those findings propose that determining changes within these T cell subsets could help identify patients at risk of renal allograft rejection and, furthermore, that they could be considered for clinical purposes due to their suppressive properties.

The depletion of eATP through its degradation into adenosine is now considered an immunomodulatory mechanism (100, 105, 107). Adenosine 2A receptor signaling attenuates kidney graft rejection and alloantigen recognition and promotes peripheral tolerance by inducing the generation of Treg cells and the anergy of conventional T cells (108–110). There is mounting evidence that the inflammatory response that accompanies rejection and chronic allograft dysfunction involves purinergic signaling. Roberts et al. report that the CD39, CD73, and A2 signaling pathways attenuate cardiac, liver, and lung ischemia–reperfusion injuries and reduce lung and kidney allograft dysfunction (111). Current studies are investigating the potential for A2 receptor agonists or molecules targeting the purinergic pathway to attenuate alloantigen recognition and transplant rejection (108, 112–114).

Given that metabolic reprogramming is an essential step to elicit an effector function, it is not surprising that interference with metabolic pathways has been attempted to control the immune response.

CONCLUDING REMARKS

This review clearly shows the cross talk between metabolism and cell-based immunity, with glycolysis, OXPHOS, and lipid metabolism playing instrumental roles in T cell response by acting on T cell motility and cytokine secretion but also regulating this response by way of eATP and adenosine production. Metabolism is also of importance in the humoral response, particularly through regulating the transition of B cells from the quiescent state to proliferation but also through interacting directly with their regulation and/or suppressive properties. The effects of current immunosuppressors on metabolism and the manipulation of metabolic pathways in transplantation thus appear to be instrumental and should clearly be taken into account in the design of future therapies.

AUTHOR CONTRIBUTIONS

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

FUNDING

This work was funded by a grant from the Fondation Centaure and was realized in the context of the IHU-Cesti project, which received French government financial support managed by the Agence Nationale de la Recherche *via* the "Investment Into The Future" program (ANR-10-IBHU-005). The IHU-Cesti project is also supported by Nantes Metropole and the Pays de la Loire Region. This work was also supported by the FP7 VISICORT project, which has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement 602470. This work was realized in the context of the LabEX IGO program, supported by the National Research Agency *via* the "Investment Into The Future" program (ANR-11-LABX-0016-01).

REFERENCES

1. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nat Rev Immunol* (2014) 14:1–12. doi:10.1038/nri3701
2. Chang C-H, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* (2013) 153:1239–51. doi:10.1016/j.cell.2013.05.016
3. Singh R, Green MR. Sequence-specific binding of transfer RNA by glyceraldehyde-3-phosphate dehydrogenase. *Science* (1993) 259:365–8. doi:10.1126/science.8420004
4. Bonafé N, Gilmore-Hebert M, Folk NL, Azodi M, Zhou Y, Chambers SK. Glyceraldehyde-3-phosphate dehydrogenase binds to the AU-rich 3' untranslated region of colony-stimulating factor-1 (CSF-1) messenger RNA in human ovarian cancer cells: possible role in CSF-1 posttranscriptional regulation and tumor phenotype. *Cancer Res* (2005) 65:3762–71. doi:10.1158/0008-5472.CAN-04-3954
5. Nagy E, Rigby WF. Glyceraldehyde-3-phosphate dehydrogenase selectively binds AU-rich RNA in the NAD(+)-binding region (Rossmann fold). *J Biol Chem* (1995) 270:2755–63. doi:10.1074/jbc.270.6.2755
6. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* (2016) 354:481–4. doi:10.1126/science.aaf6284

7. Reinhardt RL, Liang H-E, Bao K, Price AE, Mohrs M, Kelly BL, et al. A novel model for IFN-γ-mediated autoinflammatory syndromes. *J Immunol* (2015) 194:2358–68. doi:10.4049/jimmunol.1401992
8. De Rosa V, Galgani M, Porcellini A, Colamatteo A, Santopaolo M, Zuchegna C, et al. Glycolysis controls the induction of human regulatory T cells by modulating the expression of FOXP3 exon 2 splicing variants. *Nat Immunol* (2015) 16:1174–84. doi:10.1038/ni.3269
9. Pond CM, Mattacks CA. Interactions between adipose tissue around lymph nodes and lymphoid cells in vitro. *J Lipid Res* (1995) 36:2219–31.
10. Pond CM, Mattacks CA. In vivo evidence for the involvement of the adipose tissue surrounding lymph nodes in immune responses. *Immunol Lett* (1998) 63:159–67. doi:10.1016/S0165-2478(98)00074-1
11. Wolowczuk I, Verwaerde C, Viltart O, Delanoye A, Delacre M, Pot B, et al. Feeding our immune system: impact on metabolism. *Clin Dev Immunol* (2008) 2008:639803–19. doi:10.1155/2008/639803
12. van der Windt GJW, Everts B, Chang C-H, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* (2012) 36:68–78. doi:10.1016/j.immuni.2011.12.007
13. O'Sullivan D, van der Windt GJW, Huang SC-C, Curtis JD, Chang C-H, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* (2014) 41:75–88. doi:10.1016/j.immuni.2014.06.005
14. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* (2014) 20:1327–33. doi:10.1038/nm.3704
15. Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, Lu W, et al. Glucose feeds the TCA cycle via circulating lactate. *Nature* (2017) 199:499–118. doi:10.1038/nature24057
16. Brooks GA. Cell-cell and intracellular lactate shuttles. *J Physiol* (2009) 587:5591–600. doi:10.1113/jphysiol.2009.178350
17. Gladden LB. Lactate metabolism: a new paradigm for the third millennium. *J Physiol* (2004) 558:5–30. doi:10.1113/jphysiol.2003.058701
18. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, et al. Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions. *PLoS Biol* (2015) 13:e1002202. doi:10.1371/journal.pbio.1002202
19. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, et al. Inhibitory effect of tumor cell-derived lactic acid on human T cells. *Blood* (2007) 109:3812–9. doi:10.1182/blood-2006-07-035972
20. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. *J Immunol* (2013) 191:1486–95. doi:10.4049/jimmunol.1202702
21. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature* (2007) 449:819–26. doi:10.1038/nature06246
22. Balmer ML, Ma EH, Bantug GR, Grählert J, Pfister S, Glatter T, et al. Memory CD8(+) T cells require increased concentrations of acetate induced by stress for optimal function. *Immunity* (2016) 44:1312–24. doi:10.1016/j.immuni.2016.03.016
23. Yamashita H, Kaneyuki T, Tagawa K. Production of acetate in the liver and its utilization in peripheral tissues. *Biochim Biophys Acta* (2001) 1532:79–87. doi:10.1016/S1388-1981(01)00117-2
24. Knowles SE, Jarrett IG, Filsell OH, Ballard FJ. Production and utilization of acetate in mammals. *Biochem J* (1974) 142:401–11. doi:10.1042/bj1420401
25. Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F, Kroemer G. Acetyl coenzyme A: a central metabolite and second messenger. *Cell Metab* (2015) 21:805–21. doi:10.1016/j.cmet.2015.05.014
26. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol* (2015) 17:351–9. doi:10.1038/ncb3124
27. Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, et al. Regulation of cellular metabolism by protein lysine acetylation. *Science* (2010) 327:1000–4. doi:10.1126/science.1179689
28. Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, et al. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol* (2013) 14:1064–72. doi:10.1038/ni.2687
29. van der Windt GJW, O'Sullivan D, Everts B, Huang SC-C, Buck MD, Curtis JD, et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. *Proc Natl Acad Sci U S A* (2013) 110:14336–41. doi:10.1073/pnas.1221740110
30. Oronsky BT, Oronsky N, Fanger GR, Parker CW, Caroen SZ, Lybeck M, et al. Follow the ATP: tumor energy production: a perspective. *Anticancer Agents Med Chem* (2014) 14:1187–98. doi:10.2174/1871520614666140804224637
31. Gallucci S, Matzinger P. Danger signals: SOS to the immune system. *Curr Opin Immunol* (2001) 13:114–9. doi:10.1016/S0952-7915(00)00191-6
32. Bours MJL, Swennen ELR, Di Virgilio F, Cronstein BN, Dagnelie PC. Adenosine 5'-triphosphate and adenosine as endogenous signaling molecules in immunity and inflammation. *Pharmacol Ther* (2006) 112:358–404. doi:10.1016/j.pharmthera.2005.04.013
33. Faas MM, Saez T, de Vos P. Extracellular ATP and adenosine: the Yin and Yang in immune responses? *Mol Aspects Med* (2017) 55:9–19. doi:10.1016/j.mam.2017.01.002
34. Lazarowski ER. Vesicular and conductive mechanisms of nucleotide release. *Purinergic Signal* (2012) 8:359–73. doi:10.1007/s11302-012-9304-9
35. Lohman AW, Billaud M, Isakson BE. Mechanisms of ATP release and signalling in the blood vessel wall. *Cardiovasc Res* (2012) 95:269–80. doi:10.1093/cvr/cvs187
36. Gualix J, Pintor J, Miras-Portugal MT. Characterization of nucleotide transport into rat brain synaptic vesicles. *J Neurochem* (1999) 73:1098–104. doi:10.1046/j.1471-4159.1999.0731098.x
37. Dean GE, Fishkes H, Nelson PJ, Rudnick G. The hydrogen ion-pumping adenosine triphosphatase of platelet dense granule membrane. Differences from F1F0- and phosphoenzyme-type ATPases. *J Biol Chem* (1984) 259:9569–74.
38. Tokunaga A, Tsukimoto M, Harada H, Moriyama Y, Kojima S. Involvement of SLC17A9-dependent vesicular exocytosis in the mechanism of ATP release during T cell activation. *J Biol Chem* (2010) 285:17406–16. doi:10.1074/jbc.M110.112417
39. Anderson P, Rohlich P, Slorach SA, Uvnas B. Morphology and storage properties of rat mast cell granules isolated by different methods. *Acta Physiol Scand* (1974) 91:145–53. doi:10.1111/j.1748-1716.1974.tb05670.x
40. Bodin P, Burnstock G. Evidence that release of adenosine triphosphate from endothelial cells during increased shear stress is vesicular. *J Cardiovasc Pharmacol* (2001) 38:900–8. doi:10.1097/00005344-200112000-00012
41. Jacob F, Novo CP, Bachert C, Van Crombruggen K. Purinergic signaling in inflammatory cells: P2 receptor expression, functional effects, and modulation of inflammatory responses. *Purinergic Signal* (2013) 9:285–306. doi:10.1007/s11302-013-9357-4
42. Oppenheim JJ, Yang D. Alarmins: chemotactic activators of immune responses. *Curr Opin Immunol* (2005) 17:359–65. doi:10.1016/j.coi.2005.06.002
43. Dou L, Chen Y-F, Cowan PJ, Chen X-P. Extracellular ATP signaling and clinical relevance. *Clin Immunol* (2018) 188:67–73. doi:10.1016/j.clim.2017.12.006
44. Seong S-Y, Matzinger P. Hydrophobicity: an ancient damage-associated molecular pattern that initiates innate immune responses. *Nat Rev Immunol* (2004) 4:469–78. doi:10.1038/nri1372
45. Iwata M, Ota KT, Li X-Y, Sakaue F, Li N, Dutheil S, et al. Psychological stress activates the inflammasome via release of adenosine triphosphate and stimulation of the purinergic type 2X7 receptor. *Biol Psychiatry* (2016) 80:12–22. doi:10.1016/j.biopsych.2015.11.026
46. Chen Y, Corriden R, Inoue Y, Yip L, Hashiguchi N, Zinkernagel A, et al. ATP release guides neutrophil chemotaxis via P2Y2 and A3 receptors. *Science* (2006) 314:1792–5. doi:10.1126/science.1132559
47. Kronlage M, Song J, Sorokin L, Isfort K, Schwerdtle T, Leipziger J, et al. Autocrine purinergic receptor signaling is essential for macrophage chemotaxis. *Sci Signal* (2010) 3:ra55. doi:10.1126/scisignal.2000588
48. Axtell RA, Sandborg RR, Smolen JE, Ward PA, Boxer LA. Exposure of human neutrophils to exogenous nucleotides causes elevation in intracellular calcium, transmembrane calcium fluxes, and an alteration of a cytosolic factor resulting in enhanced superoxide production in response to FMLP and arachidonic acid. *Blood* (1990) 75:1324–32.
49. Schenk U, Frascoli M, Proietti M, Geffers R, Traggiai E, Buer J, et al. ATP inhibits the generation and function of regulatory T cells through the activation of purinergic P2X receptors. *Sci Signal* (2011) 4:ra12–12. doi:10.1126/scisignal.2001270
50. Corthay A. How do regulatory T cells work? *Scand J Immunol* (2009) 70:326–36. doi:10.1111/j.1365-3083.2009.02308.x
51. Killeen ME, Ferris L, Kupetsky EA, Falo L, Mathers AR. Signaling through purinergic receptors for ATP induces human cutaneous innate and adaptive Th17 responses: implications in the pathogenesis of psoriasis. *J Immunol* (2013) 190:4324–36. doi:10.4049/jimmunol.1202045

52. Tsukimoto M, Maehata M, Harada H, Ikari A, Takagi K, Degawa M. P2X7 receptor-dependent cell death is modulated during murine T cell maturation and mediated by dual signaling pathways. *J Immunol* (2006) 177:2842–50. doi:10.4049/jimmunol.177.5.2842
53. Markwardt F, Lohn M, Bohm T, Klapperstuck M. Purinoceptor-operated cationic channels in human B lymphocytes. *J Physiol* (1997) 498(Pt 1):143–51. doi:10.1113/jphysiol.1997.sp021847
54. Wiley JS, Dao-Ung LP, Gu BJ, Sluyter R, Shemon AN, Li C, et al. A loss-of-function polymorphic mutation in the cytolytic P2X7 receptor gene and chronic lymphocytic leukaemia: a molecular study. *Lancet* (2002) 359:1114–9. doi:10.1016/S0140-6736(02)08156-4
55. Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. *Biochim Biophys Acta* (2008) 1783:673–94. doi:10.1016/j.bbamcr.2008.01.024
56. Friedman DJ, Kunzli BM, A-Rahim YI, Sevigny J, Berberat PO, Enjyoji K, et al. CD39 deletion exacerbates experimental murine colitis and human polymorphisms increase susceptibility to inflammatory bowel disease. *Proc Natl Acad Sci U S A* (2009) 106:16788–93. doi:10.1073/pnas.0902869106
57. Eckle T, Fullbier L, Wehrmann M, Khoury J, Mittelbronn M, Ibla J, et al. Identification of ectonucleotidases CD39 and CD73 in innate protection during acute lung injury. *J Immunol* (2007) 178:8127–37. doi:10.4049/jimmunol.178.12.8127
58. Kowaluk EA, Jarvis MF. Therapeutic potential of adenosine kinase inhibitors. *Expert Opin Investig Drugs* (2000) 9:551–64. doi:10.1517/13543784.9.3.551
59. Boyle DL, Kowaluk EA, Jarvis MF, Lee CH, Bhagwat SS, Williams M, et al. Anti-inflammatory effects of ABT-702, a novel non-nucleoside adenosine kinase inhibitor, in rat adjuvant arthritis. *J Pharmacol Exp Ther* (2001) 296:495–500.
60. Antonioli L, Csoka B, Fornai M, Colucci R, Kokai E, Blandizzi C, et al. Adenosine and inflammation: what's new on the horizon? *Drug Discov Today* (2014) 19:1051–68. doi:10.1016/j.drudis.2014.02.010
61. Di Virgilio F, Vuerich M. Purinergic signaling in the immune system. *Auton Neurosci* (2015) 191:117–23. doi:10.1016/j.autneu.2015.04.011
62. Barletta KE, Ley K, Mehrad B. Regulation of neutrophil function by adenosine. *Arterioscler Thromb Vasc Biol* (2012) 32:856–64. doi:10.1161/ATVBAHA.111.226845
63. Haskó G, Pacher P. Regulation of macrophage function by adenosine. *Arterioscler Thromb Vasc Biol* (2012) 32:865–9. doi:10.1161/ATVBAHA.111.226852
64. Koscso B, Csoka B, Selmeczy Z, Himer L, Pacher P, Virag L, et al. Adenosine augments IL-10 production by microglial cells through an A2B adenosine receptor-mediated process. *J Immunol* (2012) 188:445–53. doi:10.4049/jimmunol.1101224
65. Ohta A, Ohta A, Madasu M, Kini R, Subramanian M, Goel N, et al. A2A adenosine receptor may allow expansion of T cells lacking effector functions in extracellular adenosine-rich microenvironments. *J Immunol* (2009) 183:5487–93. doi:10.4049/jimmunol.0901247
66. Stagg J, Smyth MJ. Extracellular adenosine triphosphate and adenosine in cancer. *Oncogene* (2010) 29:5346–58. doi:10.1038/onc.2010.292
67. Bao R, Hou J, Li Y, Bian J, Deng X, Zhu X, et al. Adenosine promotes Foxp3 expression in Treg cells in sepsis model by activating JNK/AP-1 pathway. *Am J Transl Res* (2016) 8:2284–92.
68. Ohta A, Kini R, Ohta A, Subramanian M, Madasu M, Sitkovsky M. The development and immunosuppressive functions of CD4(+) CD25(+) FoxP3(+) regulatory T cells are under influence of the adenosine-A2A adenosine receptor pathway. *Front Immunol* (2012) 3:190. doi:10.3389/fimmu.2012.00190
69. Caro-Maldonado A, Wang R, Nichols AG, Kuraoka M, Milasta S, Sun LD, et al. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. *J Immunol* (2014) 192:3626–36. doi:10.4049/jimmunol.1302062
70. Cho SH, Ahn AK, Bhargava P, Lee C-H, Eischen CM, McGuinness O, et al. Glycolytic rate and lymphomagenesis depend on PARP14, an ADP ribosyltransferase of the B aggressive lymphoma (BAL) family. *Proc Natl Acad Sci U S A* (2011) 108:15972–7. doi:10.1073/pnas.1017082108
71. Doughty CA, Bleiman BF, Wagner DJ, Dufort FJ, Mataraza JM, Roberts MF, et al. Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. *Blood* (2006) 107:4458–65. doi:10.1182/blood-2005-12-4788
72. Woodland RT, Fox CJ, Schmidt MR, Hammerman PS, Opferman JT, Korsmeyer SJ, et al. Multiple signaling pathways promote B lymphocyte stimulator dependent B-cell growth and survival. *Blood* (2008) 111:750–60. doi:10.1182/blood-2007-03-077222
73. Dufort FJ, Bleiman BF, Gumina MR, Blair D, Wagner DJ, Roberts MF, et al. Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism. *J Immunol* (2007) 179:4953–7. doi:10.4049/jimmunol.179.8.4953
74. Blair D, Dufort FJ, Chiles TC. Protein kinase Cβ is critical for the metabolic switch to glycolysis following B-cell antigen receptor engagement. *Biochem J* (2012) 448:165–9. doi:10.1042/BJ20121225
75. Cho SH, Raybuck AL, Stengel K, Wei M, Beck TC, Volanakis E, et al. Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature* (2016) 537:234–8. doi:10.1038/nature19334
76. Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemmings BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* (1995) 378:785–9. doi:10.1038/378785a0
77. Cato MH, Chintalapati SK, Yau IW, Omori SA, Rickert RC. Cyclin D3 is selectively required for proliferative expansion of germinal center B cells. *Mol Cell Biol* (2011) 31:127–37. doi:10.1128/MCB.00650-10
78. Jellusova J, Cato MH, Apgar JR, Ramezani-Rad P, Leung CR, Chen C, et al. Gsk3 is a metabolic checkpoint regulator in B cells. *Nat Immunol* (2017) 18:303–12. doi:10.1038/ni.3664
79. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* (2014) 41:14–20. doi:10.1016/j.immuni.2014.06.008
80. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* (2011) 11:723–37. doi:10.1038/nri3073
81. Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N, et al. HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell* (2003) 112:645–57. doi:10.1016/S0092-8674(03)00154-5
82. Biswas SK, Mantovani A. Orchestration of metabolism by macrophages. *Cell Metab* (2012) 15:432–7. doi:10.1016/j.cmet.2011.11.013
83. Jha AK, Huang SC-C, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* (2015) 42:419–30. doi:10.1016/j.immuni.2015.02.005
84. Michelucci A, Cordes T, Ghelfi J, Pailot A, Reiling N, Goldmann O, et al. Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc Natl Acad Sci U S A* (2013) 110:7820–5. doi:10.1073/pnas.1218599110
85. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* (2006) 4:13–24. doi:10.1016/j.cmet.2006.08.006
86. Rath M, Müller I, Kropf P, Closs EI, Munder M. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. *Front Immunol* (2014) 5:532. doi:10.3389/fimmu.2014.00532
87. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest* (2013) 123:4479–88.
88. Park M-J, Lee S-Y, Moon S-J, Son H-J, Lee S-H, Kim E-K, et al. Metformin attenuates graft-versus-host disease via restricting mammalian target of rapamycin/signal transducer and activator of transcription 3 and promoting adenosine monophosphate-activated protein kinase-autophagy for the balance between T helper 17 and Tregs. *Transl Res* (2016) 173:115–30. doi:10.1016/j.trsl.2016.03.006
89. Byersdorfer CA, Tkachev V, Opipari AW, Goodell S, Swanson J, Sandquist S, et al. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. *Blood* (2013) 122:3230–7. doi:10.1182/blood-2013-04-495515
90. Nguyen HD, Chatterjee S, Haarberg KMK, Wu Y, Bastian D, Heinrichs J, et al. Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. *J Clin Invest* (2016) 126:1337–52. doi:10.1172/JCI82587
91. Gatza E, Wahl DR, Opipari AW, Sundberg TB, Reddy P, Liu C, et al. Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft-versus-host disease. *Sci Transl Med* (2011) 3:ra8–67. doi:10.1126/scitranslmed.3001975

92. Yin Y, Choi S-C, Xu Z, Perry DJ, Seay H, Croker BP, et al. Normalization of CD4+ T cell metabolism reverses lupus. *Sci Transl Med* (2015) 7:ra18–274. doi:10.1126/scitranslmed.aaa0835
93. Yin Y, Choi S-C, Xu Z, Zeumer L, Kanda N, Croker BP, et al. Glucose oxidation is critical for CD4+ T cell activation in a mouse model of systemic lupus erythematosus. *J Immunol* (2016) 196:80–90. doi:10.4049/jimmunol.1501537
94. Nath N, Giri S, Prasad R, Salem ML, Singh AK, Singh I. 5-aminoimidazole-4-carboxamide ribonucleoside: a novel immunomodulator with therapeutic efficacy in experimental autoimmune encephalomyelitis. *J Immunol* (2005) 175:566–74. doi:10.4049/jimmunol.175.1.566
95. Lee C-F, Lo Y-C, Cheng C-H, Furtmüller GJ, Oh B, Andrade-Oliveira V, et al. Preventing allograft rejection by targeting immune metabolism. *Cell Rep* (2015) 13:760–70. doi:10.1016/j.celrep.2015.09.036
96. Yap M, Boeffard F, Clave E, Pallier A, Danger R, Giral M, et al. Expansion of highly differentiated cytotoxic terminally differentiated effector memory CD8+ T cells in a subset of clinically stable kidney transplant recipients: a potential marker for late graft dysfunction. *J Am Soc Nephrol* (2014) 25:1856–68. doi:10.1681/ASN.2013080848
97. Tilly G, Doan-Ngoc T-M, Yap M, Caristan A, Jacquemont L, Danger R, et al. IL-15 harnesses pro-inflammatory function of TEMRA CD8 in kidney-transplant recipients. *Front Immunol* (2017) 8:778. doi:10.3389/fimmu.2017.00778
98. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J Exp Med* (2007) 204:1257–65. doi:10.1084/jem.20062512
99. Leal DBR, Streher CA, Neu TN, Bittencourt FP, Leal CAM, da Silva JEP, et al. Characterization of NTPDase (NTPDase1; ecto-apyrase; ecto-diphosphohydrolase; CD39; EC 3.6.1.5) activity in human lymphocytes. *Biochim Biophys Acta* (2005) 1721:9–15. doi:10.1016/j.bbagen.2004.09.006
100. Gu J, Ni X, Pan X, Lu H, Lu Y, Zhao J, et al. Human CD39(hi) regulatory T cells present stronger stability and function under inflammatory conditions. *Cell Mol Immunol* (2017) 14:521–8. doi:10.1038/cmi.2016.30
101. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity* (2009) 30:899–911. doi:10.1016/j.immuni.2009.03.019
102. Dwyer KM, Hanidziar D, Putheti P, Hill PA, Pommey S, McRae JL, et al. Expression of CD39 by human peripheral blood CD4+ CD25+ T cells denotes a regulatory memory phenotype. *Am J Transplant* (2010) 10:2410–20. doi:10.1111/j.1600-6143.2010.03291.x
103. Schuler PJ, Saze Z, Hong C-S, Muller L, Gillespie DG, Cheng D, et al. Human CD4+ CD39+ regulatory T cells produce adenosine upon co-expression of surface CD73 or contact with CD73+ exosomes or CD73+ cells. *Clin Exp Immunol* (2014) 177:531–43. doi:10.1111/cei.12354
104. Braza F, Dugast E, Panov I, Paul C, Vogt K, Pallier A, et al. Central role of CD45RA- Foxp3hi memory regulatory T cells in clinical kidney transplantation tolerance. *J Am Soc Nephrol* (2015) 26:1795–805. doi:10.1681/ASN.2014050480
105. Durand M, Dubois F, Dejou C, Durand E, Danger R, Chesneau M, et al. Increased degradation of ATP is driven by memory regulatory T cells in kidney transplantation tolerance. *Kidney Int* (2018) 93:1154–64. doi:10.1016/j.kint.2017.12.004
106. McRae JL, Chia JS, Pommey SA, Dwyer KM. Evaluation of CD4(+) CD25(±) CD39(+) T-cell populations in peripheral blood of patients following kidney transplantation and during acute allograft rejection. *Nephrology (Carlton)* (2017) 22:505–12. doi:10.1111/nep.12894
107. Roussey-Kesler G, Giral M, Moreau A, Subra JF, Legendre C, Noël C, et al. Clinical operational tolerance after kidney transplantation. *Am J Transplant* (2006) 6:736–46. doi:10.1111/j.1600-6143.2006.01280.x
108. Sevigny CP, Li L, Awad AS, Huang L, McDuffie M, Linden J, et al. Activation of adenosine 2A receptors attenuates allograft rejection and alloantigen recognition. *J Immunol* (2007) 178:4240–9. doi:10.4049/jimmunol.178.7.4240
109. Zarek PE, Huang C-T, Lutz ER, Kowalski J, Horton MR, Linden J, et al. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. *Blood* (2008) 111:251–9. doi:10.1182/blood-2007-03-081646
110. Salcido Ochoa F, Tsang J, Tam P, Falk K, Rotzschke O. Regulatory T cells in transplantation: does extracellular adenosine triphosphate metabolism through CD39 play a crucial role? *Transplant Rev (Orlando)* (2010) 24:52–66. doi:10.1016/j.trre.2010.01.002
111. Roberts V, Stagg J, Dwyer KM. The role of ectonucleotidases CD39 and CD73 and adenosine signaling in solid organ transplantation. *Front Immunol* (2014) 5:64. doi:10.3389/fimmu.2014.00064
112. Jones KR, Kang EM. Graft versus host disease: new insights into A2A receptor agonist therapy. *Comput Struct Biotechnol J* (2015) 13:101–5. doi:10.1016/j.csbj.2014.12.003
113. Chhabra P, Linden J, Lobo P, Okusa MD, Brayman KL. The immunosuppressive role of adenosine A2A receptors in ischemia reperfusion injury and islet transplantation. *Curr Diabetes Rev* (2012) 8:419–33. doi:10.2174/157339912803529878
114. Koo TY, Lee J-G, Yan J-J, Jang JY, Ju KD, Han M, et al. The P2X7 receptor antagonist, oxidized adenosine triphosphate, ameliorates renal ischemia-reperfusion injury by expansion of regulatory T cells. *Kidney Int* (2017) 92:415–31. doi:10.1016/j.kint.2017.01.031

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 16 May 2018
doi: 10.3389/fimmu.2018.01055

Changes in Nutritional Status Impact Immune Cell Metabolism and Function

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Immune cell function and metabolism are closely linked. Many studies have now clearly demonstrated that alterations in cellular metabolism influence immune cell function and that, conversely, immune cell function determines the cellular metabolic state. Less well understood, however, are the effects of systemic metabolism or whole organism nutritional status on immune cell function and metabolism. Several studies have demonstrated that undernutrition is associated with immunosuppression, which leads to both increased susceptibility to infection and protection against several types of autoimmune disease, whereas overnutrition is associated with low-grade, chronic inflammation that increases the risk of metabolic and cardiovascular disease, promotes autoreactivity, and disrupts protective immunity. Here, we review the effects of nutritional status on immunity and highlight the effects of nutrition on circulating cytokines and immune cell populations in both human studies and mouse models. As T cells are critical members of the immune system, which direct overall immune response, we will focus this review on the influence of systemic nutritional status on T cell metabolism and function. Several cytokines and hormones have been identified which mediate the effects of nutrition on T cell metabolism and function through the expression and action of key regulatory signaling proteins. Understanding how T cells are sensitive to both inadequate and overabundant nutrients may enhance our ability to target immune cell metabolism and alter immunity in both malnutrition and obesity.

Keywords: malnutrition, obesity, T cells, cellular metabolism, inflammation, leptin

OPEN ACCESS

Edited by:
Claudio Mauro, Queen Mary University of London, United Kingdom

Reviewed by:
Jeffrey Louis Curtis, University of Michigan, United States
Hans-Dieter Volk, Charité Universitätsmedizin, Germany

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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *01 December 2017*
Accepted: *27 April 2018*
Published: *16 May 2018*

Citation:
Alwarawrah Y, Kiernan K and MacIver NJ (2018) Changes in Nutritional Status Impact Immune Cell Metabolism and Function. Front. Immunol. 9:1055. doi: 10.3389/fimmu.2018.01055

INTRODUCTION

Nutritional imbalance is a major challenge for living organisms to achieve systemic homeostasis and maintain normal physiology. Mammals have developed processes to control systemic nutrient utilization and storage. For example, excess nutrients are converted and stored in adipose tissue, liver, and muscle during times when nutrients are abundant. By contrast, stored nutrients are metabolized to provide energy and building blocks to maintain vital physiological processes when nutrient availability is low.

From these processes, adipose tissue volume changes in response to under- or overnutrition. This change in adipose tissue volume, in turn, influences the secretion of hormones and cytokines from adipose tissue (adipocytokines). Many of these adipocytokines have important immune signaling functions which can influence immune cell biology and alter immune response.

Here, we will review the effects of nutritional changes on hormones and cytokines that influence immune cell function and metabolism in mouse models, and we will specifically examine how nutritionally regulated changes in immune cells alter immunity in the context of both autoimmune disease and infection response in both human and animal studies. We will also highlight some of the promising metabolic targets that may be useful in the development of novel treatments for immunity-related disorders.

KEY SIGNALING MOLECULES ALTERED IN RESPONSE TO CHANGES IN NUTRITIONAL STATUS

Many cytokines and hormones are changed in response to over- or undernutrition. One of the earliest reports of this is with tumor necrosis factor-alpha (TNF-α). TNF-α can be secreted from adipose tissue, and its expression is increased during obesity but decreased following weight loss (1–3). TNF-α is a well-known pro-inflammatory cytokine that is essential for the acute phase reaction. Studies from the 1990s demonstrated that TNF-α was essential for the development of insulin resistance in high-fat diet-induced obesity in mice (1) and that deletion of TNF-α protected high-fat diet-induced obese mice from developing insulin resistance (4). TNF-α can be secreted from both adipose tissue-localized macrophages, which are increased in obesity, and from adipocytes (5, 6). Following these early studies showing the importance of TNF-α in mediating metabolic disease in obesity, multiple other cytokines and hormones were also found to play a similar role.

Interleukin-6 (IL-6) is another cytokine that is secreted by many immune and non-immune cells in the adipose tissue including adipocytes, macrophages, pre-adipocytes, and T cells, in response to tissue damage (7). IL-6 has broad pleiotropic functions leading to the expansion of many types of immune cells including B cells and T cells (8, 9). IL-6 can signal through binding to the IL-6 receptor dimerized with gp130 on the surface of cells; however, IL-6 effects can also be mediated through trans-signaling, in which IL-6 binds to soluble IL-6 receptor, thereby permitting IL-6 to act on any cell that expresses gp130. In that way, IL-6 receptor trans-signaling contributes to the broad pleiotropic effect of IL-6 (10). In general, IL-6 promotes T cell survival and resistance to apoptosis (11). IL-6 also has a pro-inflammatory role promoting CD4+ T cell differentiation to the Th17 or Th1 lineages, which produce the pro-inflammatory cytokines IL-17 and interferon gamma (IFN-γ), respectively (12, 13). Increased IL-6 levels have been reported in obesity in both humans and rodents (14, 15). Pan-blocking of IL-6 signaling using an anti-IL-6 antibody (MR16-1) has been shown to ameliorate insulin resistance and reduce liver-fat accumulation in high-fat diet-fed mice (16). Blocking IL-6 trans-signaling by using gp130Fc soluble protein was found to block adipose tissue macrophage recruitment in high-fat diet-fed mice, but did not inhibit insulin resistance (17). Recently, it was reported that selective blocking of IL-6 signaling in T cells improves glucose homeostasis and ameliorates liver steatosis in high-fat diet-fed mice, but only early in the development of obesity (18).

Leptin is another well-described adipocytokine known to influence immune cells. Leptin is a hormone secreted by adipocytes in proportion to adipocyte mass and is therefore increased in obesity and decreased in malnutrition. Leptin is best known for its role in influencing systemic metabolism by signaling in the hypothalamus to suppress appetite and increase energy expenditure (19). However, leptin can also communicate energy status to other systems in the body, including the immune system (20). In that role, leptin was found to have an important developmental function in the maturation of hematopoietic cells on which the leptin receptor (LepR) is expressed (21). In addition to this developmental function, leptin deficiency has also been associated with the loss of cell-mediated immunity (22). The first reported immune function for leptin was its ability to regulate macrophage phagocytosis and pro-inflammatory cytokine production: lack of leptin or its receptor was found to reduce phagocytic activity and the production of both IL-6 and TNF-α (23). In addition, LepR is expressed on the surface of T cells (24), and deletion of LepR on T cells leads to a marked decrease in T cell number and function as well as polarization to Th1 and Th17 cell subsets, ultimately leading to immune deficiency characterized by an increased susceptibility to intracellular infections (25–27).

In addition to TNF-α, IL-6, and leptin, many other hormones and cytokines are influenced by changes in systemic metabolism and are summarized in **Table 1**.

IMMUNE CELLS AFFECTED BY CHANGES IN NUTRITIONAL STATUS

The hormone and cytokine changes seen in response to obesity and malnutrition are closely linked to changes in immune cell populations. Several types of immune cells residing in the adipose tissue are affected by changes in the above-listed cytokine and hormone levels and in turn contribute to altered cytokine production in states of under- or overnutrition. These adipose tissue-localized immune cells can be affected by changes in nutritional status through both paracrine effects (due to their proximity to adipocytes) and systemic/endocrine effects of secreted adipose factors.

Macrophages comprise more than 50% of adipose tissue-resident immune cells and are, therefore, the most abundant immune cells in the adipose tissue (52). During obesity, an influx of macrophages into the adipose tissue takes place in response to the secretion of monocyte chemoattractant protein-1 from the adipose tissue (53). These macrophages become polarized into pro-inflammatory, classically activated macrophages (previously termed M1 macrophages) in response to IFN-γ, which is secreted by effector T cells (Teff cells) and other immune cells in the adipose tissue (54). This increase in inflammatory macrophage population leads to an increase in TNF-α secretion in addition to other inflammatory molecules secreted by inflammatory macrophages, including IL-1β, IL-6, and IL-12. Adipose tissue macrophages in obesity contribute to the formation of the crown-like structure that forms around necrotic adipocytes, a very distinctive histological feature of the adipose tissue during obesity. In lean individuals, alternatively activated macrophages (previously termed M2 macrophages) secrete anti-inflammatory

TABLE 1 | Key immune signaling molecules that change in response to nutritional status.

Molecule	Class	Obesity	Malnutrition	Secreted by	Immune function	Reference
Leptin	Adipocytokine	Increased	Decreased	Adipocytes	Pleiotropic hormone with many targets and many functions in immune cells Induces Th1 and Th17 polarization	(28)
Adiponectin	Adipocytokine	Decreased	Conflicting reports—may be context-dependent	Adipocytes	Polarization of monocytes and macrophages toward M2 phenotype Suppresses NK cell, eosinophil, neutrophil, γδ T cell, and dendritic cell activation and inflammatory cytokine production	(29)
Resistin	Adipocytokine	Increased	No clear correlation; more investigation needed	Adipocytes, macrophages	Stimulates the production of TNF-α and IL-12 in macrophages	(30)
Visfatin	Adipocytokine	Increased	No clear correlation; more investigation needed	Adipocytes	Stimulates the production of TNF-α, IL-1β, and IL-6	(30, 31)
TNF-α	Cytokine	Increased	Mixed results, may depend on context	Adipocytes	Neutrophil chemotaxis	(4, 15, 32–34)
IL-1β	Cytokine	Increased	Mixed results, may depend on context	Non-adipocyte cells in adipose tissue	Stimulates macrophage activity	(35, 36)
IL-6	Cytokine	Increased	Decreased	Adipocytes, macrophages	Recruitment of macrophages; polarization toward pro-inflammatory classically activated macrophages	(15, 37)
IL-8	Cytokine	Increased	Decreased	Adipocytes, macrophages	Induces neutrophil chemotaxis	(38–40)
IL-10	Cytokine	Mixed—may be context-dependent	Increased	Treg cells, iNKT cells, DCs, adipocytes, macrophages	Broad anti-inflammatory function	(41–43)
IL-33	Cytokine	Decreased	Increased	DCs, macrophages, epithelial cells	Maintains adipose tissue-resident Treg cell function, promotes Th2 response, promotes alternatively activated macrophage polarization	(44, 45)
IL-1RA	Cytokine	Increased	Unknown	Macrophages, epithelial cells	Inhibits IL-1α and IL-1β activity	(46–48)
MCP-1	Chemokine	Increased	Increased	Macrophages, adipocytes	Macrophage recruitment	(39, 49)
MIF	Chemokine	Increased	Decreased	Adipocytes, lymphocytes	Inhibits macrophage migration	(50)
MIP-1α	Chemokine	Increased	Unknown	Adipocytes	Enhances macrophage migration	(51)
MIP-1β	Chemokine	Increased	Unknown	Adipocytes	Enhances macrophage migration	(51)

cytokines including IL-10, IL-4, and IL-1 receptor agonists which promote immune modulatory functions (55). The shift in macrophage populations during obesity plays a central role in the maintenance of inflammation and the rise of obesity-associated pathologies including insulin resistance and non-alcoholic fatty liver disease.

In addition to macrophages, other smaller populations of innate immune cells are found in the adipose tissue and change in number and function in response to obesity. Neutrophils and mast cells are found to increase and become activated in the adipose tissue during obesity (56). By contrast, eosinophil numbers decrease in adipose tissue during obesity (57), which is relevant because eosinophils secrete IL-4, a cytokine that helps maintain the alternatively activated population of macrophages within the adipose tissue (57).

Several lymphocyte populations are also found in the adipose tissue. B lymphocytes (B cells) have been found to accumulate in the adipose tissue during obesity (58). Although B cells are best known for the production of antibodies, they also express inflammatory cytokines, such as IL-2 and IL-12, which influence T cell differentiation into Th1 versus Th2 cells (59). Adipose tissue also harbors a large population of natural killer T (NKT) cells (60); these cells are known for the expression of an invariant form of the T cell receptor (TCR) that interacts with a lipid antigen presenting protein, CD1d, which is highly expressed on adipocytes (61). NKT cells secrete different types of cytokines depending on the lipid antigen presented by CD1d. During normal weight conditions, NKT cells modulate inflammation by secreting anti-inflammatory cytokines such as IL-4 and IL-10 (62). In obesity, NKT cells decrease in number, at the same time adipocytes express lower levels of CD1d. This change altogether reduces the amount of anti-inflammatory cytokines secreted by NKT cells and contributes to the complications of obesity (62).

T lymphocytes (T cells) represent the most abundant lymphocyte population and second most abundant immune cell in the adipose tissue behind macrophages (63). Both CD4+ and CD8+ T cells are found in the adipose tissue. During obesity, the proportion of adipose CD8+ T cells to CD4+ T cells increases (64).

In addition, proportions of inflammatory CD4+ T cell subsets increase in obesity, whereas regulatory T cells (Treg cells) decrease (64, 65). This change in T cell populations during obesity contributes to the pro-inflammatory state of the adipose tissue: both CD8+ T cells and pro-inflammatory CD4+ Th1 cells express the pro-inflammatory cytokine IFN-γ, whereas effector CD4+ Th17 cells express the pro-inflammatory cytokine IL-17.

During normal physiological conditions, the adipose tissue represents a major depot of Treg cells in the body (66, 67). These cells represent more than 50% of CD4+ T cells in lean adipose tissue (66). Treg cells are responsible for suppressing inflammation through the secretion of anti-inflammatory cytokines such as TGF-β and IL-10 (58, 63). During obesity, the proportion of adipose tissue Treg cells decreases dramatically as adipose tissue volume increases. Due to their role in maintaining self-tolerance and in dampening excessive inflammatory response, the reduction in Treg cell number during obesity (68) induces a significant shift in immune cell populations and cytokine production toward a pro-inflammatory state (65). Paradoxically, adipose tissue Treg cells were also found to contribute to age-associated insulin resistance, and deletion of Treg cells could protect against age-associated, but not obesity-associated, insulin resistance (69).

In contrast to the effects of obesity on immune cells, malnutrition leads to a *decrease* in immune cell number. This has been shown particularly in the case of T cells: mice fasted 48 h had large and significantly decreased thymocyte and splenocyte counts compared to fed control mice (26, 70, 71). Both total T cell and CD4+ T cell numbers from spleens of fasted mice were decreased by 40–50% compared to fed control animals (26, 71). Other studies have shown that mice fed a protein-deficient diet had atrophic spleens and decreased T cell numbers compared to chow-fed control mice (72, 73). A similar finding was seen in human studies. Malnourished children had decreased CD4+ and CD8+ T cell numbers in whole blood compared to well-nourished children (74). Moreover, childhood malnutrition causes atrophy of primary lymphoid organs, leading to reduced T and B cell numbers and a generalized state of leukopenia (75). These reductions in immune cell number in malnutrition contribute to functional deficiencies, which will be discussed in further detail below.

EFFECT OF NUTRITIONAL STATUS ON IMMUNE CELL METABOLISM

Although it is clear that systemic metabolism influences immune cell function, we are only just starting to understand how changes in nutrition can influence metabolism at the cellular level. This is an important consideration, as immune cell metabolism and immune cell function are intrinsically tied. Previous studies have demonstrated a link between cellular metabolism and function for several types of immune cells (76, 77), but we will focus our discussion here on T cells. Multiple studies have now shown that changes in T cell metabolism can influence T cell differentiation and function, whereas changes in T cell function can likewise influence T cell metabolism.

The energy requirement of naïve T cells performing immune surveillance is satisfied through oxidative phosphorylation of lipids, amino acids, and glucose-derived pyruvate to ATP in the mitochondria (78). This process is highly efficient at producing ATP, but does not provide biosynthetic precursors that are necessary for proliferation or growth. Naïve T cells are arrested in the G_0 stage of the cell cycle and this state of homeostatic quiescence is actively maintained (79). Without TCR stimulation, CD4+ T cells fail to undergo homeostatic proliferation, downregulate Glut1, and die from apoptosis (80, 81). Following activation, however, T cells need to rapidly grow, proliferate, and generate cytokines to direct a functional immune response. Given the growth and proliferation requirement of an activated T cell, these cells must be prepared to increase the biosynthesis of cellular products including lipids, proteins, and nucleotides which are needed for rapid cell division (78), and for these reasons, a metabolic switch is required.

Upon activation, the metabolic state of T cells resembles that of cancer cells (82). These rapidly proliferating cells increase glucose uptake, glycolysis, and reduction of pyruvate to lactate even in the presence of oxygen, a process aptly named aerobic glycolysis (83). Warburg noticed this effect in his early studies of blood leukocytes, and more recent studies have confirmed the "Warburg effect" in thymocytes and T cells (84, 85). A state of rapid ATP usage and massive biosynthetic requirement make the process of glycolysis a more efficient way for cancer cells and activated T cells to proliferate. TCA cycle intermediates can be used as precursors in biosynthetic pathways to support the growing need for lipid, protein, and nucleotide synthesis that precedes cellular division (78). Conversion of pyruvate to lactate ensures that reducing equivalents of NAD+ are restored, allowing the process of glycolysis to continue (83).

The upregulation of the glycolytic metabolic program in activated T cells is controlled by several key signaling pathways and transcription factors. Both TCR signaling and signaling through CD28 induce the activation of the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/Akt pathway, which is partially responsible for the upregulation of a glycolytic metabolic program (80, 86). The PI3K/Akt pathway leads to the activation of mammalian target of rapamycin (mTOR) which forms two functional complexes: mammalian target of rapamycin complex 1 (mTORC1) and mammalian target of rapamycin complex 2 (mTORC2). mTOR is an important integrator of environmental signals, allowing the cell to respond to signals from the TCR, co-stimulation, cytokines, and nutrient availability (87–89). The activation of Akt and mTOR has been shown to lead to an increase in aerobic glycolysis by increasing the transcription of glycolytic genes and transcription factors including c-Myc and hypoxia-inducible factor (HIF)-1α (87, 88). In addition, constitutive Akt expression leads to an increase in surface expression of the glucose transporter Glut1 and thereby increases glucose uptake, whereas blocking Akt or PI3K decreases T cell glucose uptake. The inhibition of mTORC1 by treatment with rapamycin has been shown to prevent T cell growth and proliferation (90, 91). Knockout of the mTOR inhibitor AMPKα1 decreases the ability of T cells to respond to metabolic stress and decreases their ability to transition between anabolic and catabolic metabolisms (92). mTOR also regulates protein translation as part of mTORC1 through interaction with the translation initiation factor 4E-binding

protein (4E-BP) and p70s6K. By phosphorylating 4E-BP and p70s6K, mTOR activates these two proteins to increase protein translation in the cell (88).

At the transcriptional level, c-Myc is responsible for regulating glucose and glutamine metabolism. Specifically, c-Myc has been shown to upregulate multiple glycolytic genes upon T cell activation, including Glut1, hexokinase2 (HK2), and pyruvate kinase muscle isozyme 2 (93). Glut1 is a critical glucose transporter expressed in T cells and is upregulated upon activation. Glut1 can be stored in vesicles intracellularly and transported to the cell surface to increase glucose uptake following T cell activation, and Glut1 transcription and translation are increased following T cell activation (94). In cancer cells, c-Myc has been shown to control the transition to glycolytic metabolism in hypoxic conditions along with HIF-1-α (95). c-Myc is upregulated following T cell activation and has been demonstrated by several groups to induce cell cycle progression and glycolytic metabolism (96, 97). Without this transition, T cells would not be able to exit G_0 and enter the rapid expansion phase of the immune response. This has been confirmed in a T cell specific knockout of c-Myc, in which T cells fail to proliferate and were unable to upregulate glycolytic metabolism (95). c-Myc also upregulates glutaminolysis which can feed into anaplerosis, so that TCA cycle intermediates can be used for biosynthesis. For example, citrate is a TCA cycle intermediate that can be used as a precursor for lipid synthesis (95). HIF-1-α is also increased upon T cell activation and has been shown to increase the expression of glycolytic genes along with c-Myc (95, 98). HIF-1-α is also important in the differentiation of T cells, particularly toward Th17 cells.

Differentiation of CD4+ T cells into functionally distinct subsets is associated with alterations in the cellular metabolic phenotype. In general, Teff cells are pro-glycolytic and depend heavily upon an increased glucose uptake, as well as glutamine metabolism, to fuel effector function, as described above. Inhibition of glycolysis prevents differentiation into these pro-inflammatory subsets (99). Regulatory T cells (Treg) and memory T cells (Tmem), however, have a decreased glucose metabolism and predominantly utilize lipid oxidation to fuel suppressive and memory function, respectively (100–102). Although Treg cells have a decreased glucose metabolism in comparison to Teff cells, glycolysis is essential for Treg cell migration into sites of inflammation (103).

The first evidence of T cell differentiation depending on metabolism came from studies using the drug rapamycin. Treatment with rapamycin prevented T cell growth and proliferation and promoted differentiation of Treg cells, rather than Teff cells (90). Further evidence came from studies using whole-body or T cell-specific knockout of mTOR, in which only Treg cells were produced under activation conditions (104). Failure to produce Teff cells in the absence of mTORC1 demonstrates that glycolytic metabolism, as driven by mTORC1, is required for Teff cell differentiation and cytokine production. Moreover, increasing the activity of AMPK by treatment with metformin increased Treg cell numbers and decreased Teff cells, further demonstrating that blocking glycolytic metabolism promotes the Treg cell lineage (100). Metformin-treated T cells also had lower Glut1 levels upon activation, demonstrating a decreased glycolytic state compared to untreated T cells in these studies (100).

Not only does the activity of mTORC1 promote Teff cell differentiation over Treg cells but differential activity of mTORC1 and mTORC2 promotes distinct Teff subsets (78, 98, 105). mTORC1 activity is upregulated in Th1 and Th17 cells, whereas Th2 cells show an increased activity of mTORC2. Treg cells also demonstrate an increased AMPK activity, which leads to inhibition of mTOR. Tmem express both TRAF6 and AMPK, which promote lipid oxidation and thereby suppress the glycolytic phenotype of activated Teff cells (102).

Leptin Promotes Glycolytic Metabolism in Activated T Cells

One well-established connection between systemic nutritional status and immune cell metabolism is through leptin. As mentioned above, leptin is secreted by adipocytes in proportion to adipocyte mass and leptin levels, thereby trending with nutritional status (106). In states of malnutrition or following fasting, circulating leptin levels are decreased, whereas in obesity, leptin levels are increased. Leptin acts directly on CD4+ T cells through the LepR to direct changes in T cell metabolism and function (26, 27, 107–110). Since T cell metabolism and function are intimately linked, any change in immune cell metabolism can lead to a change in the function of that cell, altering cellular proliferation, differentiation, and cytokine production. Leptin has been shown to promote both CD4+ T cell inflammatory cytokine production and glucose metabolism. Indeed, T cells unable to respond to leptin had impaired upregulation of glucose uptake and glycolysis following T cell activation (26). In the context of malnutrition, fasting-induced hypoleptinemia caused activated CD4+ T cells to produce less inflammatory cytokines IFN-γ and IL-2 (26, 27). That functional defect did not extend to naïve T cells or Treg cells, however, presumably because those cells do not depend on increased glycolytic metabolism to fuel immune surveillance or regulatory function. In subsequent studies, Th17 cells from fasted mice were found to be functionally deficient and metabolically less glycolytic, whereas Treg cells from fasted mice did not experience a functional or a metabolic defect (27). The metabolic status of Th17 cells derived from fasted mice was assessed by extracellular flux analysis, and these cells were found to have a decreased lactate production as well as a decreased mitochondrial respiration compared to Th17 cells from *ad libitum* fed mice. The functional and metabolic defects of Th17 cells were restored when fasted mice received leptin injections or when T cells isolated from fasted mice were activated in the presence of leptin *in vitro*. Leptin can affect many types of immune system; however, these effects of leptin on T cells were shown to be cell-intrinsic, as Th17 cells from T cell-specific LepR conditional knockout mice showed a decreased glucose uptake and glycolysis as well as a decreased glycolytic enzyme expression, whereas glucose metabolism and function of Treg cells from T cell-specific LepR conditional knockout mice were unaffected. Altogether, these studies show that leptin is a systemic hormone that communicates nutritional status to immune cells by directly increasing Teff cell glucose metabolism and thereby fueling Teff cell function.

The effect of leptin on immune cells is now well documented; however, other nutritionally regulated hormones may also

mediate the communication between systemic nutritional status and immune cell function. One such candidate hormone is insulin. Insulin is secreted from pancreatic beta cells following an increase in blood glucose; however, insulin levels also become elevated in states of obesity due to insulin resistance of metabolic tissues including muscle and adipose tissue. Insulin is best known for its role in promoting glucose uptake and glycolysis in metabolic tissues through its ability to upregulate surface expression of the glucose transporter Glut4 and increase the activities of the glycolytic enzymes hexokinase and phosphofructokinase (111–116). Insulin is also able to regulate lipid and protein metabolism. Interestingly, insulin receptors are expressed on activated CD4+ T cells (117). One recent study in rats showed that inducible knockdown of insulin receptor in T cells led to a decreased T cell glucose metabolism and cytokine production (118). Further studies are needed to define the mechanism by which changes in systemic nutrition alter circulating levels of insulin and thereby influence immune cell metabolic and functional processes.

EFFECT OF NUTRITIONAL STATUS ON IMMUNE-MEDIATED DISEASE

Autoimmunity

Given the role of leptin in regulating the balance between Teff and Treg cells, multiple studies have examined the effect of leptin on autoimmune diseases. One well-studied example is the autoimmune disease multiple sclerosis (MS). The autoimmunity of MS depends upon the activation of Teff, particularly Th1 and Th17 cells, which produce inflammatory cytokines that promote inflammation. Conversely, Treg cells influence immune response by suppressing inflammation caused by Teff and other inflammatory immune cells and thereby protect against autoimmunity. The balance of Teff (Th1, Th17) to Treg cells in MS is, therefore, a critical determinant of inflammation and autoimmune disease. Multiple human studies have shown that active MS is associated with a decreased Treg cell number and proliferation (119–121).

Nutritional status appears to influence MS susceptibility. Clinical studies have shown an association between adolescent obesity and an increased risk of developing MS, particularly in women (122–125). Consistent with that observation, leptin levels have been found to be increased in patients with MS in both serum and cerebrospinal fluid and are associated with increased inflammatory cytokines (119, 120, 126). In addition, LepR expression was increased on the surface of CD8+ T cells from relapsing–remitting MS patients in relapse, as compared to patients in remission or controls (127).

The role of leptin in promoting T cell inflammation has also been well described in a mouse model of MS, experimental autoimmune encephalomyelitis (EAE). Serum leptin levels have been shown to be increased in EAE and correlate with disease severity (128, 129). Early studies in EAE showed that leptin injections worsened EAE disease in female mice and increased disease susceptibility in male mice, while promoting inflammatory cytokine release (130). Moreover, leptin-deficient mice were found to be resistant to EAE, but this protection was lost when mice were treated with recombinant leptin protein (128, 131). Leptin-neutralizing antibodies likewise protected against T cell response and EAE disease in mice (107).

Since leptin levels decrease with fasting and calorie restriction, the effect of fasting on EAE disease severity has been examined by several groups. Fasting-induced hypoleptinemia resulted in a reduced EAE disease severity (27, 129, 132, 133). This fasting-induced protection against EAE was reversed, in part, when fasted mice received leptin injections (27). Moreover, T cells recovered from draining lymph nodes in fasted EAE mice showed a decreased production of the Th1 and Th17 cytokines IFN-γ and IL-17, respectively, as well as a decrease in the expression of glycolytic proteins Glut1 and HK2 (27); again, these fasting-induced changes in Teff cell cytokine production and glucose metabolism were reversed in T cells from fasted mice receiving leptin injections.

Leptin signaling has also been implicated in several other autoimmune diseases. In systemic lupus erythematosus (SLE), leptin levels have been reported to be elevated in human patients and correlate with severity in a mouse model of the disease (134, 135). Decreasing leptin signaling through genetic knockout or antibody blockade protected against disease and increased Treg cell numbers in SLE mice (134). Additional studies in SLE models demonstrated an increased Th17 response, which could be attenuated with the neutralization of leptin (110). Leptin has also been reported to increase Th17 activity in Hashimoto's thyroiditis and collagen-induced arthritis (108, 109). This provides further evidence for the role of leptin in promoting Th17 differentiation and activation, thus promoting autoimmune pathology. Although not strictly an autoimmune disease, it is also notable that in an allogeneic skin-transplant model, leptin-deficient mice showed an increase in graft survival relative to wild-type mice (136). Altogether, leptin signaling appears to provide an important link between nutritional status and autoimmune disease through its effects on T cell metabolism and function.

Protective Immunity

Like any other physiological system, the development of the immune system is affected by nutritional status. One salient example for this is the thymic atrophy and increase in thymocyte apoptosis observed during malnutrition early in life (137–139). This has a devastating effect on the ability of the immune system to mount a successful immune response to infection. Moreover, many epidemiological studies have shown dysfunction in both innate and adaptive immunity during malnutrition (138). This explains the increased susceptibility to many kinds of infection in the malnourished individuals, such as influenza, tuberculosis, *Streptococcus pneumonia* and gastrointestinal infections (140–143), and the poor response to vaccines (138).

Obesity, however, is also associated with susceptibility to a number of infections (144). One example of this is with influenza. This was first reported when studies on the H1N1 strain of influenza showed a connection between obesity and poor disease outcome (145). Indeed, obesity was found to be a risk factor for developing H1N1 infection and was associated with a longer length of stay in the intensive care unit and with higher rates of mortality (146–148). Following the discovery that obesity increased risk and mortality from H1N1 flu, obesity that

was subsequently found to be is an independent risk factor for increased morbidity and mortality from *all* strains of influenza.

In addition to increased susceptibility to influenza, individuals with obesity are also at an increased risk from other infections: obese individuals have an increased risk of developing complications such as sepsis, pneumonia, and bacteremia following surgical procedures (149); they are more prone to *Helicobacter pylori* infection (150), and obese children were found to have three times greater risk of being asymptomatic carriers of *Neisseria meningitides* (151). In addition, obesity is associated with a lower antibody response to select vaccinations including influenza, hepatitis B, and tetanus (152).

In the case of malnutrition, the lack of protective immunity can be easily traced back to the developmental defects associated with inadequate nutrients and the lack of nutritional signals such as leptin that are critical for fueling immune cell proliferation and function. On the other hand, the susceptibility to infection and poor vaccine response associated with obesity seems unexpected when factoring in that obesity is accompanied with a low-grade inflammation and constant activation of immune cells. In the case of influenza, obesity has been found to be associated with impaired memory response (153). One possible explanation for this is that the systemic metabolic environment in obesity promotes a cellular metabolism in immune cells which supports short-lived effector cells over the generation of long-term memory cells.

TARGETING IMMUNE METABOLISM FOR DISEASE TREATMENT

As we have documented here, many signaling cascades are affected by nutritional status and subsequently have an effect on immune cell metabolism and function. For that reason, immune cell metabolism represents an attractive target to improve response in both malnutrition and obesity. Here, we will highlight several signaling molecules and metabolic enzymes that play central roles in the metabolic reprogramming of immune cells and are critical for mounting an immune response and initiating inflammatory reaction. These metabolic molecules serve as potential targets to reverse the effects of obesity and malnutrition on immunodeficiency and inflammation, respectively.

Glut1

Activated immune cells are dependent on a glycolytic metabolism to fuel rapid ATP production and provide biosynthetic materials for growth and proliferation. For that reason, activated immune cells require a large influx of glucose to fuel glycolysis (154). This suggests the glycolysis pathway as a potential target for the control of inflammation. Multiple reports have shown that the inhibition of glycolysis by 2-deoxyglucose (2-DG) can block CD4+ T cell proliferation, inflammatory macrophage polarization, and B cell survival (155–157). Although 2-DG shows a potent anti-inflammatory activity and has shown tolerability in clinical trials for the treatment of prostate cancer, cardiac adverse reactions to 2-DG were reported (158, 159), and alternative targets for the inhibition of glucose metabolism are required.

Glucose transport represents the most upstream, rate-limiting step for glycolysis. There are approximately 13 members of the glucose transporter family expressed to various extents in different tissues. The best-described glucose transporter is Glut4, which is an insulin-sensitive glucose transporter expressed on metabolic tissues including muscle, adipose tissue, and liver. However, Glut4 is not expressed on T cells (160). Rather, T cell glucose uptake is largely dependent on the ubiquitously expressed glucose transporter Glut1. Glut1 expression is upregulated in classically activated macrophages, activated Teff cells, and B cells, all of which depend on Glut1 for an increased glucose uptake during activation (154, 160, 161). Glut1 may, therefore, provide a more appropriate target for blocking glycolysis in activated immune cells than 2-DG. Treating macrophages with the Glut1 inhibitor Fasentin was found to inhibit the production of IL-1β (162). Currently, there are several trials to synthesize more selective small molecule inhibitors for Glut1 (163–165). The bioavailable Glut1 inhibitor BAY-876 represents one of the most potent Glut1 inhibitors (IC50 = 2nM) and shows more than 100-fold selectivity against the other glucose transporters (164). Unpublished data from our laboratory showed the ability of BAY-876 to selectively inhibit glucose uptake in activated CD4+ T cells. Although there is limited information about targeting Glut1 in immune cells, the new generation of selective Glut1 inhibitors may have the potential to be used as a therapy for the control of inflammation. Caution must be taken with this class of inhibitors, though, as Glut1 is critical for glucose transport to the brain and Glut1 mutations have been described in disorders of seizures and developmental delay (166, 167).

PFKFB3

One mechanism by which glycolysis is regulated is through the metabolite fructose-2,6-bisphosphate (F26BP), which is an allosteric activator of 6-phosphofructo-1-kinase: a rate-limiting enzyme in the glycolysis pathway that phosphorylates fructose 6-phosphate (F6P) to yield fructose 1,6-bisphosphate (168). The intracellular levels of F26BP are regulated by a family of bifunctional enzymes known as 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase enzymes (PFKFB 1 through 4) which can convert F6P into F26BP and *vice versa* based on the phosphorylation levels of the enzyme (169). PFKFB3 expression is upregulated in activated macrophages and T cells (170, 171). In T cells, treatment with the PFKFB inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO) has been shown to block both glycolysis and activation (171). *In vivo*, 3PO treatment of BALB/c mice challenged with methylated BSA was found to inhibit delayed-type hypersensitivity, highlighting the viability of targeting PFKFB activity for the control of inflammation (171). Unfortunately, the number of selective PFKFB small molecule inhibitors is limited; the only other PFKFB inhibitor, in addition to 3PO, is PFK15. PFK15 is a more potent PFKFB inhibitor than 3PO and showed antiproliferative activity in Jurkat T cell leukemia cells and several solid tumor-derived cell lines, but its effect on primary immune cells has yet to be investigated (172). Similar to targeting Glut1, targeting PFKFB activity may provide an additional tool to block glycolysis; however, more potent and selective inhibitors are needed, as are additional studies to investigate the effect of targeting such enzymes on immune function.

BCAT1

Most activated immune cells are dependent on glycolysis for the rapid generation of energy, which limits the flow of pyruvate to the TCA cycle. However, TCA cycle intermediates are essential for proliferation and immune cell functions, and activated immune cells generally overcome this by metabolizing amino acids. This requires the upregulation of many genes involved in amino acid transport and catabolism.

Branched-chain aminotransferase (BCAT) is the enzyme responsible for the transamination of branched-chain amino

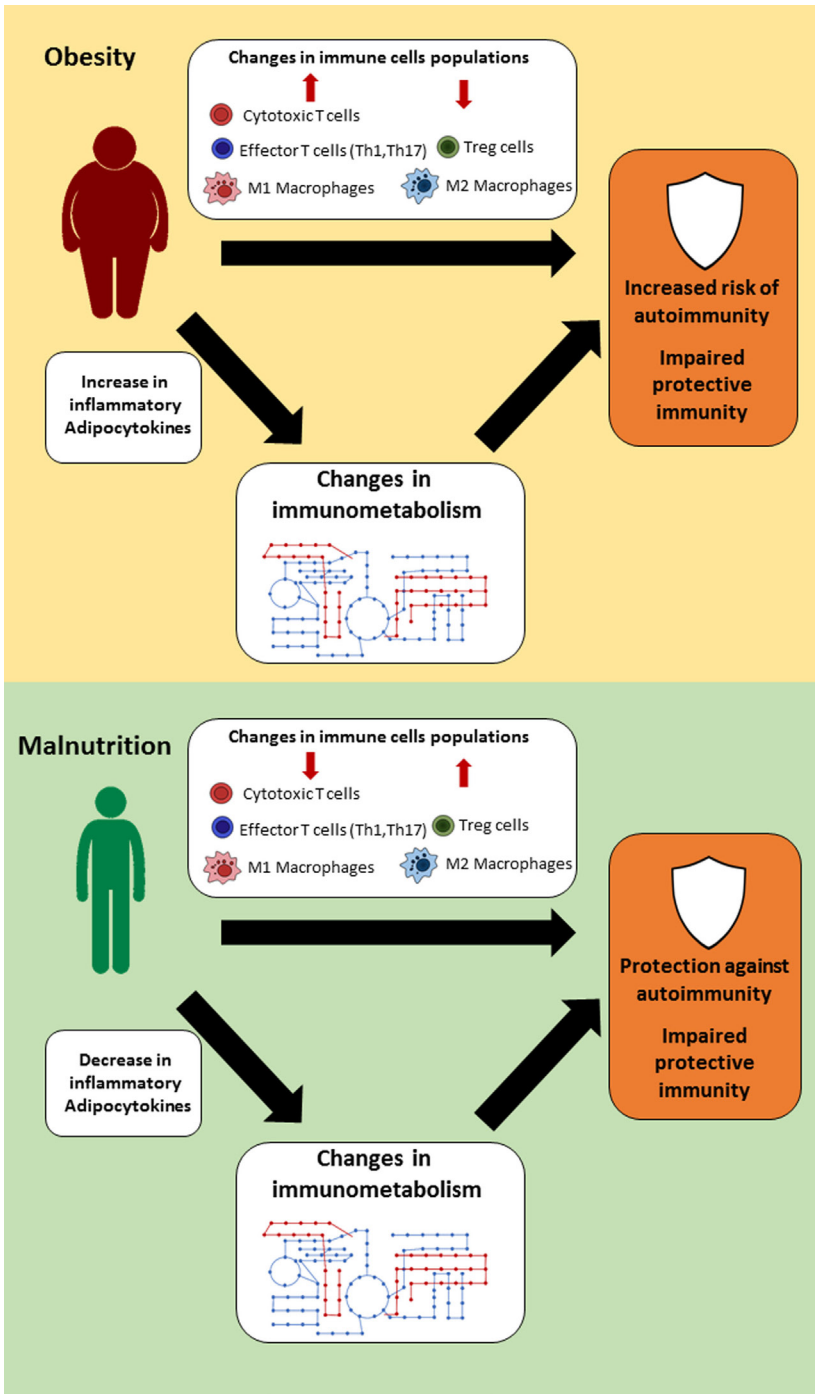

FIGURE 1 | Suggested links between nutritional status, immune metabolism, and immune function. In settings of extreme nutritional status (obesity or malnutrition), changes in immune cell populations, hormones, and cytokine levels lead to alternations in immune cell metabolism, which thereby influence immune function.

acids (BCAAs): leucine, isoleucine, and valine. There are two isoforms of the BCAT enzymes: cytosolic BCAT1 and mitochondrial BCAT2. Transamination of BCAAs is the initial reaction toward the formation of branched-chain α-keto acids, which are decarboxylated to produce coenzyme A (CoA) derivatives (173). Leucine transamination leads to the formation of glutamate and α-ketoisocaproate. The α-ketoisocaproate molecule is subsequently metabolized to form acetoacetate and acetyl-CoA, which is then oxidized in the TCA cycle, similar to glutamate which enters the TCA cycle in the form of α-ketoglutarate.

Branched-chain aminotransferase 2 is highly expressed in many tissues including the kidneys, skeletal muscle, and tissues of the digestive system, while BCAT1 is expressed in a small number of tissues including placenta, adult brain, peripheral neurons, and a limited number of embryonic tissues (174). BCAT1 was found to be highly expressed in human monocyte-derived macrophages compared to BCAT2 (175). Blocking the activity of BCAT1 by the leucine analog ERG240 was found to inhibit the induction of cis-aconitate decarboxylase (IRG1) expression by LPS which is a crucial step for the activation for macrophages (175). ERG240 treatment was able to reduce oxygen consumption and glycolysis in human macrophages. Consistent with this phenotype, the administration of ERG240 ameliorated the severity of crescentic glomerulonephritis in rats and collagen-induced arthritis in mice (175).

F1F0-ATPase

In contrast to acutely activated T cells being dependent on glycolysis, chronically activated T cells involved in autoimmune disease are more dependent on oxidative phosphorylation for energy production (176–178). This suggests that components of the oxidative phosphorylation pathway may be targets to block the expansion of autoreactive T cells in autoimmune diseases. The ATP synthase F1F0-ATPase catalyzes the final step of energy production of the respiratory chain in the mitochondria. F1F0-ATPase was found to be the target for the 1,4-benzodiazepine (Bz-423) which is reported to induce apoptosis of pathogenic lymphoid cells in lupus mouse models (179–181). Bz-423 was also reported to induce apoptosis of alloreactive T cells in a graft-versus-host disease model (182). Based on this, F1F0-ATPase gained special attention as a viable drug target for the treatment of autoimmune diseases. Currently, there are ongoing clinical trials for the treatment of inflammatory bowel disease and ulcerative colitis using the F1F0-ATPase small molecule inhibitor LYC-30937.

CONCLUSION

Changes in nutritional status have a wide range of effects on the body, which can influence organ size, hormone, and cytokine levels, and immune cell populations and function. This link between nutrition and immunity is mediated, in part, by a select group of adipocytokines, such as leptin, which can influence immune cell number and function through its effects on cellular metabolism (**Figure 1**). For that reason, leptin has been identified as a key regulator of both protective immunity and autoimmunity in the context of nutritional disorders. Other cytokines and hormones likely play a similar key role in linking nutrition and immunity. In understanding the mechanisms by which nutrition influences immunity, we can identify targets to improve or normalize immunity in cases of under- or overnutrition. Currently, there are several small molecules under investigation that show promising preclinical results and the potential for restoring immune response in both malnutrition and obesity. In summary, more studies are needed to clarify the link between nutritional status, immune metabolism, and immune function; such knowledge may pave the way for the development of novel classes of therapies that can reverse the detrimental effects of the extremes of nutritional status on immunity.

AUTHOR CONTRIBUTIONS

All authors participated in the writing of this manuscript.

FUNDING

This work was supported by the National Institutes of Health (R01-DK106090 and T32-AI052077) and the National Multiple Sclerosis Society (RG-5333-A-1).

REFERENCES

1. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* (1993) 259(5091):87–91. doi:10.1126/science.7678183
2. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* (1995) 95(5):2409–15. doi:10.1172/JCI117936
3. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* (1995) 95(5):2111–9. doi:10.1172/JCI117899
4. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* (1997) 389(6651):610–4. doi:10.1038/39335
5. Sewter CP, Digby JE, Blows F, Prins J, O'Rahilly S. Regulation of tumour necrosis factor-alpha release from human adipose tissue *in vitro*. *J Endocrinol* (1999) 163(1):33–8. doi:10.1677/joe.0.1630033
6. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* (2005) 115(5):1111–9. doi:10.1172/JCI200525102
7. Kopp A, Buechler C, Neumeier M, Weigert J, Aslanidis C, Scholmerich J, et al. Innate immunity and adipocyte function: ligand-specific activation of multiple toll-like receptors modulates cytokine, adipokine, and chemokine secretion in adipocytes. *Obesity (Silver Spring)* (2009) 17(4):648–56. doi:10.1038/oby.2008.607
8. Yasukawa K, Hirano T, Watanabe Y, Muratani K, Matsuda T, Nakai S, et al. Structure and expression of human B cell stimulatory factor-2 (BSF-2/IL-6) gene. *EMBO J* (1987) 6(10):2939–45.
9. Woloski BM, Fuller GM. Identification and partial characterization of hepatocyte-stimulating factor from leukemia cell lines: comparison with interleukin 1. *Proc Natl Acad Sci U S A* (1985) 82(5):1443–7. doi:10.1073/pnas.82.5.1443
10. Rose-John S. IL-6 trans-signaling *via* the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci* (2012) 8(9):1237–47. doi:10.7150/ijbs.4989
11. Atreya R, Mudter J, Finotto S, Mullberg J, Jostock T, Wirtz S, et al. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in

chronic intestinal inflammation: evidence in Crohn disease and experimental colitis *in vivo*. *Nat Med* (2000) 6(5):583–8. doi:10.1038/75068
12. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, et al. IL-6 controls Th17 immunity *in vivo* by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. *Proc Natl Acad Sci U S A* (2008) 105(47):18460–5. doi:10.1073/pnas.0809850105
13. Romani L, Mencacci A, Cenci E, Spaccapelo R, Toniatti C, Puccetti P, et al. Impaired neutrophil response and CD4+ T helper cell 1 development in interleukin 6-deficient mice infected with *Candida albicans*. *J Exp Med* (1996) 183(4):1345–55. doi:10.1084/jem.183.4.1345
14. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* (1997) 40(11):1286–92. doi:10.1007/s001250050822
15. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am J Physiol Endocrinol Metab* (2001) 280(5):E745–51. doi:10.1152/ajpendo.2001.280.5.E745
16. Yamaguchi K, Nishimura T, Ishiba H, Seko Y, Okajima A, Fujii H, et al. Blockade of interleukin 6 signalling ameliorates systemic insulin resistance through upregulation of glucose uptake in skeletal muscle and improves hepatic steatosis in high-fat diet fed mice. *Liver Int* (2015) 35(2):550–61. doi:10.1111/liv.12645
17. Kraakman MJ, Kammoun HL, Allen TL, Deswaerte V, Henstridge DC, Estevez E, et al. Blocking IL-6 trans-signaling prevents high-fat diet-induced adipose tissue macrophage recruitment but does not improve insulin resistance. *Cell Metab* (2015) 21(3):403–16. doi:10.1016/j.cmet.2015.02.006
18. Xu E, Pereira MMA, Karakasilioti I, Theurich S, Al-Maarri M, Rappl G, et al. Temporal and tissue-specific requirements for T-lymphocyte IL-6 signalling in obesity-associated inflammation and insulin resistance. *Nat Commun* (2017) 8:14803. doi:10.1038/ncomms14803
19. Friedman JM. The function of leptin in nutrition, weight, and physiology. *Nutr Rev* (2002) 60(10 Pt 2):S1–14; discussion S68–84, 5–7. doi:10.1301/002966402320634878
20. Naylor C, Petri WA Jr. Leptin regulation of immune responses. *Trends Mol Med* (2016) 22(2):88–98. doi:10.1016/j.molmed.2015.12.001
21. Gainsford T, Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci U S A* (1996) 93(25):14564–8. doi:10.1073/pnas.93.25.14564
22. Mandel MA, Mahmoud AA. Impairment of cell-mediated immunity in mutation diabetic mice (db/db). *J Immunol* (1978) 120(4):1375–7.
23. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. *FASEB J* (1998) 12(1):57–65. doi:10.1096/fasebj.12.1.57
24. Cioffi JA, Shafer AW, Zupancic TJ, Smith-Gbur J, Mikhail A, Platika D, et al. Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* (1996) 2(5):585–9. doi:10.1038/nm0596-585
25. Procaccini C, Jirillo E, Matarese G. Leptin as an immunomodulator. *Mol Aspects Med* (2012) 33(1):35–45. doi:10.1016/j.mam.2011.10.012
26. Saucillo DC, Gerriets VA, Sheng J, Rathmell JC, Maciver NJ. Leptin metabolically licenses T cells for activation to link nutrition and immunity. *J Immunol* (2014) 192(1):136–44. doi:10.4049/jimmunol.1301158
27. Gerriets VA, Danzaki K, Kishton RJ, Eisner W, Nichols AG, Saucillo DC, et al. Leptin directly promotes T-cell glycolytic metabolism to drive effector T-cell differentiation in a mouse model of autoimmunity. *Eur J Immunol* (2016) 46(8):1970–83. doi:10.1002/eji.201545861
28. van Stijn CM, Kim J, Lusis AJ, Barish GD, Tangirala RK. Macrophage polarization phenotype regulates adiponectin receptor expression and adiponectin anti-inflammatory response. *FASEB J* (2015) 29(2):636–49. doi:10.1096/fj.14-253831
29. Luo Y, Liu M. Adiponectin: a versatile player of innate immunity. *J Mol Cell Biol* (2016) 8(2):120–8. doi:10.1093/jmcb/mjw012
30. Al-Suhaimi EA, Shehzad A. Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. *Eur J Med Res* (2013) 18(1):12. doi:10.1186/2047-783X-18-12
31. Dostálová I, Sedlácková D, Papezova H, Nedvídková J, Haluzik M. Serum visfatin levels in patients with anorexia nervosa and bulimia nervosa. *Physiol Res* (2009) 58:903–7.
32. Azevedo ZMA, Luz RA, Victal SH, Kurdian B, Fonseca VM, Fitting C, et al. Increased production of tumor necrosis factor-a in whole blood cultures from children with primary malnutrition. *Braz J Med Biol Res* (2005) 38:171–83. doi:10.1590/S0100-879X2005000200005
33. Fock RA, Vinolo MAR, de Moura Sá Rocha V, de Sá Rocha LC, Borelli P. Protein-energy malnutrition decreases the expression of TLR-4/MD-2 and CD14 receptors in peritoneal macrophages and reduces the synthesis of TNF-α in response to lipopolysaccharide (LPS) in mice. *Cytokine* (2007) 40(2):105–14. doi:10.1016/j.cyto.2007.08.007
34. Kalliolias GD, Ivashkiv LB. TNF biology, pathogenic mechanisms and emerging therapeutic strategies. *Nat Rev Rheumatol* (2016) 12(1):49–62. doi:10.1038/nrrheum.2015.169
35. Bing C. Is interleukin-1β a culprit in macrophage-adipocyte crosstalk in obesity? *Adipocyte* (2015) 4(2):149–52. doi:10.4161/21623945.2014.979661
36. Muñoz C, Arévalo M, López M, Schlesinger L. Impaired interleukin-1 and tumor necrosis factor production in protein-calorie malnutrition. *Nutr Res* (1994) 14(3):347–52. doi:10.1016/S0271-5317(05)80173-3
37. Eder K, Baffy N, Falus A, Fulop AK. The major inflammatory mediator interleukin-6 and obesity. *Inflamm Res* (2009) 58(11):727. doi:10.1007/s00011-009-0060-4
38. Straczkowski M, Dzienis-Straczkowska S, Stêpień A, Kowalska I, Szelachowska M, Kinalska I. Plasma interleukin-8 concentrations are increased in obese subjects and related to fat mass and tumor necrosis factor-α system. *J Clin Endocrinol Metab* (2002) 87(10):4602–6. doi:10.1210/jc.2002-020135
39. Kim CS, Park HS, Kawada T, Kim JH, Lim D, Hubbard NE, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. *Int J Obes* (2006) 30:1347. doi:10.1038/sj.ijo.0803259
40. Makki K, Froguel P, Wolowczuk I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. *ISRN Inflamm* (2013) 2013:139239. doi:10.1155/2013/139239
41. Calcaterra V, De Amici M, Klersy C, Torre C, Brizzi V, Scaglia F, et al. Adiponectin, IL-10 and metabolic syndrome in obese children and adolescents. *Acta Biomed* (2009) 80:117–23.
42. Esposito K, Pontillo A, Giugliano F, Giugliano G, Marfella R, Nicoletti G, et al. Association of low interleukin-10 levels with the metabolic syndrome in obese women. *J Clin Endocrinol Metab* (2003) 88(3):1055–8. doi:10.1210/jc.2002-021437
43. Mello AS, de Oliveira DC, Bizzarro B, Sá-Nunes A, Hastreiter AA, de Oliveira Beltran JS, et al. Protein malnutrition alters spleen cell proliferation and IL-2 and IL-10 production by affecting the STAT-1 and STAT-3 balance. *Inflammation* (2014) 37(6):2125–38. doi:10.1007/s10753-014-9947-5
44. Hasan A, Al-Ghimlas F, Warsame S, Al-Hubail A, Ahmad R, Bennakhi A, et al. IL-33 is negatively associated with the BMI and confers a protective lipid/metabolic profile in non-diabetic but not diabetic subjects. *BMC Immunol* (2014) 15(1):19. doi:10.1186/1471-2172-15-19
45. Ragusa R, Cabiati M, Guzzardi MA, D'Amico A, Giannessi D, Del Ry S, et al. Effects of obesity on IL-33/ST2 system in heart, adipose tissue and liver: study in the experimental model of Zucker rats. *Exp Mol Pathol* (2017) 102(2):354–9. doi:10.1016/j.yexmp.2017.03.002
46. Jung C, Gerdes N, Fritzenwanger M, Figulla HR. Circulating levels of interleukin-1 family cytokines in overweight adolescents. *Mediators Inflamm* (2010) 2010:6. doi:10.1155/2010/958403
47. Meier CA, Bobbioni E, Gabay C, Assimacopoulos-Jeannet F, Golay A, Dayer J-M. IL-1 receptor antagonist serum levels are increased in human obesity: a possible link to the resistance to leptin? *J Clin Endocrinol Metab* (2002) 87(3):1184–8. doi:10.1210/jcem.87.3.8351
48. Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* (2004) 323(2):630–5. doi:10.1016/j.bbrc.2004.08.145
49. Bartz S, Mody A, Hornik C, Bain J, Muehlbauer M, Kiyimba T, et al. Severe acute malnutrition in childhood: hormonal and metabolic status at presentation, response to treatment, and predictors of mortality. *J Clin Endocrinol Metab* (2014) 99(6):2128–37. doi:10.1210/jc.2013-4018
50. Hambor JE, Fleck L, Stevenson JR. Impairment of macrophage migration inhibitory factor synthesis and macrophage migration in protein-malnourished mice. *Cell Immunol* (1983) 81(2):306–12. doi:10.1016/0008-8749(83)90238-1

51. Surmi BK, Hasty AH. Macrophage infiltration into adipose tissue: initiation, propagation and remodeling. *Future Lipidol* (2008) 3(5):545–56. doi:10.2217/17460875.3.5.545
52. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112(12):1796–808. doi:10.1172/JCI200319246
53. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) 116(6):1494–505. doi:10.1172/JCI26498
54. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* (2009) 15(8):914–20. doi:10.1038/nm.1964
55. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) 117(1):175–84. doi:10.1172/JCI29881
56. Huh JY, Park YJ, Ham M, Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue inflammation and metabolic dysregulation in obesity. *Mol Cells* (2014) 37(5):365–71. doi:10.14348/molcells.2014.0074
57. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* (2011) 332(6026):243–7. doi:10.1126/science.1201475
58. Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelson SE, et al. PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* (2012) 486(7404):549–53. doi:10.1038/nature11132
59. Lund FE. Cytokine-producing B lymphocytes-key regulators of immunity. *Curr Opin Immunol* (2008) 20(3):332–8. doi:10.1016/j.coi.2008.03.003
60. Lynch L, O'Shea D, Winter DC, Geoghegan J, Doherty DG, O'Farrelly C. Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur J Immunol* (2009) 39(7):1893–901. doi:10.1002/eji.200939349
61. Huh JY, Kim JI, Park YJ, Hwang IJ, Lee YS, Sohn JH, et al. A novel function of adipocytes in lipid antigen presentation to iNKT cells. *Mol Cell Biol* (2013) 33(2):328–39. doi:10.1128/MCB.00552-12
62. Lynch L, Nowak M, Varghese B, Clark J, Hogan AE, Toxavidis V, et al. Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* (2012) 37(3):574–87. doi:10.1016/j.immuni.2012.06.016
63. Gerriets VA, MacIver NJ. Role of T cells in malnutrition and obesity. *Front Immunol* (2014) 5:379. doi:10.3389/fimmu.2014.00379
64. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* (2009) 15(8):921–9. doi:10.1038/nm.2001
65. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* (2009) 15(8):930–9. doi:10.1038/nm.2002
66. Kolodin D, van Panhuys N, Li C, Magnuson AM, Cipolletta D, Miller CM, et al. Antigen-and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. *Cell Metab* (2015) 21(4):543–57. doi:10.1016/j.cmet.2015.03.005
67. Panduro M, Benoist C, Mathis D. Tissue Tregs. *Annu Rev Immunol* (2016) 34:609–33. doi:10.1146/annurev-immunol-032712-095948
68. Cipolletta D, Cohen P, Spiegelman BM, Benoist C, Mathis D. Appearance and disappearance of the mRNA signature characteristic of Treg cells in visceral adipose tissue: age, diet, and PPARgamma effects. *Proc Natl Acad Sci U S A* (2015) 112(2):482–7. doi:10.1073/pnas.1423486112
69. Bapat SP, Myoung Suh J, Fang S, Liu S, Zhang Y, Cheng A, et al. Depletion of fat-resident Treg cells prevents age-associated insulin resistance. *Nature* (2015) 528(7580):137–41. doi:10.1038/nature16151
70. Howard JK, Lord GM, Matarese G, Vendetti S, Ghatei MA, Ritter MA, et al. Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. *J Clin Invest* (1999) 104(8):1051–9. doi:10.1172/JCI6762
71. Procaccini C, De Rosa V, Galgani M, Carbone F, Cassano S, Greco D, et al. Leptin-induced mTOR activation defines a specific molecular and transcriptional signature controlling CD4+ effector T cell responses. *J Immunol* (2012) 189(6):2941–53. doi:10.4049/jimmunol.1200935
72. Pena-Cruz V, Reiss CS, McIntosh K. Sendai virus infection of mice with protein malnutrition. *J Virol* (1989) 63(8):3541–4.
73. Taylor AK, Cao W, Vora KP, De La Cruz J, Shieh WJ, Zaki SR, et al. Protein energy malnutrition decreases immunity and increases susceptibility to influenza infection in mice. *J Infect Dis* (2013) 207(3):501–10. doi:10.1093/infdis/jis527
74. Najera O, Gonzalez C, Toledo G, Lopez L, Ortiz R. Flow cytometry study of lymphocyte subsets in malnourished and well-nourished children with bacterial infections. *Clin Diagn Lab Immunol* (2004) 11(3):577–80. doi:10.1128/CDLI.11.3.577-580.2004
75. Savino W, Dardenne M, Velloso LA, Dayse Silva-Barbosa S. The thymus is a common target in malnutrition and infection. *Br J Nutr* (2007) 98(Suppl 1):S11–6. doi:10.1017/S0007114507832880
76. Saha S, Shalova IN, Biswas SK. Metabolic regulation of macrophage phenotype and function. *Immunol Rev* (2017) 280(1):102–11. doi:10.1111/imr.12603
77. Boothby M, Rickert RC. Metabolic regulation of the immune humoral response. *Immunity* (2017) 46(5):743–55. doi:10.1016/j.immuni.2017.04.009
78. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) 31:259–83. doi:10.1146/annurev-immunol-032712-095956
79. Yusuf I, Fruman DA. Regulation of quiescence in lymphocytes. *Trends Immunol* (2003) 24(7):380–6. doi:10.1016/S1471-4906(03)00141-8
80. Rathmell JC, Vander Heiden MG, Harris MH, Frauwirth KA, Thompson CB. In the absence of extrinsic signals, nutrient utilization by lymphocytes is insufficient to maintain either cell size or viability. *Mol Cell* (2000) 6(3):683–92. doi:10.1016/S1097-2765(00)00066-6
81. Jameson SC. Maintaining the norm: T-cell homeostasis. *Nat Rev Immunol* (2002) 2(8):547–56. doi:10.1038/nri853
82. Warburg O. On the origin of cancer cells. *Science* (1956) 123(3191):309–14. doi:10.1126/science.123.3191.309
83. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? *Trends Biochem Sci* (2016) 41(3):211–8. doi:10.1016/j.tibs.2015.12.001
84. Bental M, Deutsch C. Metabolic changes in activated T cells: an NMR study of human peripheral blood lymphocytes. *Magn Reson Med* (1993) 29(3):317–26. doi:10.1002/mrm.1910290307
85. Greiner EF, Guppy M, Brand K. Glucose is essential for proliferation and the glycolytic enzyme-induction that provokes a transition to glycolytic energy-production. *J Biol Chem* (1994) 269(50):31484–90.
86. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* (2002) 16(6):769–77. doi:10.1016/S1074-7613(02)00323-0
87. Keating R, McGargill MA. mTOR regulation of lymphoid cells in immunity to pathogens. *Front Immunol* (2016) 7:180. doi:10.3389/fimmu.2016.00180
88. Chi H. Regulation and function of mTOR signalling in T cell fate decisions. *Nat Rev Immunol* (2012) 12(5):325–38. doi:10.1038/nri3198
89. Powell JD, Delgoffe GM. The mammalian target of rapamycin: linking T cell differentiation, function, and metabolism. *Immunity* (2010) 33(3):301–11. doi:10.1016/j.immuni.2010.09.002
90. Kopf H, de la Rosa GM, Howard OM, Chen X. Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. *Int Immunopharmacol* (2007) 7(13):1819–24. doi:10.1016/j.intimp.2007.08.027
91. Zheng Y, Collins SL, Lutz MA, Allen AN, Kole TP, Zarek PE, et al. A role for mammalian target of rapamycin in regulating T cell activation versus anergy. *J Immunol* (2007) 178(4):2163–70. doi:10.4049/jimmunol.178.4.2163
92. MacIver NJ, Blagih J, Saucillo DC, Tonelli L, Griss T, Rathmell JC, et al. The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J Immunol* (2011) 187(8):4187–98. doi:10.4049/jimmunol.1100367
93. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. *Clin Cancer Res* (2009) 15(21):6479–83. doi:10.1158/1078-0432.CCR-09-0889
94. Widnell CC. Control of glucose transport by GLUT1: regulated secretion in an unexpected environment. *Biosci Rep* (1995) 15(6):427–43. doi:10.1007/BF01204347
95. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* (2011) 35(6):871–82. doi:10.1016/j.immuni.2011.09.021

96. Lindsten T, June CH, Thompson CB. Multiple mechanisms regulate c-Myc gene expression during normal T cell activation. *EMBO J* (1988) 7(9):2787–94.
97. Kelly K, Cochran BH, Stiles CD, Leder P. Cell-specific regulation of the c-Myc gene by lymphocyte mitogens and platelet-derived growth factor. *Cell* (1983) 35(3 Pt 2):603–10. doi:10.1016/0092-8674(83)90092-2
98. Park BV, Pan F. Metabolic regulation of T cell differentiation and function. *Mol Immunol* (2015) 68(2 Pt C):497–506. doi:10.1016/j.molimm.2015.07.027
99. Palmer CS, Ostrowski M, Balderson B, Christian N, Crowe SM. Glucose metabolism regulates T cell activation, differentiation, and functions. *Front Immunol* (2015) 6:1. doi:10.3389/fimmu.2015.00001
100. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* (2011) 186(6):3299–303. doi:10.4049/jimmunol.1003613
101. Michalek RD, Rathmell JC. The metabolic life and times of a T-cell. *Immunol Rev* (2010) 236(1):190–202. doi:10.1111/j.1600-065X.2010.00911.x
102. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* (2009) 460(7251):103–7. doi:10.1038/nature08097
103. Kishore M, Cheung KCP, Fu H, Bonacina F, Wang G, Coe D, et al. Regulatory T cell migration is dependent on glucokinase-mediated glycolysis. *Immunity* (2017) 47(5):875–89.e10. doi:10.1016/j.immuni.2017.10.017
104. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* (2009) 30(6):832–44. doi:10.1016/j.immuni.2009.04.014
105. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* (2011) 12(4):295–303. doi:10.1038/ni.2005
106. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* (1995) 1(11):1155–61. doi:10.1038/nm1195-1155
107. De Rosa V, Procaccini C, La Cava A, Chieffi P, Nicoletti GF, Fontana S, et al. Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis. *J Clin Invest* (2006) 116(2):447–55. doi:10.1172/JCI26523
108. Wang S, Baidoo SE, Liu Y, Zhu C, Tian J, Ma J, et al. T cell-derived leptin contributes to increased frequency of T helper type 17 cells in female patients with Hashimoto's thyroiditis. *Clin Exp Immunol* (2013) 171(1):63–8. doi:10.1111/j.1365-2249.2012.04670.x
109. Deng J, Liu Y, Yang M, Wang S, Zhang M, Wang X, et al. Leptin exacerbates collagen-induced arthritis *via* enhancement of Th17 cell response. *Arthritis Rheum* (2012) 64(11):3564–73. doi:10.1002/art.34637
110. Yu Y, Liu Y, Shi FD, Zou H, Matarese G, La Cava A. Cutting edge: leptin-induced RORgammat expression in CD4+ T cells promotes Th17 responses in systemic lupus erythematosus. *J Immunol* (2013) 190(7):3054–8. doi:10.4049/jimmunol.1203275
111. Shepherd PR, Kahn BB. Glucose transporters and insulin action—implications for insulin resistance and diabetes mellitus. *N Engl J Med* (1999) 341(4):248–57. doi:10.1056/NEJM199907223410406
112. Mandarino LJ, Printz RL, Cusi KA, Kinchington P, O'Doherty RM, Osawa H, et al. Regulation of hexokinase II and glycogen synthase mRNA, protein, and activity in human muscle. *Am J Physiol* (1995) 269(4 Pt 1):E701–8.
113. Dimitriadis G, Parry-Billings M, Bevan S, Leighton B, Krause U, Piva T, et al. The effects of insulin on transport and metabolism of glucose in skeletal muscle from hyperthyroid and hypothyroid rats. *Eur J Clin Invest* (1997) 27(6):475–83. doi:10.1046/j.1365-2362.1997.1380688.x
114. Dimitriadis G, Parry-Billings M, Bevan S, Dunger D, Piva T, Krause U, et al. Effects of insulin-like growth factor I on the rates of glucose transport and utilization in rat skeletal muscle *in vitro*. *Biochem J* (1992) 285(Pt 1):269–74. doi:10.1042/bj2850269
115. Dimitriadis G, Mitrou P, Lambadiari V, Maratou E, Raptis SA. Insulin effects in muscle and adipose tissue. *Diabetes Res Clin Pract* (2011) 93(Suppl 1):S52–9. doi:10.1016/S0168-8227(11)70014-6
116. Dimitriadis GD, Leighton B, Vlachonikolis IG, Parry-Billings M, Challiss RA, West D, et al. Effects of hyperthyroidism on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat. *Biochem J* (1988) 253(1):87–92. doi:10.1042/bj2530087
117. Helderman JH, Reynolds TC, Strom TB. The insulin receptor as a universal marker of activated lymphocytes. *Eur J Immunol* (1978) 8(8):589–95. doi:10.1002/eji.1830080810
118. Fischer HJ, Sie C, Schumann E, Witte AK, Dressel R, van den Brandt J, et al. The insulin receptor plays a critical role in T cell function and adaptive immunity. *J Immunol* (2017) 198(5):1910–20. doi:10.4049/jimmunol.1601011
119. Matarese G, Carrieri PB, La Cava A, Perna F, Sanna V, De Rosa V, et al. Leptin increase in multiple sclerosis associates with reduced number of CD4(+) CD25+ regulatory T cells. *Proc Natl Acad Sci U S A* (2005) 102(14):5150–5. doi:10.1073/pnas.0408995102
120. Kraszula L, Jasinska A, Eusebio M, Kuna P, Glabinski A, Pietruczuk M. Evaluation of the relationship between leptin, resistin, adiponectin and natural regulatory T cells in relapsing-remitting multiple sclerosis. *Neurol Neurochir Pol* (2012) 46(1):22–8. doi:10.5114/ninp.2012.27211
121. Carbone F, De Rosa V, Carrieri PB, Montella S, Bruzzese D, Porcellini A, et al. Regulatory T cell proliferative potential is impaired in human autoimmune disease. *Nat Med* (2014) 20(1):69–74. doi:10.1038/nm.3411
122. Hedstrom AK, Lima Bomfim I, Barcellos L, Gianfrancesco M, Schaefer C, Kockum I, et al. Interaction between adolescent obesity and HLA risk genes in the etiology of multiple sclerosis. *Neurology* (2014) 82(10):865–72. doi:10.1212/WNL.0000000000000203
123. Kavak KS, Teter BE, Hagemeier J, Zakalik K, Weinstock-Guttman B; New York State Multiple Sclerosis Consortium. Higher weight in adolescence and young adulthood is associated with an earlier age at multiple sclerosis onset. *Mult Scler* (2015) 21(7):858–65. doi:10.1177/1352458514555787
124. Gianfrancesco MA, Acuna B, Shen L, Briggs FB, Quach H, Bellesis KH, et al. Obesity during childhood and adolescence increases susceptibility to multiple sclerosis after accounting for established genetic and environmental risk factors. *Obes Res Clin Pract* (2014) 8(5):e435–47. doi:10.1016/j.orcp.2014.01.002
125. Langer-Gould A, Brara SM, Beaber BE, Koebnick C. Childhood obesity and risk of pediatric multiple sclerosis and clinically isolated syndrome. *Neurology* (2013) 80(6):548–52. doi:10.1212/WNL.0b013e31828154f3
126. Emamgholipour S, Eshaghi SM, Hossein-nezhad A, Mirzaei K, Maghbooli Z, Sahraian MA. Adipocytokine profile, cytokine levels and foxp3 expression in multiple sclerosis: a possible link to susceptibility and clinical course of disease. *PLoS One* (2013) 8(10):e76555. doi:10.1371/journal.pone.0076555
127. Frisullo G, Mirabella M, Angelucci F, Caggiula M, Morosetti R, Sancricca C, et al. The effect of disease activity on leptin, leptin receptor and suppressor of cytokine signalling-3 expression in relapsing-remitting multiple sclerosis. *J Neuroimmunol* (2007) 192(1–2):174–83. doi:10.1016/j.jneuroim.2007.08.008
128. Matarese G, Di Giacomo A, Sanna V, Lord GM, Howard JK, Di Tuoro A, et al. Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. *J Immunol* (2001) 166(10):5909–16. doi:10.4049/jimmunol.166.10.5909
129. Sanna V, Di Giacomo A, La Cava A, Lechler RI, Fontana S, Zappacosta S, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. *J Clin Invest* (2003) 111(2):241–50. doi:10.1172/JCI200316721
130. Matarese G, Sanna V, Di Giacomo A, Lord GM, Howard JK, Bloom SR, et al. Leptin potentiates experimental autoimmune encephalomyelitis in SJL female mice and confers susceptibility to males. *Eur J Immunol* (2001) 31(5):1324–32. doi:10.1002/1521-4141(200105)31:5<1324::AID-IMMU1324>3.0.CO;2-Y
131. Galgani M, Procaccini C, De Rosa V, Carbone F, Chieffi P, La Cava A, et al. Leptin modulates the survival of autoreactive CD4+ T cells through the nutrient/energy-sensing mammalian target of rapamycin signaling pathway. *J Immunol* (2010) 185(12):7474–9. doi:10.4049/jimmunol.1001674
132. Piccio L, Stark JL, Cross AH. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J Leukoc Biol* (2008) 84(4):940–8. doi:10.1189/jlb.0208133
133. Kafami L, Raza M, Razavi A, Mirshafiey A, Movahedian M, Khorramizadeh MR. Intermittent feeding attenuates clinical course of experimental autoimmune encephalomyelitis in C57BL/6 mice. *Avicenna J Med Biotechnol* (2010) 2(1):47–52.
134. Lourenco EV, Liu A, Matarese G, La Cava A. Leptin promotes systemic lupus erythematosus by increasing autoantibody production and inhibiting

immune regulation. *Proc Natl Acad Sci U S A* (2016) 113(38):10637–42. doi:10.1073/pnas.1607101113
135. Wang X, Qiao Y, Yang L, Song S, Han Y, Tian Y, et al. Leptin levels in patients with systemic lupus erythematosus inversely correlate with regulatory T cell frequency. *Lupus* (2017) 26(13):1401–6. doi:10.1177/0961203317703497
136. Moraes-Vieira PM, Bassi EJ, Larocca RA, Castoldi A, Burghos M, Lepique AP, et al. Leptin deficiency modulates allograft survival by favoring a Th2 and a regulatory immune profile. [corrected]. *Am J Transplant* (2013) 13(1):36–44. doi:10.1111/j.1600-6143.2012.04283.x
137. Prentice AM. The thymus: a barometer of malnutrition. *Br J Nutr* (1999) 81(5):345–7.
138. Rytter MJ, Kolte L, Briend A, Friis H, Christensen VB. The immune system in children with malnutrition—a systematic review. *PLoS One* (2014) 9(8):e105017. doi:10.1371/journal.pone.0105017
139. Ortiz R, Cortes L, Cortes E, Medina H. Malnutrition alters the rates of apoptosis in splenocytes and thymocyte subpopulations of rats. *Clin Exp Immunol* (2009) 155(1):96–106. doi:10.1111/j.1365-2249.2008.03796.x
140. Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int J Tuberc Lung Dis* (2004) 8(3):286–98.
141. Verhagen LM, Gomez-Castellano K, Snelders E, Rivera-Olivero I, Pocaterra L, Melchers WJ, et al. Respiratory infections in Enepa Amerindians are related to malnutrition and *Streptococcus pneumoniae* carriage. *J Infect* (2013) 67(4):273–81. doi:10.1016/j.jinf.2013.06.010
142. Ritz BW, Aktan I, Nogusa S, Gardner EM. Energy restriction impairs natural killer cell function and increases the severity of influenza infection in young adult male C57BL/6 mice. *J Nutr* (2008) 138(11):2269–75. doi:10.3945/jn.108.093633
143. Petri WA Jr, Mondal D, Peterson KM, Duggal P, Haque R. Association of malnutrition with amebiasis. *Nutr Rev* (2009) 67(Suppl 2):S207–15. doi:10.1111/j.1753-4887.2009.00242.x
144. Huttunen R, Syrjanen J. Obesity and the risk and outcome of infection. *Int J Obes (Lond)* (2013) 37(3):333–40. doi:10.1038/ijo.2012.62
145. Van Kerkhove MD, Vandemaele KA, Shinde V, Jaramillo-Gutierrez G, Koukounari A, Donnelly CA, et al. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. *PLoS Med* (2011) 8(7):e1001053. doi:10.1371/journal.pmed.1001053
146. Louie JK, Acosta M, Samuel MC, Schechter R, Vugia DJ, Harriman K, et al. A novel risk factor for a novel virus: obesity and 2009 pandemic influenza A (H1N1). *Clin Infect Dis* (2011) 52(3):301–12. doi:10.1093/cid/ciq152
147. Diaz E, Rodriguez A, Martin-Loeches I, Lorente L, Del Mar Martin M, Pozo JC, et al. Impact of obesity in patients infected with 2009 influenza A(H1N1). *Chest* (2011) 139(2):382–6. doi:10.1378/chest.10-1160
148. Riquelme R, Jimenez P, Videla AJ, Lopez H, Chalmers J, Singanayagam A, et al. Predicting mortality in hospitalized patients with 2009 H1N1 influenza pneumonia. *Int J Tuberc Lung Dis* (2011) 15(4):542–6. doi:10.5588/ijtld.10.0539
149. Choban PS, Flancbaum L. The impact of obesity on surgical outcomes: a review. *J Am Coll Surg* (1997) 185(6):593–603. doi:10.1016/S1072-7515(97)00109-9
150. Arslan E, Atilgan H, Yavasoglu I. The prevalence of *Helicobacter pylori* in obese subjects. *Eur J Intern Med* (2009) 20(7):695–7. doi:10.1016/j.ejim.2009.07.013
151. Uberos J, Molina-Carballo A, Fernandez-Puentes V, Rodriguez-Belmonte R, Munoz-Hoyos A. Overweight and obesity as risk factors for the asymptomatic carrier state of *Neisseria meningitidis* among a paediatric population. *Eur J Clin Microbiol Infect Dis* (2010) 29(3):333–4. doi:10.1007/s10096-009-0849-7
152. Hemalatha Rajkumar PB. The impact of obesity on immune response to infection and vaccine: an insight into plausible mechanisms. *Endocrinol Metab Synd* (2013) 2(2):113. doi:10.4172/2161-1017.1000113
153. Karlsson EA, Sheridan PA, Beck MA. Diet-induced obesity impairs the T cell memory response to influenza virus infection. *J Immunol* (2010) 184(6):3127–33. doi:10.4049/jimmunol.0903220
154. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, et al. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem* (2014) 289(11):7884–96. doi:10.1074/jbc.M113.522037
155. Blair D, Dufort FJ, Chiles TC. Protein kinase Cbeta is critical for the metabolic switch to glycolysis following B-cell antigen receptor engagement. *Biochem J* (2012) 448(1):165–9. doi:10.1042/BJ20121225
156. Cao Y, Rathmell JC, Macintyre AN. Metabolic reprogramming towards aerobic glycolysis correlates with greater proliferative ability and resistance to metabolic inhibition in CD8 versus CD4 T cells. *PLoS One* (2014) 9(8):e104104. doi:10.1371/journal.pone.0104104
157. Zhao Q, Chu Z, Zhu L, Yang T, Wang P, Liu F, et al. 2-Deoxy-d-glucose treatment decreases anti-inflammatory M2 macrophage polarization in mice with tumor and allergic airway inflammation. *Front Immunol* (2017) 8:637. doi:10.3389/fimmu.2017.00637
158. Raez LE, Papadopoulos K, Ricart AD, Chiorean EG, Dipaola RS, Stein MN, et al. A phase I dose-escalation trial of 2-deoxy-D-glucose alone or combined with docetaxel in patients with advanced solid tumors. *Cancer Chemother Pharmacol* (2013) 71(2):523–30. doi:10.1007/s00280-012-2045-1
159. Stein M, Lin H, Jeyamohan C, Dvorzhinski D, Gounder M, Bray K, et al. Targeting tumor metabolism with 2-deoxyglucose in patients with castrate-resistant prostate cancer and advanced malignancies. *Prostate* (2010) 70(13):1388–94. doi:10.1002/pros.21172
160. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20(1):61–72. doi:10.1016/j.cmet.2014.05.004
161. Caro-Maldonado A, Wang R, Nichols AG, Kuraoka M, Milasta S, Sun LD, et al. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. *J Immunol* (2014) 192(8):3626–36. doi:10.4049/jimmunol.1302062
162. Dror E, Dalmas E, Meier DT, Wueest S, Thevenet J, Thienel C, et al. Postprandial macrophage-derived IL-1beta stimulates insulin, and both synergistically promote glucose disposal and inflammation. *Nat Immunol* (2017) 18(3):283–92. doi:10.1038/ni.3659
163. Ung PM, Song W, Cheng L, Zhao X, Hu H, Chen L, et al. Inhibitor discovery for the human GLUT1 from homology modeling and virtual screening. *ACS Chem Biol* (2016) 11(7):1908–16. doi:10.1021/acschembio.6b00304
164. Siebeneicher H, Cleve A, Rehwinkel H, Neuhaus R, Heisler I, Muller T, et al. Identification and optimization of the first highly selective GLUT1 inhibitor BAY-876. *ChemMedChem* (2016) 11(20):2261–71. doi:10.1002/cmdc.201600276
165. Liu Y, Cao Y, Zhang W, Bergmeier S, Qian Y, Akbar H, et al. A small-molecule inhibitor of glucose transporter 1 downregulates glycolysis, induces cell-cycle arrest, and inhibits cancer cell growth *in vitro* and *in vivo*. *Mol Cancer Ther* (2012) 11(8):1672–82. doi:10.1158/1535-7163.MCT-12-0131
166. De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI. Defective glucose transport across the blood–brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. *N Engl J Med* (1991) 325(10):703–9. doi:10.1056/NEJM199109053251006
167. Brockmann K, Wang D, Korenke CG, von Moers A, Ho YY, Pascual JM, et al. Autosomal dominant glut-1 deficiency syndrome and familial epilepsy. *Ann Neurol* (2001) 50(4):476–85. doi:10.1002/ana.1222
168. Van Schaftingen E, Jett MF, Hue L, Hers HG. Control of liver 6-phosphofructokinase by fructose 2,6-bisphosphate and other effectors. *Proc Natl Acad Sci U S A* (1981) 78(6):3483–6. doi:10.1073/pnas.78.6.3483
169. Yalcin A, Telang S, Clem B, Chesney J. Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer. *Exp Mol Pathol* (2009) 86(3):174–9. doi:10.1016/j.yexmp.2009.01.003
170. Rodriguez-Prados JC, Traves PG, Cuenca J, Rico D, Aragones J, Martin-Sanz P, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol* (2010) 185(1):605–14. doi:10.4049/jimmunol.0901698
171. Telang S, Clem BF, Klarer AC, Clem AL, Trent JO, Bucala R, et al. Small molecule inhibition of 6-phosphofructo-2-kinase suppresses t cell activation. *J Transl Med* (2012) 10:95. doi:10.1186/1479-5876-10-95
172. Clem BF, O'Neal J, Tapolsky G, Clem AL, Imbert-Fernandez Y, Kerr DA II, et al. Targeting 6-phosphofructo-2-kinase (PFKFB3) as a therapeutic strategy against cancer. *Mol Cancer Ther* (2013) 12(8):1461–70. doi:10.1158/1535-7163.MCT-13-0097
173. Tom A, Nair KS. Assessment of branched-chain amino acid status and potential for biomarkers. *J Nutr* (2006) 136(1 Suppl):324S–30S. doi:10.1093/jn/136.1.324S
174. Sweatt AJ, Wood M, Suryawan A, Wallin R, Willingham MC, Hutson SM. Branched-chain amino acid catabolism: unique segregation of pathway

enzymes in organ systems and peripheral nerves. *Am J Physiol Endocrinol Metab* (2004) 286(1):E64–76. doi:10.1152/ajpendo.00276.2003

175. Papathanassiu AE, Ko JH, Imprialou M, Bagnati M, Srivastava PK, Vu HA, et al. BCAT1 controls metabolic reprogramming in activated human macrophages and is associated with inflammatory diseases. *Nat Commun* (2017) 8:16040. doi:10.1038/ncomms16040
176. Benke PJ, Drisko J, Ahmad P. Increased oxidative metabolism in phytohemagglutinin-stimulated lymphocytes from patients with systemic lupus erythematosus is associated with serum SSA antibody. *Biochem Med Metab Biol* (1991) 45(1):28–40. doi:10.1016/0885-4505(91)90005-6
177. Kuhnke A, Burmester GR, Krauss S, Buttgereit F. Bioenergetics of immune cells to assess rheumatic disease activity and efficacy of glucocorticoid treatment. *Ann Rheum Dis* (2003) 62(2):133–9. doi:10.1136/ard.62.2.133
178. Wahl DR, Petersen B, Warner R, Richardson BC, Glick GD, Opipari AW. Characterization of the metabolic phenotype of chronically activated lymphocytes. *Lupus* (2010) 19(13):1492–501. doi:10.1177/0961203310373109
179. Johnson KM, Chen X, Boitano A, Swenson L, Opipari AW Jr, Glick GD. Identification and validation of the mitochondrial F1F0-ATPase as the molecular target of the immunomodulatory benzodiazepine Bz-423. *Chem Biol* (2005) 12(4):485–96. doi:10.1016/j.chembiol.2005.02.012
180. Bednarski JJ, Warner RE, Rao T, Leonetti F, Yung R, Richardson BC, et al. Attenuation of autoimmune disease in Fas-deficient mice by treatment with a cytotoxic benzodiazepine. *Arthritis Rheum* (2003) 48(3):757–66. doi:10.1002/art.10968
181. Blatt NB, Bednarski JJ, Warner RE, Leonetti F, Johnson KM, Boitano A, et al. Benzodiazepine-induced superoxide signals B cell apoptosis: mechanistic insight and potential therapeutic utility. *J Clin Invest* (2002) 110(8):1123–32. doi:10.1172/JCI0216029
182. Gatza E, Wahl DR, Opipari AW, Sundberg TB, Reddy P, Liu C, et al. Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft-versus-host disease. *Sci Transl Med* (2011) 3(67):67ra8. doi:10.1126/scitranslmed.3001975

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 01 March 2018
doi: 10.3389/fimmu.2018.00352

Serial Monitoring of Immune Markers Being Represented Regulatory T Cell/T Helper 17 Cell Ratio: Indicating Tolerance for Tapering Immunosuppression after Liver Transplantation

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Recipients of liver transplantation (LT) require long-term immunosuppressive drug treatment, but lifelong immunosuppressive treatment has severe side effects. It is known that some LT recipients develop immune tolerance, and although the development of such operational tolerance should allow a decrease in the burden of immunosuppressive drug treatment, the factors that indicate operational tolerance are not clear. This study aimed to monitor immunological markers over time in LT recipients to identify those markers indicating the development of operational tolerance. We performed a prospective pilot study measuring immune markers, including the ratio of regulatory T (Treg) and T helper (Th) 17 cells in peripheral blood in the 14 most immunologically stable patients among 70 clinically stable LT recipients. The doses of immunosuppressive drugs given to these 14 LT recipients were tapered over time and they were monitored for immunological markers related to the development of immune tolerance. As the doses of immunosuppressive drugs were reduced, the Treg/Th17, Th1/Th17, and CD8/Th17 ratio in tolerant recipients was significantly increased compared with that of nontolerant recipients. These results suggest that monitoring of changes in the immune makers, including Treg/Th17 ratio during tapering of immunosuppression may allow prediction of the development of tolerance.

Keywords: regulatory T cell, T helper 17 cell, liver transplantation, tolerance, immunosuppression

OPEN ACCESS

Edited by:
Duncan Howie, University of Oxford, United Kingdom

Reviewed by:
Alberto Baroja-Mazo, Instituto Murciano de Investigación Biosanitaria, Spain
Nick David Jones, University of Birmingham, United Kingdom

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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *23 November 2017*
Accepted: *07 February 2018*
Published: *01 March 2018*

Citation:
Jhun J, Lee SH, Lee SK, Kim HY, Jung ES, Kim DG, Choi J, Bae SH, Yoon SK, Chung BH, Yang CW, Cho M-L and Choi JY (2018) Serial Monitoring of Immune Markers Being Represented Regulatory T Cell/T Helper 17 Cell Ratio: Indicating Tolerance for Tapering Immunosuppression after Liver Transplantation. Front. Immunol. 9:352. doi: 10.3389/fimmu.2018.00352

Abbreviations: Treg, regulatory T cell; Th17, T helper 17 cell; LT, liver transplantation; IL, interleukin; HBV, hepatitis B virus; HCV, hepatitis C virus.

INTRODUCTION

Liver transplantation (LT) has a lower rate of rejection than transplantation of other solid organs. Indeed, operational tolerance has been reported to occur in approximately 5% of LT recipients, with the most optimistic study reporting up to 50% (1–3). Although there have been many studies of immune tolerance after LT that have used different inclusion criteria to identify clinically stable patients (4–6), there is no clinically useful indicator that predicts tolerance. A recent study suggested that immune tolerance after LT is associated with time after surgery (>10 years) (7). Because LT recipients are given long-term immunosuppression, it is likely that undesirable side effects of immunosuppressive drug treatment occur in LT recipients who may actually be tolerant. Thus, there is a requirement to define immune markers in peripheral blood that can identify those LT recipients who will develop tolerance, especially during tapering of immunosuppressive drug treatment.

Immune monitoring after LT using biomarkers is an important method for identifying rejection and immune tolerance. There is evidence that the levels of inflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-6 are biomarkers of rejection after LT (8, 9). Since, T helper 17 (Th17) cells cause chronic and excessive inflammation (10, 11), Th17 cells also play a critical role in the prognosis of LT. It is well known that Th17 cells induce liver allograft rejection in a rat model (12), while LT recipients experiencing acute rejection had significantly increased peripheral blood Th17 cells compared with LT recipients without rejection (13). The population of $CD4^+IL\text{-}17^+$ T helper (Th17) cells in peripheral blood has been reported to be increased in LT recipients experiencing rejection compared to those without rejection (13). Moreover, the differentiation of Th17 cells in peripheral blood was correlated positively with the histological score of liver tissue in LT recipients experiencing rejection (14). In contrast, T regulatory (Treg) cells have immunosuppressive activity and can reduce inflammatory responses (15). Numerous studies have demonstrated the anti-inflammatory function of Treg cells and their ability to enhance LT tolerance (16–18). It is also well documented that the levels of circulating $CD4^+CD25^{high}FoxP3^+$ Treg cells were downregulated in LT patients undergoing rejection and were negatively correlated with rejection severity (19). Indeed, $CD4^+CD25^{high}$ Treg cells perform a significant role in maintaining tolerance (20).

Previously, we demonstrated an imbalance between Th17 and Treg cells in peripheral blood mononuclear cells (PBMCs) from LT recipients (21); immunosuppressive treatment had no significant effect on Th17 cells, but significantly reduced the frequency of Treg cells. Therefore, we designed this study to undertake long-term monitoring of LT recipients. Based on the overall function of Th17 and Treg cells in LT and in immune inflammatory responses, we hypothesized that the frequencies of Th17 and Treg cells in the peripheral blood of LT recipients could be an indicator of LT prognosis. We sought to demonstrate whether the Treg/Th17 ratio in peripheral blood of LT recipients was positively correlated with the development of tolerance and whether it could be an effective diagnostic marker of LT tolerance.

PATIENTS AND METHODS

Patients

For this study, patients were prospectively enrolled from a single LT clinic at Seoul St. Mary's Hospital. The inclusion criteria were: liver transplanted more than 3 years ago; a high level of liver function with no history of rejection; no history of significant change in the dosage of immunosuppressive drugs for at least 1 year; age ≥20 years and ≤65 years; Eastern Cooperative Oncology Group performance score 0–1; Child–Pugh score A; and patients agreed to participate. Exclusion criteria were: hepatitis C virus positive; a history of biliary complications or infection; other severe medical comorbidities; or a history of malignancy. Patients who met the inclusion criteria were examined for immunologic markers, including Treg cells (CD4, CD25, and Foxp3), Th17 cells (CD4, IL-17), and various cytokines [IL-4, IL-10, IL-17, IL-33, and interferon (IFN)-γ]. The 20% most immunologically stable patients were finally included as the tapering group. Informed and written consent was provided by all patients and the study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC11SISI0340).

Tapering Protocol

Schematic timeline of tapering immunosuppressant is shown in **Figure 1**. Enrolled patients were followed up every 3 months if possible. At every visit, laboratory tests including those for liver function, routine blood counts, and immunologic markers were performed. The doses of immunosuppressive agents were reduced by 25–30% every 6 months until they reached half the recommended dose. From half-dose to discontinuation, the doses were tapered more cautiously and slowly by 10–12.5% every 6 months depending on the patient's clinical status. The target time to discontinuation of immunosuppressive drug treatment was 30–36 months. After 1 year from stopping immunosuppression, liver biopsy was performed in consented tolerant patients.

Definition of Nontolerance and Operational Tolerance

During tapering of the doses of immunosuppressive drugs, an increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels to greater than or equal to twice the upper baseline value without any other cause, such as infection, drugs, or biliary complications was considered to indicate nontolerance/rejection. If the patients agreed, a liver biopsy was performed to confirm nontolerance by histology. Operational

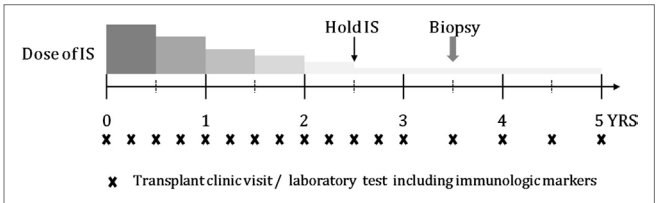

FIGURE 1 | Schematic timeline of tapering IS and follow-up, including transplant clinic visit and laboratory test.

tolerance was defined as having continuously stable liver function tests for 12 months after discontinuation of immunosuppressive drugs.

PBMC Isolation and Flow Cytometric Analysis

Peripheral blood mononuclear cells were isolated from heparinized venous blood by standard density gradient centrifugation over Ficoll-Paque (GE Healthcare Biosciences, Uppsala, Sweden). Cell cultures were performed in RPMI-1640 medium (Gibco BRL, Carlsbad, CA, USA) containing penicillin (100 U/mL), streptomycin (100 μg/mL), and 10% fetal bovine serum (Gibco BRL) that had been inactivated by heating to 55°C for 30 min. The cell suspensions were dispensed into 48-well plates (Nunc, Roskilde, Denmark).

Preparation of PBMCs for flow cytometric analysis was completed within 1 h after the sampling of peripheral blood. For analysis of intracellular cytokine production, PBMCs were stimulated with 50 ng/mL phorbol myristate acetate (Sigma Aldrich, St. Louis, MO, USA), 500 ng/mL ionomycin (Sigma Aldrich), and GolgiStop (BD Biosciences, San Diego, CA, USA) for 4 h. The cells were then washed and 5×10^5 cells per sample were incubated with antibodies against surface markers for 30 min at 4°C in the dark. The cells were then permeabilized using a Cytofix/Cytoperm Plus kit (BD Biosciences) and stained with antibodies specific for intracellular markers for 30 min at 4°C in the dark. For analysis of Treg cells, PBMCs were surface-labeled with phycoerythrin (PE)/cyanine 7-conjugated anti-CD4 (BioLegend, San Diego, CA, USA) and allophycocyanin (APC)-conjugated anti-CD25, followed by fixation, permeabilization, and intracellular staining with anti-Foxp3, performed using the eBioscience Foxp3 staining kit (eBioscience, San Diego, CA, USA). For intracellular staining, PE-conjugated anti-IL-17 (BD Biosciences), FITC-conjugated anti-IFN-γ (eBioscience), and APC-conjugated anti-IL-4 (BD Biosciences) were used. Appropriate isotype controls were used to set gates for analysis of cytokine expression. Cells were analyzed using a FACSCalibur flow cytometry system (BD Biosciences) and FlowJo software (Tree Star, Ashland, OR, USA).

Enzyme-Linked Immunosorbent Assays for Cytokines

In brief, a 96-well plate (Nunc) was coated with 4 μg/mL monoclonal antibody against IL-4, IL-10, IL-17, IL-21, IL-33, or IFN-γ (R&D Systems) at 4°C overnight. After blocking with phosphate-buffered saline (PBS)/1% bovine serum albumin/0.05% Tween 20 (PBS/Tween) for 2 h at room temperature (22–25°C), test samples and the standard recombinant IL-4, IL-10, IL-17, IL-21, IL-33, and IFN-γ were added to the 96-well plate and incubated for 2 h at room temperature. Plates were washed four times with PBS/Tween and then incubated with 500 ng/mL biotinylated mouse monoclonal antibodies against IL-4, IL-10, IL-17, IL-21, IL-33, and IFN-γ for 2 h at room temperature. After washing, streptavidin-alkaline phosphatase-horseradish peroxidase conjugate (Sigma) was added and the plate was incubated for 2 h. The plate was again washed and incubated with 1 mg/mL p-nitrophenyl phosphate (Sigma) dissolved in diethanolamine (Sigma) to develop the color reaction. The reaction was stopped by the addition of 1 M NaOH and the optical density of each well was read at 405 nm. The lower limit of detection for recombinant IL-4, IL-10, IL-17, IL-21, IL-33, and IFN-γ was 10 pg/mL. Recombinant human IL-4, IL-10, IL-17, IL-21, IL-33, and IFN-γ diluted in culture medium were used as the calibration standards at concentrations in the range of 10–2000 pg/mL. A standard curve was drawn by plotting optical density against the log of the concentration of recombinant cytokines and was used to calculate the IL-4, IL-10, IL-17, IL-21, IL-33, and IFN-γ concentrations in the test samples.

Hematoxylin–Eosin Staining and Immunohistochemistry

Liver needle biopsies were performed and the tissue was fixed in 4% (vol/vol) paraformaldehyde and embedded in paraffin. The sections were dewaxed using xylene and dehydrated in a gradient of alcohols. Liver tissue sections (5 μm thick) were stained with hematoxylin and eosin and monoclonal antibodies to human IL-17 (Abcam, Cambridge, UK) and Foxp3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Immunohistochemistry was performed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). Tissues were incubated first with the primary anti-IL-17 and anti-Foxp3 antibodies overnight at 4°C, followed by incubation with a biotinylated secondary linking Ab and a streptavidin-peroxidase complex for 1 h. The final color product was developed using diaminobenzidine chromogen (Dako, Carpinteria, CA, USA). The sections were counter-stained with hematoxylin and photographed with an Olympus photomicroscope (Tokyo, Japan).

Statistical Analysis

The characteristics of patients are presented as the mean ± SD (range) or numbers, as appropriate. Spearman correlation analysis was performed to identify relationships between drug dosage and the Treg/Th17 ratio. To evaluate the differences between the regulation and non-regulation groups, repeated-measures analysis of variance was used. Comparison of continuous baseline characteristics and immunologic markers between the regulation and non-regulation groups were analyzed by Student's *t*-test for normally distributed variables and the Mann–Whitney *U* test for nonnormally distributed variables. Categorical variables were evaluated using the chi-square test with Fisher's exact test. All statistical analyses were performed using SPSS ver. 15.0 (SPSS, Chicago, IL, USA).

RESULTS

Selection of LT Recipients for Tapering

A total of 194 patients were enrolled in this study. Of these, 109 were excluded for criteria, including a history of biliary complications ($n = 70$), a history of rejection ($n = 10$), or malignancy ($n = 4$). Of the 85 patients eligible for inclusion, 70 agreed to participate in this study (**Figure 2A**). The baseline characteristics of these 70 patients are shown in **Table 1**. They comprised 53 men

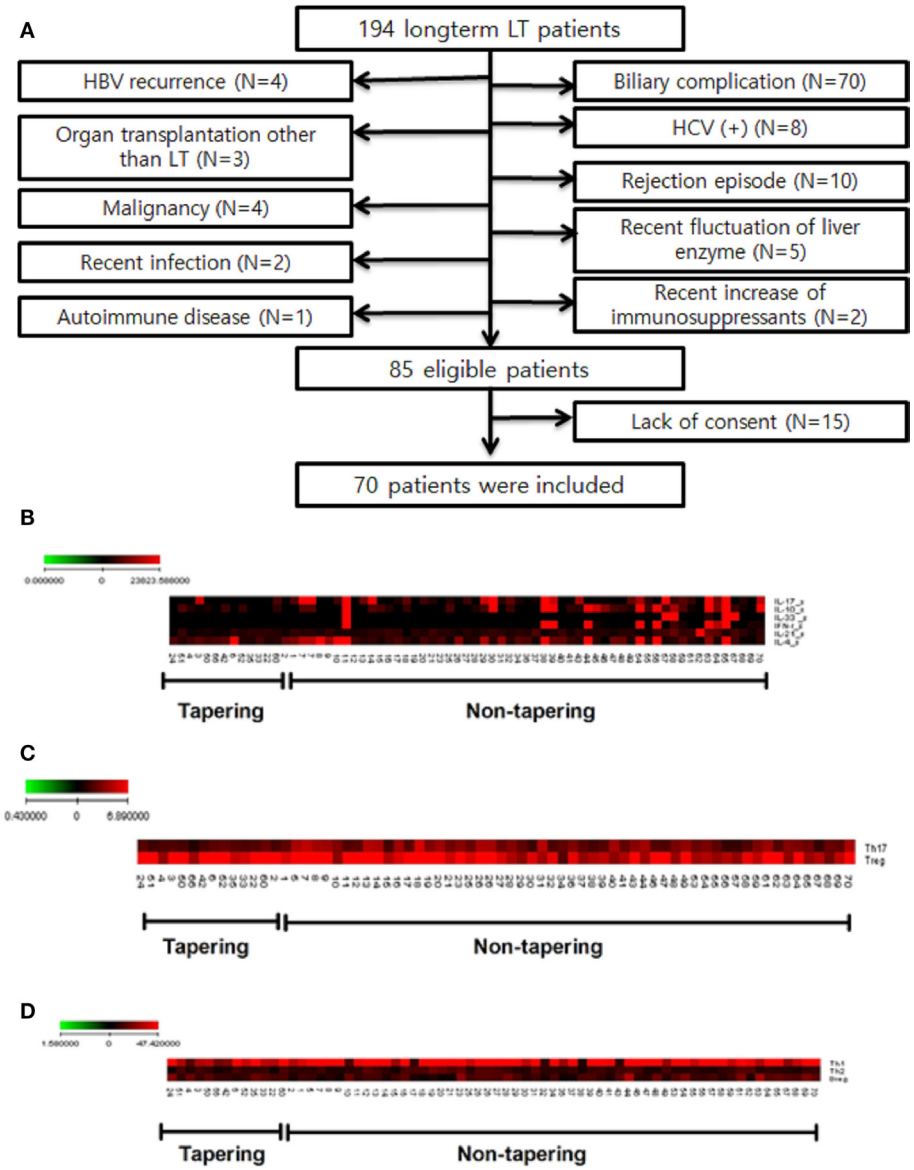

FIGURE 2 | Study flow chart and selection of tapering patients based on difference of cytokines and T cell populations of patients (n = 70). Heat map of T cell differentiation and cytokine expression data from peripheral blood mononuclear cells (PBMC) samples of patients undergoing tapering (n = 14) and those not undergoing tapering (n = 56). The legend on the horizontal line indicates the number of patients involved in the investigation. **(A)** Flow chart of patients included in the study. LT, liver transplantation; HBV, hepatitis B virus; HCV, hepatitis C virus. **(B)** ELISA of patient plasma was performed to identify cytokines that were differentially expressed between the two groups. **(C,D)** Flow cytometry of patient PBMCs was performed to evaluate whether T helper (Th1), Th2, Th17 cell, and regulatory T cells differed between the two groups. The expression of cytokines (pg/mL) and differentiation of cells (%) are illustrated according to the color scale on the left. Red indicates high expression and black indicates low expression.

and 17 women with a mean age of 57.7 ± 8.3 (35–71) years. The most common reason for LT was liver cirrhosis caused by hepatitis B (n = 35), and the time, since LT was 85.1 ± 28.6 (41–181) months. Fifty of the patients (71.5%) took tacrolimus (n = 30) or cyclosporine (n = 20) for immunosuppression. We analyzed the immunological markers in the PBMCs and plasma from all these LT recipients. Based on low expression of proinflammatory cytokines, Th17 and high expression of Treg, we identified the 14 most immunologically stable patients who were included as the tapering group (**Figures 2B,C**). The levels of Th1, Th2, and regulatory B cells were similar in the tapering and nontapering groups (**Figure 2D**).

Baseline Characteristics and Clinical Results of the Tapering Group

The tapering group, which consisted of 14 immunologically stable patients, was treated with tapering doses of immunosuppressive drugs. The clinical information for these LT recipients is

TABLE 1 | Baseline characteristics of patients ($n = 70$).

Variable	
Age (years)	57.7 ± 8.3 (35–71)
Sex (M/F)	53 (75.7%)/17 (24.3%)
LDLT/DDLT	54 (77.1%)/16 (22.9%)
Time since LT (months)	85.1 ± 28.6 (41–181)
Type of immunosuppression	
Tacrolimus	30 (42.9%)
Cyclosporine	20 (28.6%)
MMF	1 (1.4%)
Tacrolimus + MMF	4 (5.7%)
Cyclosporine + MMF	10 (14.3%)
Other (changed because of side effects)	5 (7.1%)
Reason for LT	
LC-B	35 (50.0%)
Alcohol	5 (7.1%)
Hepatocellular carcinoma	23 (32.9%)
Combined	7 (10.0%)

LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation; LT, liver transplantation; MMF, mycophenolate mofetil; LC-B, liver cirrhosis caused by hepatitis B.

TABLE 2 | Characteristics at the time of rejection in patients undergoing tapering ($n = 7$).

Variable	
Intolerant (number)	7 (50%)
Dosage of IS	29.0 ± 17.6% (0–50%)
Peak AST/ALT	86.1 ± 45.4 (41–164)/145.4 ± 84.4 (51–258)
Liver biopsy	4/7 (57.2%)
RAI score	3.25 ± 1.3 (2–5)
Immune markers (% of initial value)	
Treg/T helper 17 cell (Th17)	210.1 ± 84.8 (136–364)
T regulatory cell (Treg)	146.9 ± 45.6 (69–214)
Th17	68.0 ± 25.4 (32–102)
Th1	81.4 ± 26.6 (40–108)
$CD8^{+EM}$/interferon (IFN)-γ	86.5 ± 34.8 (42–138)
$CD8^{+CM}$/IFN-γ	80.8 ± 37.4 (38–148)
Result after rejection	
Recovery	7/7 (100%)
Dose reduction retrial	5/7 (57.2%)
Rejection after retrial	0/5 (0%)
Dosage of IS after retrial	41.0 ± 26.1 (10–75%)

IS, immunosuppressant; AST, asparate aminotransferase; ALT, alanine aminotransferase; RAI, rejection activity score; AP, alkaline phosphatase; GGP, gamma-glutamyl transpeptidase.

documented in Table S1 in Supplementary Material. They were initially being treated with tacrolimus ($n = 7$) or cyclosporine ($n = 7$). The mean duration of tapering to 50% of the initial immunosuppressive drug dose was 11.3 ± 2.1 (8–15) months and the mean time to discontinuation (0% dose) was 32.8 ± 3.2 (30–36) months. The mean tapered dose of immunosuppression (% of initial dose) was 23.4 ± 21.3% (0–75%).

Of the 14 tapering group patients, 7 (50%) had been stable during minimization and/or stopping immunosuppression in the study. These seven patients were named as regulation group and the group subclassified into two groups: tolerance group (group 1, $n = 3$), patients who were able to discontinue and maintain without immunosuppressive drug treatment; minimization group (group 2, $n = 4$), patients who achieved 70% ($n = 2$) or 80% ($n = 2$) reduction in the dose of immunosuppressive drugs. The other seven patients had experienced rejection during tapering period and were named as non-regulation group. All non-regulation group ($n = 7$) patients were successfully recovered from nontolerance after increasing dose of immunosuppression. After recovering from nontolerance, five patients could be re-tapered to the dose right before the rejection occurred and the other two were not re-tapered. According to re-tapering or not, non-regulation group were subclassified into two groups: re-tapering group (group 3, $n = 5$), no re-tapering group (group 4, $n = 2$).

At the time that rejection occurred in the non-regulation group, the mean dosage of immunosuppressive drugs was 29.0 ± 17.6% (0–50%) of the initial dosage (Figure S1 in Supplementary Material; **Table 2**). Peak AST and ALT levels were 86.1 ± 45.4 (41–164) and 145.4 ± 84.4 (51–258), respectively. Four of the non-regulation group underwent liver biopsy and the mean rejection activity score was 3.25 ± 1.3 (2–5).

A comparison of the baseline characteristics of the regulation and non-regulation groups showed no significant differences in age, sex, type of LT, type of immunosuppressant, or reason for LT (**Table 3**). Although the difference with other groups was not significant, the group 4 patients were younger (51.0 ± 11.3 years) and had a shorter follow-up after LT (43.5 ± 0.7 months).

Failure to Increasing of the Treg/Th17 Ratio Can Be Used to Predict Nontolerance of Drug Tapering

During tapering immunosuppressive drugs, we checked the level of immune markers and calculated the ratio in each dosage of immunosuppressive drugs. The ratio at the 100% dosage was defined as the reference point (1.0) and the other ratios in each dosage were converted according to the reference point. As the doses of immunosuppressive drugs were tapered, the Treg/Th17 ratio increased significantly for the tapering group as a whole ($\rho = 0.586$, $P < 0.005$, **Figure 3A**). In the regulation group, the Treg/Th17 ratio remained increased at doses tapered to 75 and 50% of the initial dose. In contrast, in the non-regulation group, the Treg/Th17 ratio at 50% dosage was higher than that at 100% dosage, but lower than that at 75% dosage (**Figure 3B**). Comparison of the Treg/Th17 ratios in the regulation and non-regulation groups showed that those in the non-regulation group did not increase as much as those in the regulation group ($P < 0.001$). At the time when the immunosuppression dose was 50% of the initial dose, the Treg/Th17 ratio differed significantly between the regulation and non-regulation groups ($P = 0.025$, **Figure 3C**). In the regulation group, as tapering immunosuppressive drugs, both tolerance group (group 1) and minimization group (group 2) had increased Treg/Th17 ratio with much higher ratio in group 1 than that in group 2 (**Figure 3D**). When all groups were compared, the Treg/Th17 ratio in group 1 was increased compared to those of the other three groups (**Figure 3E**). At the time when the dose

TABLE 3 | Comparison of baseline characteristics in tolerant and nontolerant groups.

Variable	Regulation group ($n = 7$)		Non-regulation group ($n = 7$)		*P* value
	group 1 ($n = 3$)	group 2 ($n = 4$)	group 3 ($n = 5$)	group 4 ($n = 2$)	
Age (years)	59.7 ± 13.3	57.3 ± 12.3	61.8 ± 10.3	51.0 ± 11.3	0.60
Sex (M/F)	3/0	4/0	5/0	2/0	1.00
LDLT/DDLT	2/1	2/2	1/1	4/1	0.94
Time since LT (months)	72.3 ± 25.5	84.5 ± 29.9	94.6 ± 51.4	43.5 ± 0.7	0.17
Type of immunosuppression					0.50
Tacrolimus	1	2	3	1	
Cyclosporine	2	2	2	1	
Reason for LT					0.89
LC-B	1	3	2	1	
Alcohol	1	0	1	0	
HCC	1	0	1	0	
Combined	0	1	1	1	

LDLT, living donor liver transplantation; DDLT, deceased donor liver transplantation; LT, liver transplantation; LC, liver cirrhosis caused by hepatitis B; HCC, hepatocellular carcinoma.

was 50% of the initial dose, the increase in Treg/Th17 ratio was greatest in group 1, followed by group 2, group 3, and group 4 (**Figure 3F**).

Variation in Th1/Th17, $CD8^{+EM}$IFN-γ^+/Th17, and $CD8^{+CM}$IFN-γ^+/Th17 May Be an Indicator of Nontolerance during Drug Tapering

We also analyzed the variation in Th1/Th17, $CD8^+$ effector memory ($CD8^{+EM}$)IFN-γ^+/Th17, and $CD8^+$ central memory ($CD8^{+CM}$)IFN-γ^+/Th17 ratios as the immunosuppressive drug doses were tapered to half the initial dose.

Comparison of the changes in the Th1/Th17 ratio showed it was dramatically increased in the regulation group compared to the non-regulation group, similar to the Treg/Th17 ratio (**Figure 4A**). At the time of 50% of the initial dose, the Th1/Th17 ratio was significantly higher in the regulation group ($P = 0.038$, **Figure 4B**), and a four-group comparison showed that the Th1/Th17 ratio increased much more in group 1 than in the other three groups (Figure S2A in Supplementary Material). The difference in the ratio did not reach significance at the 75% dose, but group 1 demonstrated a significantly higher ratio than the other three groups at the 50% dose, at which point the magnitude of the ratio decreased from group 2 to group 3, but did not differ significantly (Figure S2B in Supplementary Material). In group 4, the ratio was actually decreased compared with the ratio at the initial dose.

The regulation group demonstrated a higher $CD8^{EM+}$IFN-γ^+/Th17 ratio than the non-regulation group during the tapering of immunosuppressive drug dose (**Figure 4C**). The $CD8^{+EM}$IFN-γ^+/Th17 ratio was higher in the regulation group at 50% dosage, but this increase was not significant ($P = 0.259$, **Figure 4D**). In the four-group analysis, the ratios of $CD8^{+EM}$IFN-γ^+/Th17 decreased in order from group 1 to group 2, group 3, and group 4. In group 1, the $CD8^{+EM}$IFN-γ^+/Th17 ratio increased more than in the other three groups. However, in group 4, the ratio consistently decreased from the initial ratio (Figures S2C,D in Supplementary Material). Similarly to other markers, the $CD8^{+CM}$IFN-γ^+/Th17 ratio was higher in the regulation group than in the non-regulation group, especially at 50% dosage (**Figures 4E,F**). Subgroup analysis which showed the ratio in group 1 was much higher than those in the other groups, which did not differ significantly (Figures S2E,F in Supplementary Material).

Alteration of Immune Markers during Tapering to 0% (Discontinuation of Treatment)

During tapering of immunosuppression, the Th1/Th17, $CD8^{+EM}$IFN-γ^+/Th17, and $CD8^{+CM}$IFN-γ^+/Th17 ratios all increased. The Th1/Th17 (Figure S3A in Supplementary Material), $CD8^{+EM}$IFN-γ^+/Th17 (Supplementary Figure 3B), and $CD8^{+CM}$IFN-γ^+/Th17 (Figure S3C in Supplementary Material) ratios were negatively correlated with the degree of tapering.

Of 14 whole tapering patients, 4 patients were successfully tapered to 0% dosage of immunosuppressive drugs. As we mentioned above, three patients were tolerance group (group 1) patients. The other one patient was in re-tapering group (group 3) which is one of non-regulation group, because he had experienced nontolerance at the dose of 0%. The Treg/Th17 ratio was increased in both these groups, but in tolerance group (group 1) was more consistent and higher than in re-tapering group (group 3) (Figure S4A in Supplementary Material). The Th1/Th17 ratio showed similar alterations in both groups with a slightly higher ratio in group 1, especially at 50% dosage (Figure S4B in Supplementary Material). The $CD8^{+EM}$IFN-γ^+/Th17 ratio also demonstrated similar changes in both groups with a higher ratio in group 1 (Figure S4C in Supplementary Material). The changes in the $CD8^{+CM}$IFN-γ^+/Th17 ratio were similar to but not as clear as those in the Treg/Th17 ratio. The greatest difference between the two groups was found at 50% dosage, where group 1 demonstrated a higher ratio than group 3 (Figure S4D in Supplementary Material).

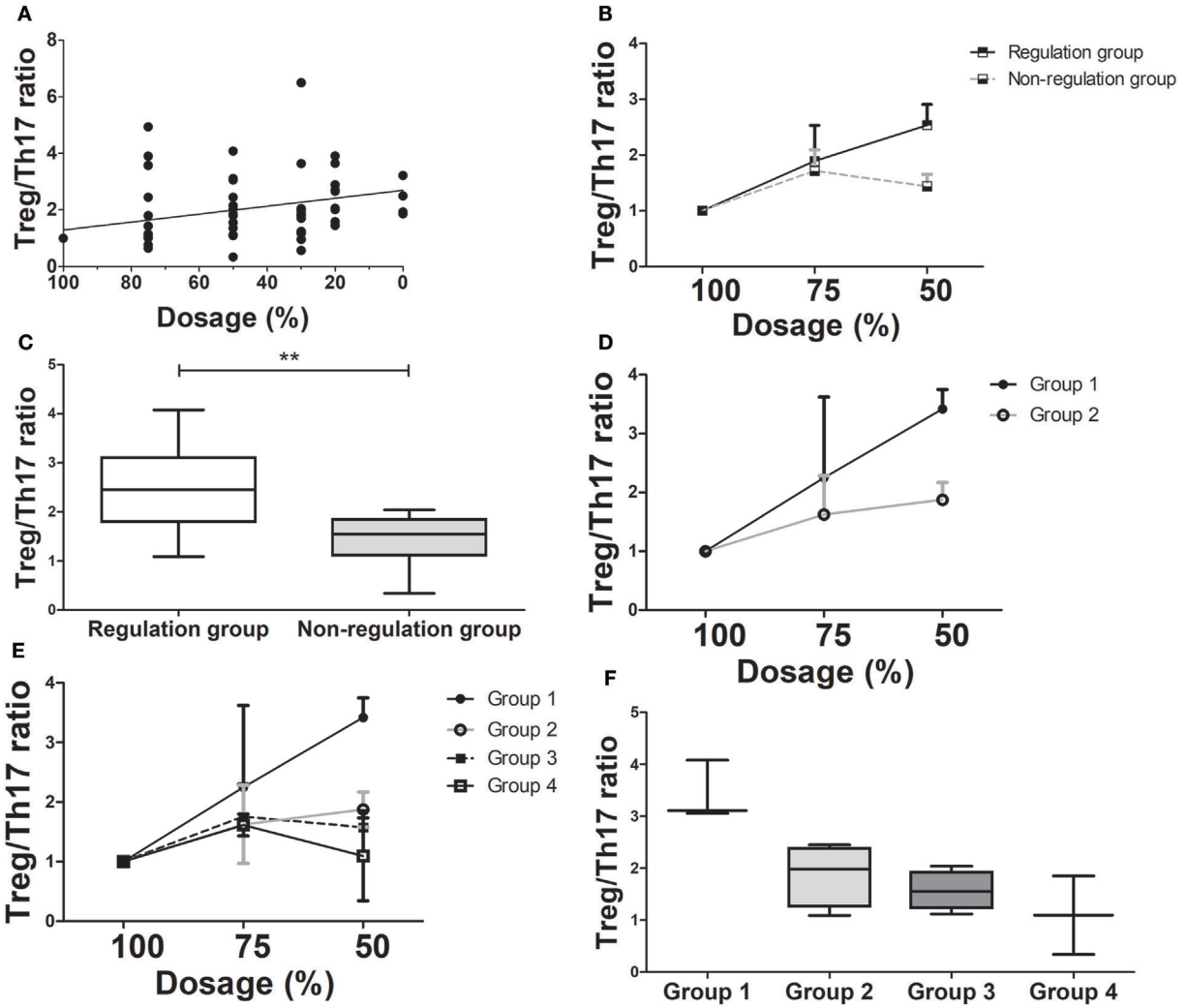

FIGURE 3 | Changes in T regulatory cell (Treg)/T helper 17 (Th17) cell ratio as immunosuppressive drug doses are tapered ($n = 14$). **(A)** There has been a moderate positive correlation between dose reduction and Treg/Th17 ($\rho = 0.586$, $P < 0.001$). **(B)** The Treg/Th17 ratio between regulation and non-regulation groups during tapering of immunosuppressive drug doses. **(C)** The Treg/Th17 ratios in the regulation and non-regulation groups after 50% reduction of immunosuppressive drugs (**$P < 0.03$). **(D)** The comparison of changing in Treg/Th17 ratio between group 1 and group 2 which are sub-groups of regulation group during tapering of immunosuppressive drugs. **(E)** The changing of Treg/Th17 ratio in all four groups. **(F)** The Treg/Th17 ratio of four groups at 50% reduction of immunosuppressive drugs. Group 1 had the highest Treg/Th17 ratio than that of other three groups.

Differences in Histopathologic Findings in Regulation and Non-regulation Groups

In regulation group, two patients who were subclassified into group 1 (tolerance group) agreed to undergo liver biopsy. Four patients in non-regulation group agreed to take liver biopsy. In the regulation group, the biopsies showed mild portal inflammation, but in the non-regulation group, there was moderate portal inflammation and even vasculitis in the liver tissues that were biopsied at the time of rejection (**Figure 5A**). We also observed an alteration in the ratio of IL-17 and Foxp3 in liver tissue. IL-17 expression was also increased in liver tissue from the non-regulation group compared with that of the regulation group ($P = 0.003$, **Figure 5B**). However, Foxp3 expression was decreased in liver tissue from the non-regulation group compared with that of the regulation group ($P = 0.046$, **Figure 5C**).

Representative Flow Chart of 60 Months Follow-up in Each Group

The data at the 60-month follow-up for each group are shown in **Figure 5**. In tolerance group (group 1) patient, 30 months was taken to taper the immunosuppressive drug doses and there was no significant increases in liver enzymes for the whole 60 months. Immunologic markers were maintained at increased levels during follow-up even after complete tapering/discontinuation of immunosuppressive agents (**Figure 6A**). These increased ratio of immunologic markers in tolerance group was quite higher

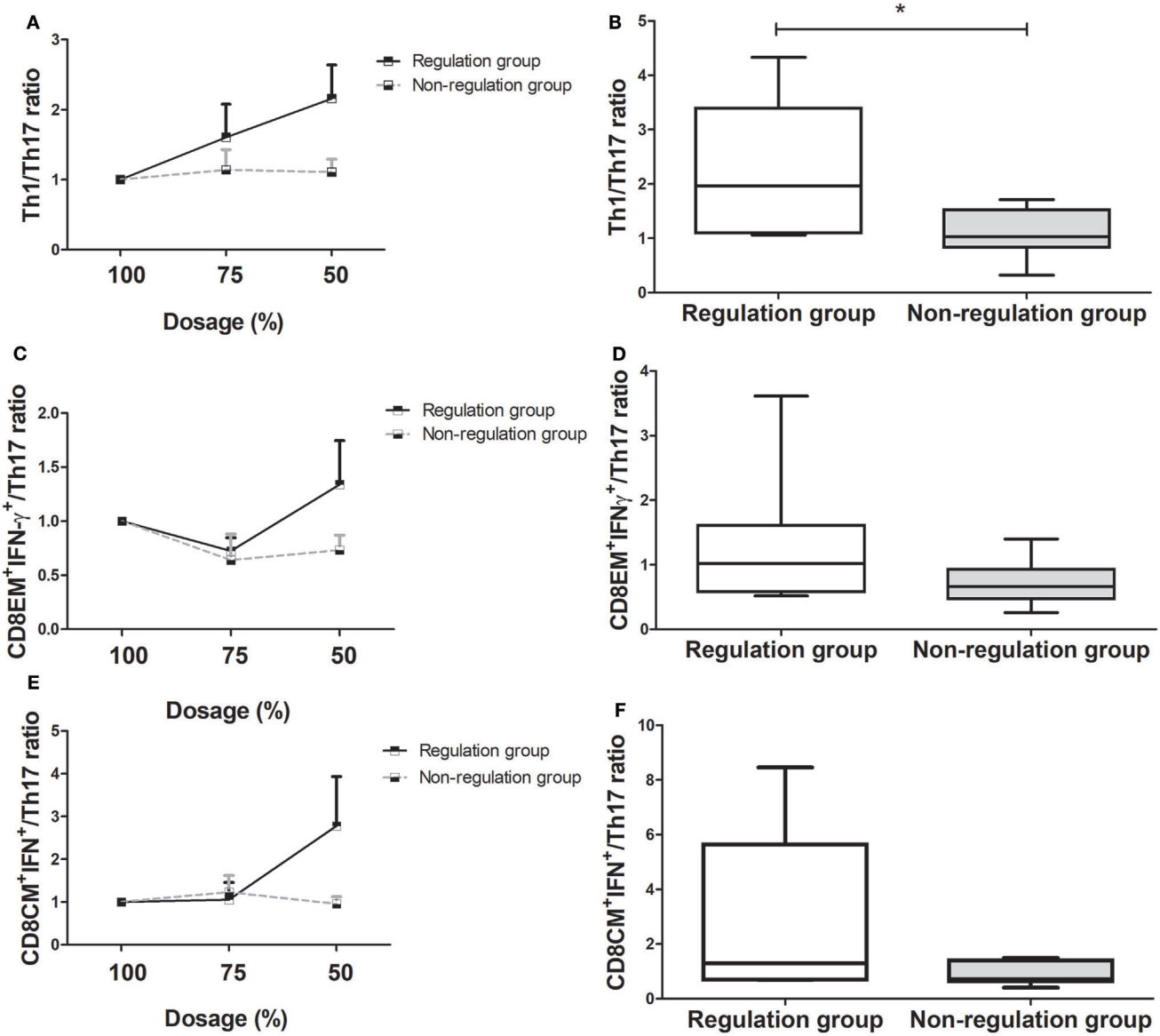

FIGURE 4 | Changes in T helper (Th)1/Th17 cell, CD8EM$^+$interferon(IFN)-γ^+/Th17, and CD8CM$^+$IFN-γ^+/Th17 ratio as immunosuppressive drug doses are tapered ($n = 14$). **(A)** Th1/Th17 ratio of the regulation group increased, but that of the non-regulation group did not increase more. **(B)** At 50% of immunosuppressive drug dosage, Th1/Th17 ratio of regulation group increased compared with that of non-regulation group (*$P < 0.05$). **(C)** CD8EM$^+$IFN-γ^+/Th17 ratio of the regulation group had been more increased than that of non-regulation group. **(D)** At 50% dosage, the increase in the CD8EM$^+$IFN-γ^+/Th17 ratio was higher in regulation group than non-regulation group without significance. **(E)** CD8CM$^+$IFN-γ^+/Th17 ratio of the tolerance group increased, but that of the non-regulation group decreased. **(F)** The comparison of the CD8CM$^+$IFN-γ^+/Th17 ratio at 50% dosage between regulation and non-regulation group.

and remarkable than that of other 3 groups. Minimization group (group 2) demonstrated no increase in liver enzymes and a smaller increase in Treg/Th17 and Th1/Th17 ratios than tolerance group (**Figure 6B**). The re-tapering group (group 3) patient who had experienced nontoleracne at 50% tapering was re-tapered after recovery from the rejection episode. During the tapering process, his immunologic markers did not increase as much as those of groups 1 and 2. At the time of the rejection episode, the immunologic markers had decreased; during the re-tapering and follow-up periods, the immunologic markers showed a fluctuation with sustained mild increases (**Figure 6C**). However, in the no re-tapering group (group 4), there were no increase in immunologic markers including Treg/Th17 during tapering or rejection episodes and follow-up (**Figure 6D**).

DISCUSSION

In this prospective pilot study, we identified potential immune markers, particularly the Treg/Th17 ratio, for prediction of operational tolerance during immunosuppressive drug dose tapering

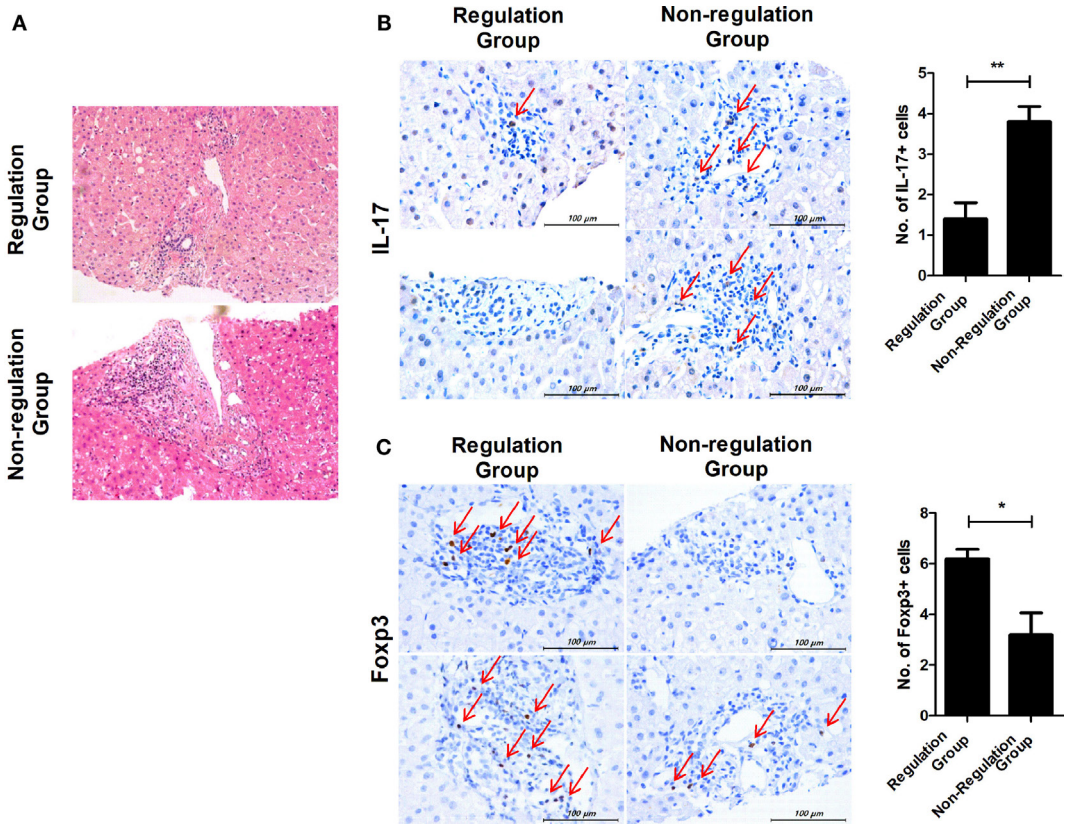

FIGURE 5 | Comparison of histopathologic findings in regulation and non-regulation groups. Two patients in the regulation group and four patients in the non-regulation group agreed to undergo liver biopsy. **(A)** Only mild portal inflammation was found in liver tissue from the regulation group. In contrast, moderate portal inflammation and even vasculitis was detected in liver tissue from the non-regulation group. **(B,C)** Biopsy tissues were stained with antibodies specific for IL-17 and Foxp3. Brown staining indicates positive for IL-17 and Foxp3, and positive cells are pointed with thin arrow. The number of positive cells was counted in six-independent liver tissues. **(B)** Regulation group $n = 2$, Non-regulation group $n = 4$, $P = 0.003$ C. Regulation group $n = 2$, Non-regulation group $n = 4$, $P = 0.046$.

in long-term stable LT recipients. We documented that the ratio of Treg/Th17 increased much more in regulation patients than in non-regulation patients during tapering of immunosuppressive drug treatment. Moreover, the Treg/Th17 ratio remained increased over the 60-month follow-up in tolerant patients.

The rate of operational tolerance in our study was about 21% (3/14). This result is similar to those of previous studies that suggested rates of operational tolerance from 5 to 50% (1–3). All the non-regulation (nontoleracne) patients ($n = 7$) in the tapering group recovered after tapering was stopped and the dosage of immunosuppressive drugs was increased. This result was consistent with that of a previous study (2). Evaluation of the time at which rejection occurred showed that it manifested at an average immunosuppressive drug dose of 29.0 ± 17.6% (0–50%) of the initial dose and more than 12 months after initiating tapering. In a previous study in children, rejection was documented at a dose of 38.4 ± 26.9% (0–75%) (2). Comparison of the clinical parameters of the regulation and non-regulation groups showed that group 4 patients had a shorter time, since LT, although this was not significant. Time since transplantation is the only established clinical factor associated with tolerance. Recipients with a longer time since LT had a higher tolerance rate than those with shorter times since LT (22). In our study, although the difference was not significant, the shorter time since LT in the group 4 could have contributed to their nontolerance.

The most meaningful finding of this research is that the ratio of Treg/Th17 in PBMCs can be used to predict tolerance after LT. We demonstrated that the Treg/Th17 ratio increased as the immunosuppressive drug doses were reduced. Moreover, the Treg/Th17 ratio increased much more with tapering in the regulation group than in the non-regulation group. The non-regulation, especially group 4, demonstrated decreased Treg/Th17 ratios as tapering progressed from 75 to 50% of the initial drug dose. These findings are consistent with those of Pons et al. who reported that Treg cells increased in tolerant patients (23), but contrast with those of García et al. who reported that Treg cells were not altered in tolerant patients (24). Our finding that the Treg/Th17 ratio decreased in the non-regulation group (groups 3 and 4) also could be explained by the previous report suggesting that Th17 cells are a critical marker of acute rejection after LT (25). In our study, tolerant patients (group 1) maintained consistently high Treg/Th17 ratios until complete withdrawal of immunosuppression. Thus, operational tolerance may be predicted by

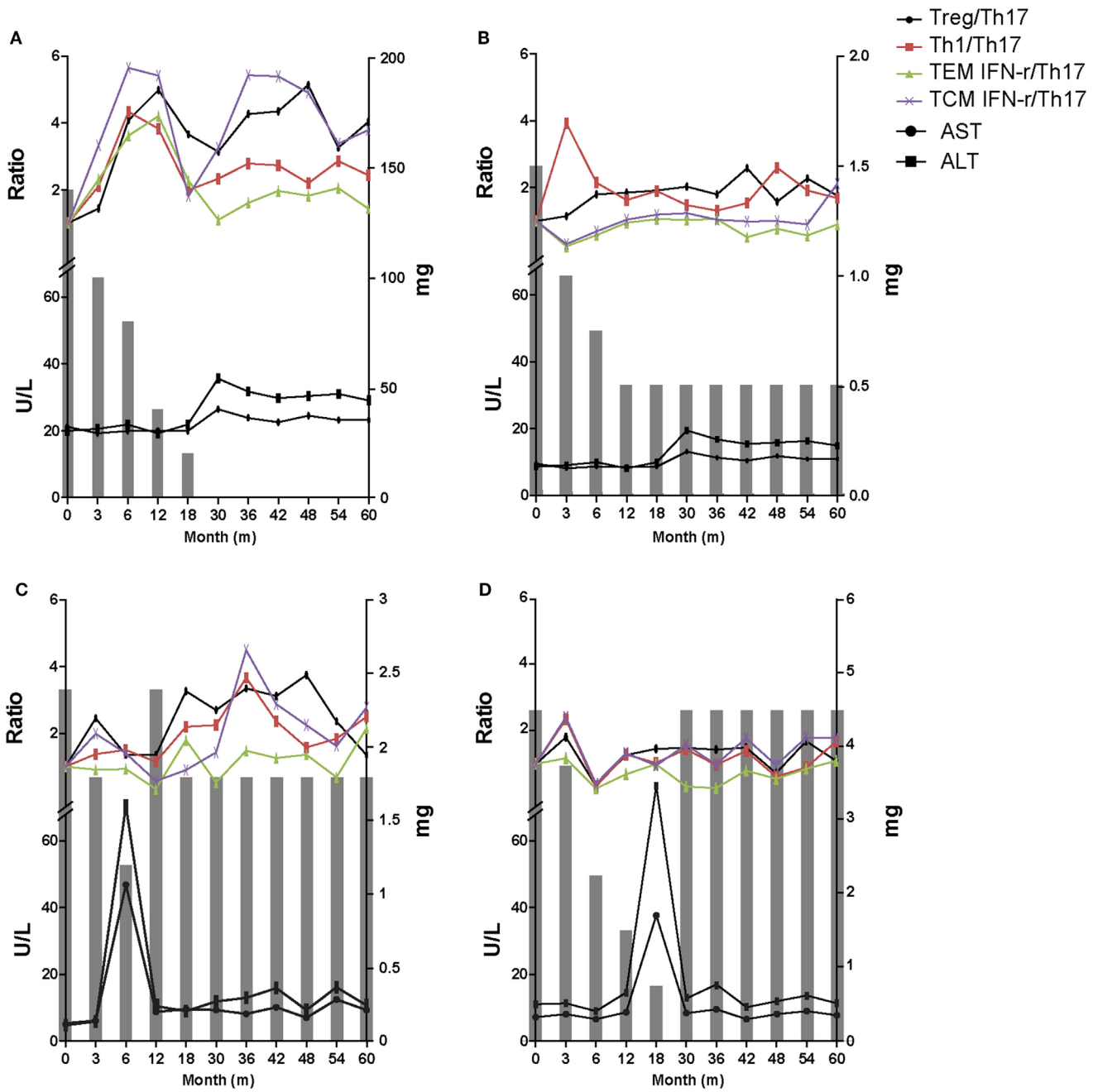

FIGURE 6 | Representative 60-month flow chart of all four groups. The gray bar means the dosage of immunosuppressive drugs at the time of each tapering time. **(A)** In tolerant group (group 1) patients, immunosuppressive drug treatment was discontinued over a period of 30 months and there was no significant increase in liver enzymes for the whole 60 months. Immunologic markers were maintained at an increased level. **(B)** Minimization group (group 2) demonstrated no increase in liver enzymes and smaller increase in T regulatory (Treg)/T helper 17 (Th17) cell and Th1/Th17 compared with group 1. **(C)** The re-tapering group (group 3) patient experienced rejection at 50% of the initial immunosuppressant dose. After recovery his immunosuppressive drug doses were re-tapered. During retapering, his immunologic markers were sustained at increased but fluctuating levels. **(D)** The no re-tapering group (group 4) patients experienced rejection at 20% of the initial immunosuppressant dose and recovered after increasing the dose to the initial amount. There was no increase in immunologic markers including Treg/Th17 during follow-up while taking the initial dose of immunosuppressive drugs.

checking the changes in the ratio of Treg/Th17 in blood. To our knowledge, this is the first study to suggest that the reciprocal balance between Th17 and Treg in the peripheral blood of LT recipients is correlated with immune tolerance. These results also suggest that immunosuppressive drug treatment can inhibit the development of tolerance in LT recipients.

Interferon-γ, which is expressed by Th1 and $CD8^+$ T cells, is an important cytokine for allograft survival. It is well documented

that IFN-γ deficiency induces rejection in experimental LT (26). It has been suggested that activation of $CD8^{+EM}$ T cells through the T-cell antigen receptor and the costimulatory receptor CD28 induces rapid IFN-γ expression (27). Currently, IFN-γ can regulate necroptosis and loss of IFN-γ aggravated experimental autoimmune disorder through upregulation of Th17 cells differentiation (28). Since, necroptosis has been observed in liver-related injury and disease indicating that necroptosis can be a target of the pathogenesis of several liver diseases (29, 30), induction of IFN-γ level can improve inflammatory response. In this study, we found that the Th1/Th17 and $CD8^{+EM}IFN\text{-}\gamma^{+}$/Th17 ratios were increased in the tapering groups. These results suggest that tapering can induce tolerance by increasing the number of cells releasing IFN-γ.

With respect to histopathology, although only a few patients underwent liver biopsies, the regulation group showed less portal inflammation than the non-regulation group. Our results are consistent with those of a previous study in children showing that the tolerant group maintained stable histology during a 5-year follow-up after withdrawal of immunosuppressive drugs (31). Moreover, we detected that IL-17 expression was enhanced, whereas Foxp3 expression was reduced in the liver of non-regulation patients compared with regulation patients. These histopathologic results were correlated with those from blood samples using confocal microscopy. Therefore, it is possible that the blood Treg/Th17 ratio may reflect histologic changes in the liver.

We also conducted a 60-month follow-up of clinical data, immunologic markers, and histologic findings. In tolerant patients with stable liver enzymes, elevated levels of immunologic markers including the Treg/Th17 ratio were maintained during the whole follow-up period. However, nontolerant patients had lower levels of the immunologic markers, including the Treg/Th17 ratio. Therefore, we could speculate that high Treg/Th17, Th1/Th17, and CD8/Th17 ratios may contribute to not only establishment of tolerance, but also its continuation.

Because this was a pilot study it had several limitations. First, only a small number of patients underwent tapering of immunosuppressive drug doses. For the safety of patients, we conducted the tapering study in the 20% most immunologically stable patients who also had stable laboratory measurements and clinical history. For this reason, only 14 patients underwent tapering of immunosuppressive drugs. Second, there were only a few liver biopsy samples and no baseline liver biopsies, because only a small number of the patients agreed to undergo liver biopsy. Despite these limitations, this is the first study to serially monitor the Treg/Th17 ratio in PBMCs from LT recipients undergoing tapering of immunosuppression over 60 months of follow-up and to compare parameters indicating immune tolerance in the regulation and non-regulation groups of LT recipients. This point is strength for the clinical application of these data to LT recipients.

In summary, the ratio of Treg/Th17 in PBMCs increased to a greater degree in tolerant patients during tapering of immunosuppressive drugs and after withdrawing immunosuppressive drugs. Our results suggest that the reciprocal balance between Th17 and Treg in the peripheral blood of LT recipients may contribute to the establishment and maintenance of tolerance and could be used as an indicator of the likelihood of operational tolerance in LT recipients.

ETHICS STATEMENT

Informed consent was provided by all patients and the study was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC11SISI0340).

AUTHOR CONTRIBUTIONS

JJ, SHL, JYC, and M-LC designed the experiments. JJ, SHL, EJ, DK, JWC, and CY performed the experiments. SHL, SKL, HK, SB, BC, CY, JYC, and M-LC analyzed the data. JJ, SHL, SKL, JYC and M-LC wrote the manuscript. JYC and M-LC supervised the study.

FUNDING

This research was supported by a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI15C3062). This study was supported by a grant of the Korean Health Technology R&D Project, Ministry for Health & Welfare, Republic of Korea (HI14C3417). This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2015R1D1A1A01058153).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fimmu.2018.00352/full#supplementary-material.

FIGURE S1 | Dosage and timing at the moment of intolerance ($n = 7$).

FIGURE S2 | Changes in Th1/Th17, $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17, and $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio of all four groups as immunosuppressive drug doses are tapered. **(A)** Th1/Th17 ratio of group 1 increased compared with that of the other three groups. **(B)** At 50% dosage, the increase in the Th1/Th17 ratio was greatest in group 1 followed by group 2, group 3, and group 4. **(C)** Group 1 had more increased $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio than that of the other three groups. **(D)** At 50% dosage, group 1 had the greatest increase in the $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio. **(E)** $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio of group 1 increased compared with that of the other three groups. **(F)** At 50% dosage, the increase in the $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio was greatest in group 1.

FIGURE S3 | Correlation between dosage of immunosuppressive drugs and Th1/Th17, $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17, and $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio as tapering immunosuppressive drug. **(A–C)** There has been mild positive correlation between the ratio of Th1/Th17, $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17, and $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 and dosage tapering.

FIGURE S4 | Changes in Treg/Th17, Th1/Th17, $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17, and $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio as immunosuppressive drug doses are tapered. **(A–D)** Treg/Th17, Th1/Th17, $CD8EM^{+}IFN\text{-}\gamma^{+}$/Th17, and $CD8CM^{+}IFN\text{-}\gamma^{+}$/Th17 ratio of the group 1 patients had been more increased with stable value than that of the group 3 patients.

REFERENCES

1. Demetris AJ, Lunz JG III, Randhawa P, Wu T, Nalesnik M, Thomson AW. Monitoring of human liver and kidney allograft tolerance: a tissue/histopathology perspective. *Transpl Int* (2009) 22(1):120–41. doi:10.1111/j.1432-2277.2008.00765.x
2. Feng S, Ekong UD, Lobritto SJ, Demetris AJ, Roberts JP, Rosenthal P, et al. Complete immunosuppression withdrawal and subsequent allograft function among pediatric recipients of parental living donor liver transplants. *JAMA* (2012) 307(3):283–93. doi:10.1001/jama.2011.2014
3. McCaughan GW. Withdrawal of immunosuppression in liver transplant recipients: is this as good as it gets? *Liver Transpl* (2002) 8(4):408–10. doi:10.1053/jlts.2002.0080408
4. Ueda M, Oike F, Ogura Y, Uryuhara K, Fujimoto Y, Kasahara M, et al. Long-term outcomes of 600 living donor liver transplants for pediatric patients at a single center. *Liver Transpl* (2006) 12(9):1326–36. doi:10.1002/lt.20826
5. Mazariegos GV, Sindhi R, Thomson AW, Marcos A. Clinical tolerance following liver transplantation: long term results and future prospects. *Transpl Immunol* (2007) 17(2):114–9. doi:10.1016/j.trim.2006.09.033
6. Ramos HC, Reyes J, Abu-Elmagd K, Zeevi A, Reinsmoen N, Tzakis A, et al. Weaning of immunosuppression in long-term liver transplant recipients. *Transplantation* (1995) 59(2):212–7. doi:10.1097/00007890-199501270-00010
7. de la Garza RG, Sarobe P, Merino J, Lasarte JJ, D'Avola D, Belsue V, et al. Trial of complete weaning from immunosuppression for liver transplant recipients: factors predictive of tolerance. *Liver Transpl* (2013) 19(9):937–44. doi:10.1002/lt.23686
8. Imagawa DK, Millis JM, Olthoff KM, Derus LJ, Chia D, Sugich LR, et al. The role of tumor necrosis factor in allograft rejection. I. Evidence that elevated levels of tumor necrosis factor-alpha predict rejection following orthotopic liver transplantation. *Transplantation* (1990) 50(2):219–25. doi:10.1097/00007890-199008000-00009
9. Kita Y, Iwaki Y, Demetris AJ, Starzl TE. Evaluation of sequential serum interleukin-6 levels in liver allograft recipients. *Transplantation* (1994) 57(7):1037–41. doi:10.1097/00007890-199404150-00009
10. Kimura A, Kishimoto T. Th17 cells in inflammation. *Int Immunopharmacol* (2011) 11(3):319–22. doi:10.1016/j.intimp.2010.10.004
11. Waite JC, Skokos D. Th17 response and inflammatory autoimmune diseases. *Int J Inflam* (2012) 2012:819467. doi:10.1155/2012/819467
12. Xie XJ, Ye YF, Zhou L, Xie HY, Jiang GP, Feng XW, et al. Th17 promotes acute rejection following liver transplantation in rats. *J Zhejiang Univ Sci B* (2010) 11(11):819–27. doi:10.1631/jzus.B1000030
13. Fan H, Li LX, Han DD, Kou JT, Li P, He Q. Increase of peripheral Th17 lymphocytes during acute cellular rejection in liver transplant recipients. *Hepatobiliary Pancreat Dis Int* (2012) 11(6):606–11. doi:10.1016/S1499-3872(12)60231-8
14. Germani G, Rodriguez-Castro K, Russo FP, Senzolo M, Zanetto A, Ferrarese A, et al. Markers of acute rejection and graft acceptance in liver transplantation. *World J Gastroenterol* (2015) 21(4):1061–8. doi:10.3748/wjg.v21.i4.1061
15. Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol* (2009) 21(10):1105–11. doi:10.1093/intimm/dxp095
16. Li W, Kuhr CS, Zheng XX, Carper K, Thomson AW, Reyes JD, et al. New insights into mechanisms of spontaneous liver transplant tolerance: the role of Foxp3-expressing CD25+CD4+ regulatory T cells. *Am J Transplant* (2008) 8(8):1639–51. doi:10.1111/j.1600-6143.2008.02300.x
17. Li W, Zheng XX, Kuhr CS, Perkins JD. CTLA4 engagement is required for induction of murine liver transplant spontaneous tolerance. *Am J Transplant* (2005) 5(5):978–86. doi:10.1111/j.1600-6143.2005.00823.x
18. Fujiki M, Esquivel CO, Martinez OM, Strober S, Uemoto S, Krams SM. Induced tolerance to rat liver allografts involves the apoptosis of intragraft T cells and the generation of CD4(+)CD25(+)FoxP3(+) T regulatory cells. *Liver Transpl* (2010) 16(2):147–54. doi:10.1002/lt.21963
19. He Q, Fan H, Li JQ, Qi HZ. Decreased circulating CD4+CD25highFoxp3+ T cells during acute rejection in liver transplant patients. *Transplant Proc* (2011) 43(5):1696–700. doi:10.1016/j.transproceed.2011.03.084
20. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* (2008) 133(5):775–87. doi:10.1016/j.cell.2008.05.009
21. Kim HY, Cho ML, Jhun JY, Byun JK, Kim EK, Yim YB, et al. The imbalance of T helper 17/regulatory T cells and memory B cells during the early post-transplantation period in peripheral blood of living donor liver transplantation recipients under calcineurin inhibitor-based immunosuppression. *Immunology* (2013) 138(2):124–33. doi:10.1111/imm.12021
22. Adams DH, Sanchez-Fueyo A, Samuel D. From immunosuppression to tolerance. *J Hepatol* (2015) 62(1 Suppl):S170–85. doi:10.1016/j.jhep.2015.02.042
23. Pons JA, Revilla-Nuin B, Baroja-Mazo A, Ramírez P, Martínez-Alarcón L, Sánchez-Bueno F, et al. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. *Transplantation* (2008) 86(10):1370–8. doi:10.1097/TP.0b013e318188d3e6
24. García de la Garza R, Sarobe P, Merino J, Lasarte JJ, D'Avola D, Belsue V, et al. Immune monitoring of immunosuppression withdrawal of liver transplant recipients. *Transpl Immunol* (2015) 33(2):110–6. doi:10.1016/j.trim.2015.07.006
25. Zhou Y, Yang X, Zhang H, Jiang J. The roles of T helper type 17/regulatory T cells in acute rejection after liver transplantation in rats. *Transplantation* (2015) 99(6):1126–31. doi:10.1097/TP.0000000000000666
26. Mele TS, Kneteman NM, Zhu LF, Ramassar V, Urmson J, Halloran B, et al. IFN-gamma is an absolute requirement for spontaneous acceptance of liver allografts. *Am J Transplant* (2003) 3(8):942–51. doi:10.1034/j.1600-6143.2003.00153.x
27. Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, et al. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol* (2013) 14(10):1064–72. doi:10.1038/ni.2687
28. Lee SH, Kwon JY, Kim SY, Jung K, Cho ML. Interferon-gamma regulates inflammatory cell death by targeting necroptosis in experimental autoimmune arthritis. *Sci Rep* (2017) 7(1):10133. doi:10.1038/s41598-017-09767-0
29. Zhao H, Jaffer T, Eguchi S, Wang Z, Linkermann A, Ma D. Role of necroptosis in the pathogenesis of solid organ injury. *Cell Death Dis* (2015) 6:e1975. doi:10.1038/cddis.2015.316
30. Saeed WK, Jun DW. Necroptosis: an emerging type of cell death in liver diseases. *World J Gastroenterol* (2014) 20(35):12526–32. doi:10.3748/wjg.v20.i35.12526
31. Feng S, Demetris AJ, Spain KM, Kanaparthi S, Burrell BE, Ekong UD, et al. Five-year histological and serological follow-up of operationally tolerant pediatric liver transplant recipients enrolled in WISP-R. *Hepatology* (2017) 65(2):647–60. doi:10.1002/hep.28681

Conflict of Interest Statement: The authors declare that there is no conflict of interest regarding the publication of this article.

frontiers in Immunology

REVIEW
published: 19 February 2018
doi: 10.3389/fimmu.2018.00270

Metabolic Modulation in Macrophage Effector Function

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Traditionally cellular respiration or metabolism has been viewed as catabolic and anabolic pathways generating energy and biosynthetic precursors required for growth and general cellular maintenance. However, growing literature provides evidence of a much broader role for metabolic reactions and processes in controlling immunological effector functions. Much of this research into immunometabolism has focused on macrophages, cells that are central in pro- as well as anti-inflammatory responses—responses that in turn are a direct result of metabolic reprogramming. As we learn more about the precise role of metabolic pathways and pathway intermediates in immune function, a novel opportunity to target immunometabolism therapeutically has emerged. Here, we review the current understanding of the regulation of macrophage function through metabolic remodeling.

Keywords: macrophage, immunometabolism, glycolysis, tricarboxylic acid cycle, electron transport chain

OPEN ACCESS

Edited by:
Duncan Howie,
University of Oxford,
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Reviewed by:
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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *30 November 2017*
Accepted: *30 January 2018*
Published: *19 February 2018*

Citation:
Diskin C and Pålsson-McDermott EM (2018) Metabolic Modulation in Macrophage Effector Function. Front. Immunol. 9:270. doi: 10.3389/fimmu.2018.00270

INTRODUCTION

All living cells rely on an organized sequence of anabolic and catabolic reactions to produce a steady supply of energy and biosynthetic precursors. In order to optimize functionality, enzymes that control these tightly regulated metabolic pathways are compartmentalized into specific organelles within the cells. Immune cells such as macrophages are no different in this aspect; however,

Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ACO2, aconitase 2; AOX, alternative oxidase; BCAA, branched-chain amino acid; BCKA, branched-chain keto acid; BMDM, bone-marrow-derived macrophage; CAD, *cis*-aconitate decarboxylase; CARKL, carbohydrate kinase-like protein; CIC, citrate carrier; CPT1, carnitine palmitoyltransferase 1; DGAT2, diacylglycerol O-acyl transferase 2; DMI, dimethyl itaconate; DMM, dimethyl malonate; ECAR, extracellular acidification rate; ECSIT, evolutionarily conserved signaling intermediate in toll pathway; EIF2AK2, eukaryotic translation initiation factor-2 alpha kinase 2; ETC, electron transport chain; FAS, fatty-acid synthase; FIH, factor-inhibiting HIF; GAIT, gamma-interferon-activated inhibitor of translation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT1, glucose transporter 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; GPCR, G-protein-coupled receptor; GPT3, glutamic-pyruvic transaminase; HIF, hypoxia-inducible factor; HMG CoA, 3-hydroxy-3methylglutaryl-coenzymeA; HO-1, heme oxygenase 1; HRE, hypoxia response element; IDH, isocitrate dehydrogenase; IFN-γ, interferon gamma; IL, interleukin; iNOS, inducible nitric-oxide synthase; LAL, lysosomal acid lipase; LDH, lactate dehydrogenase; LIF, leukemia inhibitory factor; LPS, lipopolysaccharide; MCAD, medium-chain acyl coenzyme A dehydrogenase; M-CSF, macrophage colony-stimulating factor; MDH, malate dehydrogenase; mTOR, mechanistic target of rapamycin; NLRP3, nod-like receptor family pyrin domain containing 3; NO, nitric oxide; NOX4, NADPH oxidase 4; OCAR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; PBMC, peripheral blood mononuclear cells; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1; PD-L1, programmed death-ligand 1; PGC-1β, PPAR-gamma coactivator 1β; PGE2, prostaglandin E2; PKM2, pyruvate kinase M2; PPP, pentose phosphate pathway; PRR, pattern recognition receptor; RA, rheumatoid arthritis; RET, reverse electron transfer; ROS, reactive oxygen species; SDH, succinate dehydrogenase; STAT, signal transducer and activator of transcription; SUCNR1, succinate receptor 1; TAM, tumor-associated macrophage; TCA, tricarboxylic acid; TLR, toll-like receptor; TNF-α, tumor necrosis factor-alpha; UCP, uncoupling protein; VEGF, vascular endothelial growth factor; VHL, von Hippel–Lindau protein; 2-DG, 2-deoxyglucose; 3′UTR, 3′untranslated region.

recent studies now reveal that immune effector functions such as cytokine production in response to pathogens are directly coupled to specific changes in cellular metabolism. This metabolic reprogramming of immune cells is required for both inflammatory and anti-inflammatory responses.

Macrophages are found in almost every tissue in our body, and along with dendritic cells they are at the forefront of initiating an innate immune response through phagocytosis and cytokine release, as well as an adaptive immune response through antigen presentation. Recognized nomenclature divides activated macrophages into two subgroups *in vitro*: the classically activated macrophages (M1) associated with inflammatory responses, which *in vitro* are generated by typically stimulating the resting macrophages with lipopolysaccharide (LPS) and interferon gamma (IFN-γ). Secondly, the alternatively activated macrophages (M2) are associated with tissue remodeling, resolution of inflammation, and anti-inflammatory responses, and are generated *in vitro* using anti-inflammatory stimuli including IL-4. We now know that this is an oversimplification of the actual functional diversity occurring *in vivo*. The vast spectrum of different macrophage activation statuses was clearly demonstrated in a transcriptomics study by Xue et al. who stimulated human macrophages with a range of stimuli (1). In addition, gene-set enrichment analysis was applied to sample groups from smokers and COPD patients. The data set generated, and other transciptome studies published since, proposes a spectrum model of macrophage activation rather than the dichotomous M1/M2 classification system. While useful in mapping the metabolic pathways of differentially activated macrophages, and although many of the studies described here classify macrophages as M1 or M2, we now view macrophage polarization differently. Evidenced primarily *in vivo*, macrophages respond to specific external stimuli, resulting in unique sets of macrophage phenotypes that fall between the two extremes of M1 and M2. Hence, manipulating or skewing the different macrophage phenotypes in clinical settings such as asthma, sepsis, tumor, atherosclerosis, infectious disease, and metabolic disorders may provide us with a novel therapeutic approach.

Here, we review current literature on how macrophages utilize metabolic pathways in order to generate adequate energy and biosynthetic macromolecules to meet the fluctuating needs involved in host immune responses.

GLYCOLYSIS

Overview

Glucose, fructose, pyruvate, and other small carbohydrates play key roles in energy metabolism as well as provide carbon skeletons for the synthesis of other macromolecules. Glycolysis, the process which involves the breakdown of six-carbon glucose to three-carbon pyruvate, is central in generating ATP without requiring oxygen, where the reverse process, gluconeogenesis, consumes ATP while generating polysaccharides for storage. Glycolysis involves 10 enzymatically regulated steps, overall generating two molecules of pyruvate per molecule of glucose, with a net energy gain of two ATP and two NADH (**Figure 1**). Although often illustrated as a linear reaction, in fact glycolysis branches off in order for intermediate metabolites to proceed along other metabolic pathways. These include the first intermediate of glycolysis, glucose-6-phosphate, which is required for glycogen synthesis and the pentose phosphate pathway (PPP), as well as the glycolytic intermediate glyceraldehyde-3-phosphate, which through glycerol generates triglycerides and fatty acids (**Figure 1**).

For many years, the general school of thought has been that pyruvate generated through glycolysis enters the mitochondria where it undergoes oxidative decarboxylation by pyruvate dehydrogenase (PDH) and thereby serves as the major source of acetyl-CoA, the starting point of the tricarboxylic acid (TCA) cycle (see below). During periods of high energy demand or low oxygen supply, pyruvate can instead be converted to lactate, another potential nutrient, by lactate dehydrogenase (LDH). However, new data by Hui et al. beg us to rethink the role for lactate in fueling the TCA cycle (2). Instead of pyruvate feeding the TCA cycle, circulating lactate was instead shown to be the primary source of carbon for the TCA cycle in all tissues except for brain.

Glycolysis and Macrophage Function

The upregulation of glycolysis in activated macrophages was first observed several decades ago (3–5), but it is only in recent years that the mechanisms governing the process and the functional significance of this metabolic shift have been unearthed. A sharp increase in the rate of glycolysis is now closely associated with an inflammatory phenotype in macrophages. It has been demonstrated that administration of 2-deoxyglucose (2-DG), a derivative of glucose that is taken up by the cell but cannot be further processed, blocks many aspects of the classical M1 inflammatory phenotype including opsonin-mediated phagocytosis (6, 7), secretion of pro-inflammatory cytokines, and production of reactive oxygen species (ROS) (8). In contrast, alternatively activated M2 macrophages or those associated with immune tolerance, such as alveolar macrophages, employ oxidative phosphorylation (OXPHOS) over glycolysis as their main source of ATP (5). The rapid increase in glucose uptake by classically activated M1 macrophages is facilitated by upregulation of glucose transporter 1 (GLUT1) expression (8, 9) (**Figure 1**).

It may seem counterintuitive that activated macrophages utilize glycolysis as their main source of energy, as OXPHOS generates 36 molecules of ATP compared with glycolysis, which produces a mere two molecules of ATP per molecule of glucose. However, a substantial boost in glycolysis can be achieved more rapidly than in OXPHOS, which would require concomitant mitochondrial biogenesis. Not only does glycolysis confer an advantage in terms of the speed at which it can be upregulated, but also it provides biosynthetic intermediates to be used in the PPP, among other processes, which are hugely important for classical macrophage activation and effector functions [reviewed in Ref. (10, 11)].

Multiple studies using murine and human macrophages have demonstrated that classically activated M1 macrophages are heavily dependent on glycolysis. Rodriguez-Prados et al. used a glucose tracer-based metabolomics approach to show that

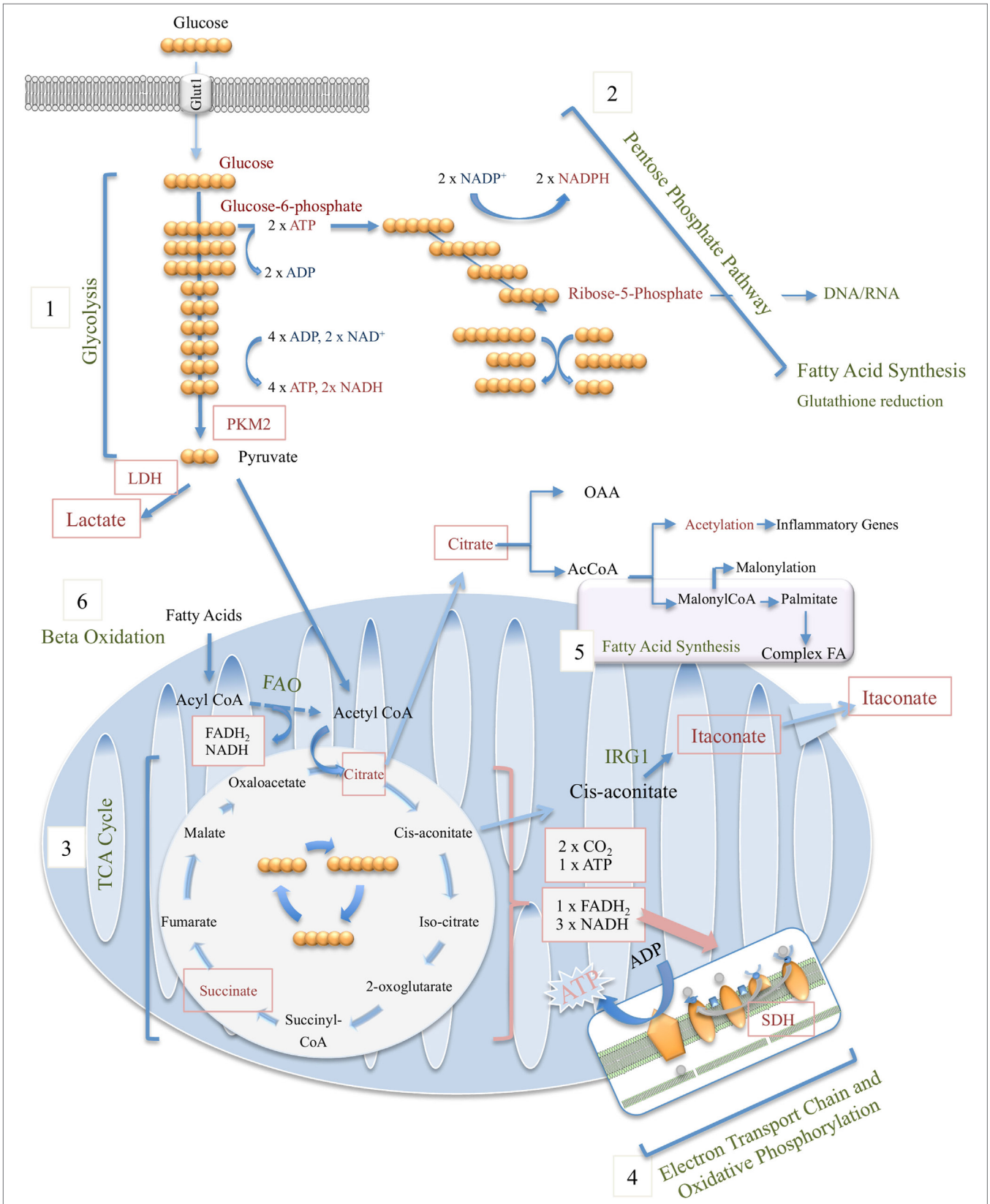

FIGURE 1 | Overview of macrophage metabolic pathways, including glycolysis (1), pentose phosphate pathway (2), tricarboxylic acid (TCA) cycle (3), electron transport chain and oxidative phosphorylation (4), fatty-acid synthesis (5), and beta (fatty acid) oxidation (6).

activation of murine peritoneal macrophages through various toll-like receptor (TLR) pathways all resulted in a highly glycolytic phenotype (12). It was also determined using extracellular flux analysis to study metabolic features of murine M1 and M2 macrophages, that M2 macrophages display enhanced mitochondrial OXPHOS, whereas M1 macrophages predominantly use glycolysis to generate ATP (13). Human studies seem to mimic observations in mice, with the leukocytes of patients suffering from sepsis undergoing a shift to aerobic glycolysis, which is reversed upon patient recovery (14). In contrast to LPS and other pro-inflammatory stimuli enhancing aerobic glycolysis in macrophages, anti-inflammatory signals have been shown to exert the opposite effect on macrophage glucose metabolism. It was recently established that interleukin (IL)-10 suppresses glycolysis in LPS-stimulated wild-type bone-marrow-derived macrophages (BMDMs). Furthermore, in contrast BMDMs derived from *Il10*$^{-/-}$ mice exhibit elevated rates of glycolysis (15). The effect of IL-10 on glycolysis may be dependent on nitric oxide (NO) (16).

The elevated glycolysis associated with inflammatory macrophages is heavily dependent on hypoxia-inducible factor-1 (HIF-1α). When oxygen levels are low, HIF-1α no longer undergoes prolyl hydroxylation, leading to a decreased binding of the interacting partner von Hippel–Lindau protein (VHL), and reduced proteosomal degradation of HIF-1α. As a result, stabilized HIF-1α can bind the constitutively expressed HIF-1β subunit, initiating the transcription of hypoxic genes, including glucose transporters and glycolytic enzymes (17–23). Blouin et al. were first to show that stimulation of macrophages with LPS increased HIF-1α protein levels, leading to a functional HIF-1 complex that bind to hypoxic response elements (HREs) in target genes (24). It was later determined that the induction of HIF-1α in the context of inflammation was dependent on NF-κB, which acts as a transcriptional activator of HIF-1α (25). HIF-1α was also found to play a role in trained immunity, which involves epigenetic remodeling of myeloid cells in response to stimuli such as β-glucan (26). β-Glucan derived from *Candida Albincans* plays a central role in the induction of innate immune memory and is known to confer protection against a range of infections. It was also found that the HIF-1α glycolytic reprogramming of activated macrophages played a significant role in monocyte-derived macrophage migration into tissues (27). HIF-1α also induces the transcription of the key pro-inflammatory cytokine IL-1β (28).

Although a Warburg-like phenomenon is predominantly associated with M1 macrophages, alternatively activated macrophages also display an upregulated rate of glycolysis in addition to augmented mitochondrial metabolism. Huang et al. found that both IL-4 and macrophage colony-stimulating factor (M-CSF) drive mechanistic target of rapamycin complex 2 (mTORC2) activation, which in turn induces interferon regulatory factor 4 (IRF4) expression, contributing to the upregulation of glycolysis (29). Another study found that M-CSF, which is associated with M2 polarization, instigated a similar expression of glucose transporters, a higher lactate production rate, and increased expression of several glycolytic enzymes in macrophages, compared with granulocyte-macrophage colony-stimulating factor (GM-CSF), which is typically associated with M1-like phenotype (30). Tan et al. found that administration of 2-DG in addition to IL-4 reduced the expression of early M2 activation markers (31). As more studies like these emerge, it may transpire that glycolysis could play a more important role in M2 macrophages than previously considered.

Multiple Roles of Glycolytic Enzymes

Specific roles for several of the glycolytic enzymes have been identified in macrophages. Some of these enzymes "moonlight" by carrying out functions in immunity separate to their glycolytic activity. One example includes hexokinase, which has been found to not only function as a pattern recognition receptor (PRR), but also, together with mTORC1, plays a critical role for nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome assembly (32). Furthermore, a component of bacterial peptidoglycan, *N*-acetylglucosamine, can bind to hexokinase, resulting in its inhibition and subsequent dissociation from the outer membrane of the mitochondria, culminating in NLRP3 activation (33).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) can bind to AU-rich RNA sequences in its Rossman fold, the site that typically binds NAD^+ (34). GAPDH takes part in formation of the gamma–interferon-activated inhibitor of translation (GAIT) complex. Upon assembly in murine macrophages, the GAIT complex binds to a specific element in the 3′untranslated region (3′UTR) of several inflammatory mRNAs and inhibits their translation. Its targets include vascular endothelial growth factor (VEGF), several chemokines, and corresponding chemokine receptors (35). GAPDH alone can bind directly to mRNA to inhibit translation of IFN-γ in T cells *via* 3′UTR binding (36) and also block translation of tumor necrosis factor-α (TNF-α) mRNA in human macrophages (37).

α-enolase is another glycolytic enzyme that was found to display non-glycolytic functions in macrophages. Bae et al. found that monocytes and macrophages in the inflamed synovium of rheumatoid arthritis (RA) patients and in a mouse model of arthritis expressed surface α-enolase. Antibodies against enolase, previously reported in RA patients, were shown to increase the production of pro-inflammatory cytokines and prostaglandins from enolase-expressing macrophages and therefore may contribute to the pathogenesis of the disease (38).

Another glycolytic regulator that is of great importance in macrophage effector functions is pyruvate kinase M2 (PKM2). PKM2 is an HIF-1α target gene that was originally found to promote the Warburg effect in tumor cells. It was also found to interact directly with HIF-1α in the nucleus and enhance the transcription of HIF-1α-responsive genes (39, 40). PKM2 was later found to play a significant role in LPS-activated macrophages. Dimeric enzymatically inactive PKM2 translocates to the nucleus where it acts as a coactivator of HIF-1α, promoting expression of pro-inflammatory as well as pro-glycolytic genes. Nuclear PKM2 together with HIF-1α binds directly to HRE sites in the IL-1β promoter in LPS-stimulated macrophages (41). Using small-molecule activators that promote a tetrameric form of PKM2, the pyruvate kinase enzymatic activity can be restored while simultaneously preventing nuclear translocation. PKM2 activators impaired M1 macrophage polarization, promoting expression of M2 genes while reducing LPS-induced glycolysis. Furthermore, these activators diminished IL-1β production *in vivo* in response

to *Salmonella typhimurium* or LPS alone and increased levels of anti-inflammatory IL-10. PKM2 was also found to play a role in NLRP3 and absent in melanoma 2 (AIM2) inflammasome activation. It was demonstrated that PKM2-dependent glycolysis promotes the phosphorylation of eukaryotic translation initiation factor-2 alpha kinase 2 (EIF2AK2, also called PKR), which was previously shown to be necessary for inflammasome activation and secretion of IL-1β, IL-18, and high-mobility group box 1 protein (HMGB1) from macrophages (42, 43). PKM2 may also contribute to the pathogenesis of coronary artery disease. Peripheral circulating monocytes differentiated *ex vivo*, as well as macrophages from the atherosclerotic plaques of patients suffering from coronary artery disease exhibit increased expression of dimeric PKM2, augmented glycolytic flux, and upregulated ROS production. PKM2 translocates to the nucleus and phosphorylates signal transducer and activator of transcription 3 (STAT3), contributing to the increase in IL-1β and IL-6 associated with these patients (44). Very recently, PKM2 was shown to regulate the expression of the checkpoint programmed death-ligand 1 (PD-L1), a ligand for the immune checkpoint receptor PD-1, in macrophages as well as other immune cells and cancer cells. Both pharmacological intervention and genetic silencing of PKM2 inhibited LPS-induced expression of PD-L1. Furthermore, PKM2 and HIF-1α bind to two HRE sites in the promoter of PD-L1 (45). This observation could have therapeutic potential as targeting immune checkpoints such as PD-L1 and PD1 has proven successful clinically [reviewed in Ref. (46)].

Tumor-Associated Macrophages (TAMs)

In cells undergoing Warburg metabolism, pyruvate resulting from glycolysis is diverted away from the TCA cycle and instead becomes converted to lactate by LDH. In addition to macrophages producing lactate, extracellular lactate from surrounding tissues also impact on macrophage function. Lactate secreted from tumor cells was found to drive M2 polarization in TAMs, which facilitated tumor growth (47). Although TAMs are often considered to be more M2-like, we now know that they have a high glycolytic rate similar to M1 macrophages; however, the effect of this on tumor progression is somewhat unclear (48). Murine TAMs exhibit diminished glycolysis through expression of REDD1, an mTORC1 inhibitor. This decrease in glycolysis is thought to facilitate metastasis and aberrant angiogenesis in tumors (49). However, a study carried out using TAMs generated *in vitro* from human monocytes yielded quite different results. They found an elevated glycolytic flux in TAMs to be associated with angiogenesis and metastasis in pancreatic cancer and showed that treatment with 2-DG was sufficient to reverse this effect (50). TAM metabolism is undoubtedly complicated and this area of research was extensively reviewed recently (51).

THE PENTOSE PHOSPHATE PATHWAY (PPP)

Overview

Glucose-6-phosphate from glycolysis feeds the anabolic PPP, which not only generates pentoses and 5-ribose phosphate for nucleic acid production but also serves as our major source of NADPH (**Figure 1**). NADPH provides the reducing power required for a range of synthetic reactions and anabolic pathways. NADPH offers reducing equivalents for generation of the antioxidant glutathione, thereby allowing for clearance of harmful ROS as well as being responsible for the respiratory burst in neutrophils and macrophages generating H_2O_2 to aid bacterial killing.

Like glycolysis, the PPP takes place in the cytosol and can be divided into an initial oxidative phase during which NADPH is generated, and a later non-oxidative phase where five-carbon sugars are synthesized.

PPP and Macrophage Function

The PPP has been shown to be upregulated in M1 macrophages (28, 52). NAPDH is likely to be of great importance for M1 macrophages as it is required by the enzyme NADPH oxidase which catalyzes the generation of ROS. As mentioned, NADPH is also used for the production of antioxidants, which may be important in the resolving phase of inflammation (53). Production of nucleotides is likely to be essential for activated macrophages. Although they display a reduced rate of proliferation, nucleotides are required for miRNAs involved in gene regulation (11). M2 macrophages, on the other hand, appear to suppress the PPP. Haschemi et al. demonstrated that regulation of the PPP in macrophages is under the control of the carbohydrate kinase-like protein (CARKL), a sedoheptulose kinase. CARKL was found to be upregulated in response to IL-4 but suppressed in response to LPS, resulting in an inhibition of the PPP in M2 macrophages (54). This conclusion was drawn from experiments using primary murine macrophages, human peripheral blood mononuclear cells (PBMC), and the macrophage cell line RAW 264.7. Employing overexpression and genetic silencing in RAW 264.7 cells, the authors found that the loss of CARKL mimicked the increase in extracellular acidification rate (ECAR) and decrease in oxygen consumption rate (OCR) that is seen upon LPS stimulation, while overexpression attenuated the effect that LPS has on the ECAR and OCAR. Therefore, the downregulation of CARKL seems to be important for the redirection of glucose from aerobic metabolism to glycolysis and the PPP that is seen in pro-inflammatory macrophages.

THE TRICARBOXYLIC ACID (TCA) CYCLE

Overview

When oxygen is readily available, glycolysis becomes the initial stage of glucose catabolism. Once pyruvate and lactate are generated, three further metabolic processes occurring in the mitochondria become responsible for potentially generating a further 36 molecules of ATP per glucose molecule. Firstly, pyruvate is oxidized through a series of reactions termed the TCA cycle. This is followed by the electron transport chain (ETC), and lastly the OXPHOS of ADP to ATP, a process that is driven by the proton gradient resulting from electron transport (**Figure 1**).

The point of entry for pyruvate formed during glycolysis into the TCA cycle comes when pyruvate is decarboxylated

to acetyl CoA by the PDH complex. Acetyl CoA then enters a series of eight enzymatically regulated oxidizing reactions where each acetyl CoA is converted into two molecules of water and carbon dioxide. Pyruvate loses one-carbon and the two-carbon acetyl group of acetyl CoA condenses with the acceptor compound oxaloacetate resulting in six-carbon citrate. In a cyclic, carefully regulated series of reactions citrate is decarboxylated and oxidized resulting in malate from which the starting oxaloacetate is regenerated, completing the cycle (**Figure 1**). Only one single ATP is directly generated by one lap around the TCA cycle (two per molecule of glucose); however, most of the energy produced is stored in the form of the reduced coenzymes NADH and $FADH_2$ which can drive the production of large amounts of ATP in the subsequent reactions of the ETC and OXPHOS (see below). In contrast to glycolysis the TCA cycle requires oxygen.

TCA Cycle and Macrophage Function

In addition to the increased glycolytic flux and reduced oxygen consumption that have been extensively studied in inflammatory macrophages for several decades (55), significant changes are also known to occur in the TCA or Krebs cycle. As with glycolysis, key intermediates of the TCA cycle serve as precursors in biosynthetic pathways. Citrate plays an important role here, fueling not only fatty-acid synthesis and histone acetylation but also acts as a precursor of itaconate, one of the most highly induced metabolites in LPS-activated macrophages. Citrate is firstly converted into *cis*-aconitate by mitochondrial aconitase 2 (ACO2), which is turned into itaconate by immune-responsive gene 1 (IRG1), also known as *cis*-aconitate decarboxylase (CAD) (**Figure 1**).

Resting macrophages and M2-like macrophages are considered to utilize an intact TCA cycle in conjunction with OXPHOS in order to generate ATP. An intact TCA cycle is thought to be important for the UDP-GlcNAc-mediated glycosylation of lectin and mannose receptors that are highly expressed on M2 macrophages (52). Pyruvate generated in M1 macrophages is converted to acetyl CoA by PDH, which is later converted to citrate. PDH activity was found to be intact in M1 macrophages, even though HIF-1α can potentially induce pyruvate dehydrogenase kinase 1 (PDK-1), an inhibitor of PDH (56). Interestingly, as mentioned, recent data have established that glucose fuels the TCA cycle indirectly *via* circulating lactate. Using ^{13}C-labeled lactate and other metabolites, the authors show that the carbons in TCA cycle intermediates in most tissues arose from circulating lactate, instead of directly from glycolysis (2). As inflammatory macrophages are known to produce large quantities of lactate, it is plausible that this lactate could be important for use in the TCA cycle in surrounding tissues or even other immune cells. However, it remains to be confirmed if these new data have any implications for macrophage metabolism.

When macrophages are stimulated with LPS or another inflammatory signal, their TCA cycle becomes disrupted at distinct points in the cycle (52, 57). Therefore, an accumulation of certain metabolites such as citrate, itaconate, and succinate occurs in M1 macrophages.

Citrate

M1 macrophages display increased levels of citrate giving a first indication to its importance in macrophage effector functions. This accumulation in citrate is likely due to a downregulation of isocitrate dehydrogenase (IDH), the enzyme that catalyzes the conversion of isocitrate to α-ketoglutarate (52). An early study also reported enhanced activity of citrate synthase, the enzyme that catalyzes the formation of citrate from acetyl CoA and oxaloacetate (3). Both mRNA levels and protein expression of the mitochondrial citrate carrier (CIC, also termed Slc25a1) were found to be elevated in LPS-stimulated macrophages. CIC exports citrate from the mitochondrial matrix while importing cytosolic malate (58). In addition, the same group later demonstrated that CIC is upregulated in response to the pro-inflammatory cytokines TNF-α and IFN-γ (59). Once in the cytosol, citrate can be used for *de novo* lipogenesis (discussed below), which is important for membrane biogenesis. This involves the conversion of citrate to acetyl CoA by ATP citrate lyase (ACLY) (60). Interestingly, ACLY activity was found to be regulated by IL-4 *via* Akt-mTORC1 signaling. This alters histone acetylation and therefore regulates the expression of a subset of M2-associated genes (61). Citrate also appears to be critical for prostaglandin production, as both pharmacological inhibition and knockdown of CIC markedly reduced prostaglandin E2 (PGE2) levels (59). Disrupting ACLY activity also led to a significant decrease in PGE2 (60). In addition to acting as a pro-inflammatory mediator itself, PGE2 was also recently shown to be essential for LPS-induced expression of pro-IL-1β (62). Perturbing the activity and/or expression of CIC or ACLY also negatively affects NO and ROS production (58–60). Genetic silencing of ACLY and the use of three different inhibitors all reduce levels of ROS and NO in the human cell line U937. Similarly, knockdown or chemical inhibition of CIC also significantly reduced levels of ROS and NO. Although the mechanism is not yet fully elucidated, authors propose that ACLY activity may play a role in ROS and NO production through indirectly boosting NADPH supplies.

Citrate accumulation in macrophages can also lead to changes in gene expression. Citrate-derived acetyl CoA is critical for histone acetylation, as this process was found to be impaired upon siRNA-induced silencing of ACLY. These epigenetic changes could be of great importance in the context of inflammation; for example, IL-6 expression has been shown to be regulated by histone acetylation in macrophages (63). Non-histone protein acetylation can also impact cytokine expression, as microtubule acetylation was found to modulate IL-10 production (64). Citrate has also been identified as an inhibitor of HIF asparaginyl hydroxylase (FIH), which acts as a negative regulator of HIF-1α activity. Hence, citrate could potentially indirectly regulate HIF-1α targeted genes (65). However, none of the studies linking citrate to acetylation of proteins have yet been carried out in macrophages.

Citrate-derived acetyl CoA can also be converted to malonyl CoA, which acts as a cofactor for a lysine modification dubbed malonylation. This modification changes a positively charged residue into a negative charge (66, 67). This modification, although only recently discovered, has already been implicated in

type-2 diabetes (68). Malonylation has not yet been documented in macrophages or other immune cells but could potentially play a role, given the accumulation of citrate observed in M1 macrophages.

Itaconate

Citrate-derived *cis*-aconitate can be converted to itaconate, one of the most highly induced metabolites in activated macrophages (52, 56, 69). Although itaconate has been gaining more interest in recent years, its antibacterial effects have been recognized since the 1970s when it was shown to inhibit the growth of *Pseudomonas indigofera* by targeting isocitrate lyase––an important enzyme in the glyoxylate cycle in bacteria (70). More recently, itaconate has been shown to exert bacteriostatic effects on *Mycobacterium tuberculosis*, *S. enterica* (71), and *Legionella pneumophila* (72). There is also evidence of bacteria evolving to combat the action of this immunometabolite, as *P. aeruginosa* and *Yersinia pestis* were both found to express three separate enzymes that function in the degradation of itaconate. These three genes were found to be critical for the pathogenicity and survival of these bacteria (73). Itaconate was also implicated in a pro-inflammatory setting in two metabolic screens: one carried out in mice infected with *M. tuberculosis* (74) and the other in the macrophage-like cell line RAW 264.7 stimulated with LPS (75).

Immune-responsive gene 1, also later known as CAD/ACOD1, was previously known to be induced upon stimulation with LPS (76), but its function was not elucidated until Michelucci et al. demonstrated that it was the enzyme responsible for catalyzing the decarboxylation of *cis*-aconitate to produce itaconate. As expected, knocking down IRG1 in a macrophage cell line resulted in impaired antibacterial activity, due to a significant drop in itaconate levels (71). In addition to studies showing an increase in IRG1 expression in murine M1 macrophages, it has also been shown to be upregulated in humans during sepsis (77). Although the bactericidal effects have been well characterized, the immunomodulatory function of itaconate is a more recent area of study. Itaconate is generally thought of as being anti-inflammatory and was shown to inhibit the production of several TLR-induced pro-inflammatory cytokines by augmenting the expression of A20, a negative regulator of NF-κB (77). IRG1 was also found to be induced by the activity of heme oxygenase 1 (HO-1), which is expressed in the lungs and associated with LPS tolerance. Induction of HO-1 with carbon monoxide was found to decrease TNF-α levels and inhibition of HO-1 had the opposite effect, providing further evidence for the anti-inflammatory effects of IRG1 (78). IRG1 has also been shown to play a role in implantation, a process generally associated with immune tolerance or immune suppression. The expression of IRG1 was demonstrated to be regulated by the progesterone receptor (79) and leukemia inhibitory factor (LIF) (80), both of which are heavily involved in the implantation process.

Itaconate was found to associate with the mitochondria (81) and was shown to reduce mitochondrial substrate-level phosphorylation, an effect that was abrogated upon siRNA-mediated silencing of IRG1 (82). Itaconate has been proposed to contribute to the second breakpoint in the TCA cycle that occurs in M1 macrophages. Through its ability to inhibit succinate dehydrogenase (SDH), the enzyme that catalyzes the oxidation of succinate to fumarate, overproduction of itaconate leads to an accumulation of succinate (83, 84). This increase in succinate levels was abolished in *Irg1*$^{-/-}$ macrophages, whereas treatment with exogenous itaconate in the form of dimethyl itaconate (DMI) was found to enhance succinate levels (83). Furthermore, by using exogenous itaconate, as well as using mice lacking IRG1, Lampropoulou et al. confirmed that itaconate acts in an anti-inflammatory manner by inhibiting SDH-mediated oxidation of succinate, impacting on mitochondrial respiration and production of pro-inflammatory cytokines in macrophages *in vitro* and *in vivo* (84). However, another study employing radiolabeling suggests that exogenous DMI does not get taken up by the cell nor processed to itaconate intracellularly. Nonetheless, the presence of DMI in the media still leads to an increase in intracellular levels of itaconate so the authors have postulated that the effects may be due to an unidentified receptor. Therefore, any results obtained using DMI as a source of exogenous itaconate must be interpreted with caution.

Succinate

Succinate was identified as an oncometabolite before its importance in macrophage metabolism became clear. In 2005, succinate was found to drive the Warburg effect by activating HIF-1α through the inhibition of cytosolic prolyl hydroxylases (85). However, the role of succinate in inflammatory macrophages did not present itself until Tannahill et al. demonstrated that the accumulation of succinate in LPS-stimulated macrophages induced HIF-1α stabilization and activation which in turn leads to an upregulation of pro-inflammatory IL-1β as a target gene (28). This study established that cytosolic HIF-1 prolyl hydroxylases are inhibited upon the accumulation of succinate in normoxic inflammatory macrophages, and confirmed that the IL-1β gene contains HREs in its promoter. The authors also observed a boost in global LPS-induced succinylation, a protein modification akin to malonylation, although the functional significance of the modification is unclear (28).

Another important discovery regarding succinate was the identification of the succinate receptor GPR91, which was previously considered an orphan G-protein coupled receptor (GPCR). A study conducted by He et al. assigned the TCA cycle intermediates succinate and α-ketoglutarate to the orphan GPCRs GPR91 and GPR99, respectively (86). GPR91, which has since been renamed the succinate receptor (SUCNR1), was found to be expressed in a wide variety of tissues (86). SUCNR1 has since been shown to be expressed on macrophages and to play a role in many inflammatory diseases (87–89). Activated M1 macrophages secrete succinate into the extracellular space while also upregulating expression of SUCNR1. This way succinate signals in an autocrine and paracrine manner to stimulate the release of IL-1β. High levels of succinate were found in synovial fluid taken from RA patients and using a *Sucnr1*$^{-/-}$ mouse model of arthritis, this succinate feed forward loop was confirmed by impaired macrophage activation as well as reduced levels of IL-1β (87). In contrast, *Sucnr1*$^{-/-}$ mice were found to exhibit exacerbated allergic contact dermatitis and in this same study the authors

also reported that SUCNR1 deficiency improved arthritis in the mouse model (88). The authors proposed that the increased severity of allergic contact dermatitis observed in the *Sucnr1*$^{-/-}$ mice was due to abnormal mast cell development, which leads to mast cell hyperactivation. Recently, the succinate receptor has been implicated in type-2 diabetes and the associated adipose tissue inflammation (89). Succinate levels were raised in both type-2 diabetic patients and mice fed a high-fat diet compared with healthy controls. *Sucnr1*$^{-/-}$ mice displayed improved glucose tolerance and had significantly fewer macrophages present in their adipose tissue. In addition, macrophages from the *Sucnr1*$^{-/-}$ mice exhibited impaired chemotaxis toward apoptotic adipocytes (89). With more research currently ongoing into the effects of succinate and its receptor in inflammation, the therapeutic potential of targeting SUCNR1 for inflammatory diseases may soon be defined.

While most work has focused on citrate, itaconate, and succinate, other TCA cycle intermediates have also been found to play significant roles in macrophages. Arts et al. demonstrated that an accumulation of fumarate in M1 macrophages, which was dependent on glutaminolysis, was of great importance in trained immunity of macrophages. Fumarate alone induced epigenetic changes akin to those observed in response to β-glucan and also augmented pro-inflammatory cytokine production upon restimulation with LPS (90). This was the first piece of evidence that an accumulation of TCA cycle intermediates can alter the macrophage epigenome but more recently α-ketoglutarate has also been shown to play a part in epigenetic reprogramming in macrophages. It was discovered that α-ketoglutarate, again produced *via* glutaminolysis, is crucial for full M2 activation and drives epigenetic changes in M2-associated genes in a Jumonji Domain Containing Protein 3 (JMJD3)-dependent manner (91). Furthermore, treatment of BMDMs with an inhibitor of glutaminolysis boosted pro-inflammatory cytokine secretion. Ratios of α-ketoglutarate to succinate in an M1 macrophage versus and M2 macrophage differ. There is a larger succinate to α-ketoglutarate ratio in M1 macrophages due to partial blockade of succinate oxidation by SDH. However, in M2 macrophages succinate oxidation proceeds as normal and α-ketoglutarate becomes more important for processes such as epigenetic changes.

As these findings are still recent, more data will no doubt emerge detailing how TCA cycle intermediates and other metabolic changes sculpt the macrophage epigenome.

ELECTRON TRANSPORT CHAIN (ETC) AND OXIDATIVE PHOSPHORYLATION

Overview

As for the TCA cycle, the reactions of the ETC occur in the mitochondria. The mitochondria have an outer permeable membrane and an inner membrane with extensive folds called cristae. Large electron-carrier complexes in the inner membrane of mitochondria reoxidize NADH and $FADH_2$ generated from the TCA cycle, and in the process electrons are passed stepwise to molecular oxygen. During this process protons are taken up from the mitochondrial matrix space and transferred to the intermembrane space. The potential energy of the NADH and $FADH_2$ generated during glycolysis and the TCA cycle is thereby used to drive the synthesis of large amounts of ATP as an electrochemical potential gradient for protons is created across the inner mitochondrial membrane (**Figure 1**). Flow of protons back into the matrix through ATP synthase drives synthesis of ATP. Four large complexes, except cytochrome c and ubiquinone, contain the electron carriers that make up the electron transfer chain. Complex I is the largest of the complexes and contains NADH dehydrogenase responsible for oxidizing NADH. Complex II is composed of four subunits of SDH, the only enzyme that participates in both the TCA cycle and the ETC. SDH is localized on the inner face of the mitochondrial inner membrane where it oxidizes succinate to fumarate through binding of succinate to SDHA, a reaction that is coupled to the reduction of ubiquinone to ubiquinol. As electrons move down the respiratory chain through complex III and complex IV to O_2, H^+ ions are transferred to the matrix creating the proton gradient required by the coupled reaction of F_0F_1 ATP synthase complex (**Figure 2**).

ETC and Macrophage Function

While classically activated macrophages are known to produce most of their ATP *via* glycolysis, alternatively activated macrophages have been shown to utilize OXPHOS. Therefore, M2 macrophages maintain forward electron flow through the ETC and predominantly generate ATP *via* ATP synthase (52). Treatment of macrophages with IL-4 was shown to upregulate OXPHOS *via* the transcription factor STAT6 and PPARγ coactivator-1β (PGC-1β). Overexpression of PGC-1β in BMDMs reduced the production of pro-inflammatory cytokines and PGC-1β knockdown impaired traits of alternative activation such as the promotion of fatty-acid oxidation and arginase activity (92). This effect on OXPHOS is not exclusive to IL-4, as IL-25 was also found to promote M2 polarization and elevate macrophage mitochondrial respiratory capacity (93). IL-10 also stimulates OXPHOS, one way in which the anti-inflammatory cytokine opposes M1 polarization (15).

However, in M1 macrophages OXPHOS is abated and the ETC becomes dysregulated (52). In one study, the downregulation of OXPHOS and the concomitant increase in mitochondrial fragmentation in response to pro-inflammatory stimuli was shown to be under the control of a microRNA–mi-R125b (94). Pro-inflammatory macrophages modify the ETC so that its primary function is ROS production, which is critical in the defense against infection. It is well established that phagosomal ROS is vital for M1 macrophages but the importance of mitochondrial ROS (mROS) has only been appreciated more recently. It was demonstrated that several TLR receptors signal through TRAF6 and evolutionarily conserved signaling intermediate in toll pathway (ECSIT) (which associates with complex I of the ETC) to promote mROS production and the recruitment of the mitochondria to the phagosomes. Perturbation of this signaling pathway was shown to impair bacterial killing by macrophages (95). These findings are not restricted to mice as immune cells isolated from patients suffering from TNF receptor-associated periodic syndrome (TRAPS) were found to have elevated mROS (96). In addition to a more direct role in bacterial killing (95), mROS was also demonstrated to contribute to NLRP3

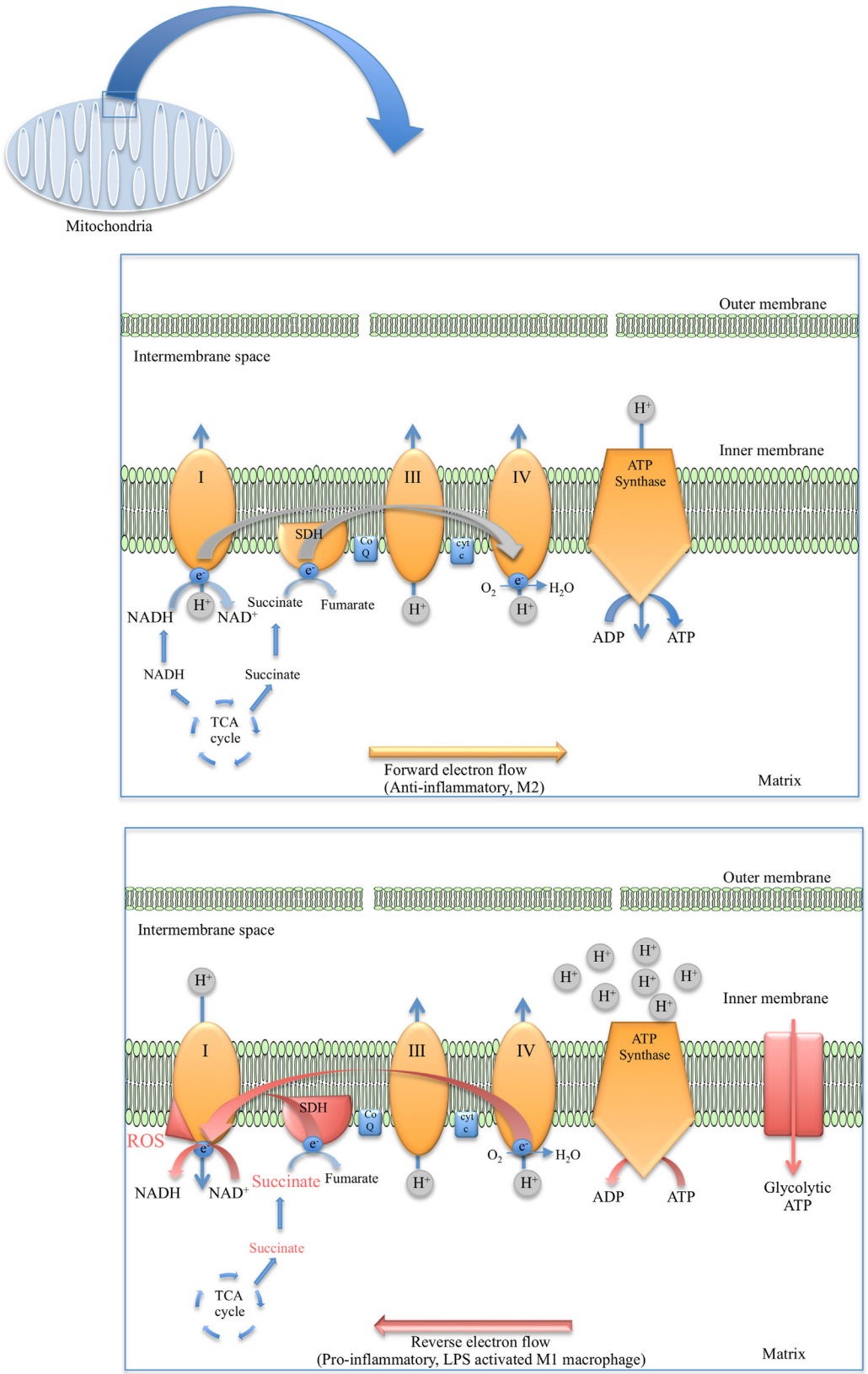

FIGURE 2 | Diagram depicting the flow of electrons in anti-inflammatory macrophages (top) versus the reverse electron transport (RET) phenomenon observed in lipopolysaccharide (LPS)-stimulated macrophages (bottom).

inflammasome activation in macrophages (97). Further evidence for the importance of mROS in macrophages arises from the role that uncoupling protein 2 (UCP2) appears to play. UCP2 is located at the inner mitochondrial membrane and unlike its homolog UCP1, which is expressed mainly in brown adipose tissue (98), UCP2 is highly expressed in macrophages along with other immune cells. UCP2 is believed to mitigate ROS levels in macrophages through uncoupling of OXPHOS. $UCP2^{-/-}$ mice were found to be more resistant to *Toxoplasma gondii* infection and macrophages from these knockout mice were found to have elevated of ROS compared with wild-type controls (99). As expected, IL-10 was found to exert the opposite effect on mROS compared with LPS and other TLR agonists. Treatment of macrophages with IL-10 promoted the abolition of dysfunctional mitochondria (characterized by elevated levels of ROS and lower mitochondrial membrane potential) through induction of mitophagy (15). The complexes of the ETC except for complex II (SDH) are known to be able to form supercomplexes in the mitochondrial inner membrane, which seems to change how the electrons are processed depending on the carbon source (100). Supercomplex formation is also thought to restrict mROS production (101). Macrophages were shown to disassemble these supercomplexes in response to bacterial detection, a process which was found to be dependent on TLR signaling and NLRP3 activation (102). Although complex II cannot form supercomplexes, it has been shown that phosphorylation of complex II by Fgr kinase is important in this disassembly (103).

In recent years, it has come to light that mROS in pro-inflammatory macrophages may be generated *via* reverse electron transport (RET) (**Figure 2**). The first evidence of RET giving rise to mROS was in oxygen sensing (104) and aging in *Drosophila melanogaster* (105). RET at complex I was also demonstrated to drive mROS production in reperfusion injury due to the accumulation of succinate and the elevated activity of SDH (106). Later RET was found to occur in macrophages when Mills et al. established that the build-up of succinate in LPS-stimulated macrophages and the oxidation of this succinate by SDH resulted in the production of mROS, seemingly from RET at complex I (107) (**Figure 2**). Definite roles have emerged for complex I and complex II in ROS production in M1 macrophages through studies in which inhibitors of these complexes were used. For example, Kelly et al. used metformin and rotenone to inhibit complex I in LPS-treated BMDMs, which markedly reduced mROS production as well as decreased IL-1β and increased IL-10 (108). However, another study used imiquimod and a similar molecule called CL097 to inhibit complex I and NAD(P)H dehydrogenase, quinone 2 (NQO2) and observed a boost in ROS production and NLRP3 activation (109). Inhibiting complex II (SDH) with dimethyl malonate (DMM) inhibits IL-1β production and raises IL-10 levels both in BMDMs and *in vivo*. Expression of alternative oxidase (AOX), which provides a different route for excess electrons so that ROS are not formed, prevents the inflammatory phenotype (107).

M1 macrophages also produce NO, which is induced upon HIF-1α activation (110) and contributes to the bactericidal and antitumor capacity of macrophages (111). NO is known to inhibit mitochondrial respiration *via* complex IV (cytochrome c oxidase) (112, 113), and it may also inhibit complex I through S-nitrosylation of thiol groups on the enzyme (114). This inhibition of mitochondrial OXPHOS has been found to prevent the repolarization of M1 macrophages to an anti-inflammatory M2 phenotype, although the reverse is possible. Inhibition of NO production was shown to significantly improve this repolarization (115). This observation could be clinically relevant as it may be desirable to repolarize M1 macrophages to M2 in the case of inflammatory diseases.

FATTY-ACID SYNTHESIS AND BETA OXIDATION

Overview

The synthesis and degradation of fatty acids occur by two separate processes in different parts of the cell. Fatty-acid synthesis takes place in the cytosol, using citrate from the TCA cycle as a substrate in a series of reactions catalyzed by fatty-acid synthase (FAS). Citrate leaves the mitochondria and the TCA cycle in exchange for malate with the help of the mitochondrial CIC. Cytosolic citrate is then broken down by ACLY into acetyl-CoA and oxaloacetate. While oxaloacetate can be converted back into malate by malate dehydrogenase (MDH) and re-enter the mitochondria, acetyl-CoA on the other hand is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl-CoA can then be polymerized by FAS in a series of repetitive reactions, growing by two carbons with each reaction until it reaches the 16 carbon length of palmitic acid. In addition, acetyl-CoA plays a central role in cholesterol synthesis through the cytosolic mevalonate pathway. Three molecules of acetyl-CoA are condensed to form 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), which in turn in converted into mevalonate by HMG-CoA reductase, followed by a series of reactions leading to cholesterol.

As beta oxidation or fatty-acid degradation takes place within the mitochondria, the first step involves transport of free cytosolic fatty acids across the mitochondrial membranes. This process starts when fatty acids are converted to acyl-CoA by acyl synthetase, and aided by carnitine palmitoyltransferase I (CPT1) in the outer membrane of the mitochondria, acyl then becomes bound to carnitine. The carnitine–acyl-CPT1 complex enters the mitochondrial matrix aided by acyl carnitine translocase and acyl is finally released with the help of carnitine palmitoyltransferase II (CPT-2), resulting in mitochondrial acyl-CoA. Acyl-CoA is then oxidized in a repetitive cyclic series of reactions with a net yield of each oxidation cycle being one NADH, one $FADH_2$, and one molecule of acetyl-CoA.

Malonyl-CoA generated during fatty-acid synthesis serves as a key regulatory feedback loop for beta oxidation as it inhibits the rate-limiting enzyme CPT1, thereby preventing cytosolic fatty acids from binding carnitine and entering the mitochondria where beta oxidation takes place.

Fatty-Acid Synthesis and Macrophage Function

Overall, fatty-acid synthesis is closely linked to pro-inflammatory effector functions of macrophages. We and others have shown an

increase in citrate and fatty acids in LPS-activated macrophages (28, 52). Feingold et al. demonstrated that glucose-derived carbons generated through an increased rate of glycolysis in LPS-activated macrophages were preferentially incorporated into fatty acids and sterols (116). In addition, LPS-activated macrophages, as well as macrophages associated with atherosclerosis, so-called foam cells, display an increased accumulation of triglycerides and cholesterol esters which may contribute to the pathogenesis of chronic inflammatory diseases (117–119). This accumulation is in large due to increased *de novo* synthesis of fatty acids, coupled to a robust increase in several of the key enzymes involved in glycerol lipid synthesis including glutamic-pyruvic transaminase (GPT3), Lipin 1, and diacylglycerol O-acyl transferase 2 (DGAT2). Paired with the marked increase in fatty-acid synthesis observed in LPS-stimulated macrophages is a marked decrease in fatty-acid oxidation, linked with suppressed expression of CPT1 (116). Differentiation of monocytes is linked with an M-CSF-stimulated upregulation of genes required for fatty-acid synthesis, and a switch in major lipid synthesis class from cholesterol in monocytes to phosphatidylcholine in macrophages. This induction of fatty-acid synthesis is critical for monocyte differentiation and phagocytic activity of macrophages. A newly identified protein named FAMIN was found to associate with FAS on peroxisomes and regulate *de novo* lipogenesis. Interestingly, FAMIN was identified through single-nucleotide polymorphisms (SNPs) associated with inflammatory diseases and was found to be essential for the production of pro-inflammatory cytokines and ROS, as well inflammasome activation in LPS-stimulated macrophages and in a murine model of sepsis (120).

Beta Oxidation and Macrophage Function

In a similar manner to fatty-acid synthesis being coupled to pro-inflammatory macrophages, beta oxidation is synonymous with anti-inflammatory macrophages. Lipolysis liberates free fatty acids, which are taken up by the macrophage by fatty-acid transporters such as CD36, thereby fueling mitochondrial OXPHOS. In IL-4-stimulated macrophages, this metabolic switch is largely mediated through STAT6 and PGC1β (92). Alternatively activated anti-inflammatory M2 macrophages display increased expression of CPT-1, CD36, and medium-chain acyl coenzyme A dehydrogenase (MCAD) (92). M2 polarization depends on lysosomal acid lipase (LAL)-mediated lipolysis as demonstrated by a blocked protective M2 response during parasitic helminth infection (121).

Malonyl-CoA from the TCA regulates fatty-acid oxidation by binding to CPT1, thereby making this the rate-limiting step in beta oxidation. Inflammatory macrophages of adipose tissue contribute to obesity-induced insulin resistance triggered by fatty acids and a range of other stimuli including ROS and pro-inflammatory cytokines. Promoting increased fatty-acid oxidation by over expressing CPT-1 in human adipose tissue macrophages promoted fatty-acid oxidation causing reduced inflammatory responses, as well as improved insulin sensitivity of adipocytes, reduced endoplasmic reticulum stress and less ROS damage in macrophages (122).

Taken together, this indicates that boosting fatty-acid oxidation in inflammatory macrophages would have beneficial anti-inflammatory effects. However, recent studies suggest that the assumption that fatty-acid oxidation is purely anti-inflammatory may be an over simplification. NLRP3 is an important component of one of the large multiprotein inflammasomes. Assembly of the NLRP3 inflammasome occurs in response to a range of different stimuli including viruses, components of bacteria as well as bacterial toxins, liposomes, and cholesterol crystals (123–128). Interestingly, NLRP3 is also activated by palmitate, which through oxidation *via* CPT1, fuels mitochondrial respiration, subsequent production of ROS, and activation of NLRP3 (129–131). Hence, in addition to LPS-activated macrophages requiring fatty-acid synthesis and FAS for adequate activation of NLRP3, oxidation of palmitate is also required for mitochondrial ROS activation of NLRP3. In addition, NLRP3 activation can be inhibited by modulating the activity of NADPH oxidase 4 (NOX4) (129). NOX4 regulates CPT1A activity and fatty-acid oxidation, and inhibition of NOX4 leads to suppressed NLRP3 activity and reduced secretion of IL-1β and IL-18 *in vitro* as well as *in vivo* (132).

Furthermore, as expected, macrophages generated from mice lacking CPT2 displayed impaired fatty-acid oxidation. Surprisingly, however, this did not affect their response to IL-4 polarization (133), implying that fatty-acid oxidation is not required for differentiation of M2 macrophages and that the role for fatty-acid oxidation here is more complex than originally proposed.

AMINO-ACID METABOLISM

Overview

The metabolism of amino acids plays an important role in many cellular processes where free amino acids are used as building blocks for not only protein synthesis but also for *de novo* synthesis of branched chain fatty acids, as in the case of valine and leucine, while glutamine and aspartate are used for purine and pyrimidine synthesis. Furthermore, cellular as well as dietary amino-acid catabolism can be used to support ATP production or as a source of citrate for fatty-acid synthesis. An initial step of transamination, resulting in α-ketoacids, allows for the carbon skeleton of the amino acid to enter the TCA cycle at one of multiple points such as α-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, pyruvate, or acetyl-CoA, thereby providing fuel in times of superfluous cellular amino acids.

Amino-Acid Metabolism and Macrophage Function

Cells, including macrophages, of higher vertebrates can synthesize 11 of the 20 amino acids. Dietary intake and protein salvage pathways are the only source of the remaining nine essential amino acids. Three of the essential amino acids, leucine, valine, and isoleucine, are the so-called branched-chain amino acids (BCAAs) with diverse roles outside of nutrition, such as regulation of protein degradation and synthesis in skeletal muscle, as well as regulation of synthesis of neurotransmitters such as serotonin in the brain, thereby affecting behavior. BCAAs also facilitate glucose uptake by the liver and skeletal muscles as well as enhance glycogen synthesis.

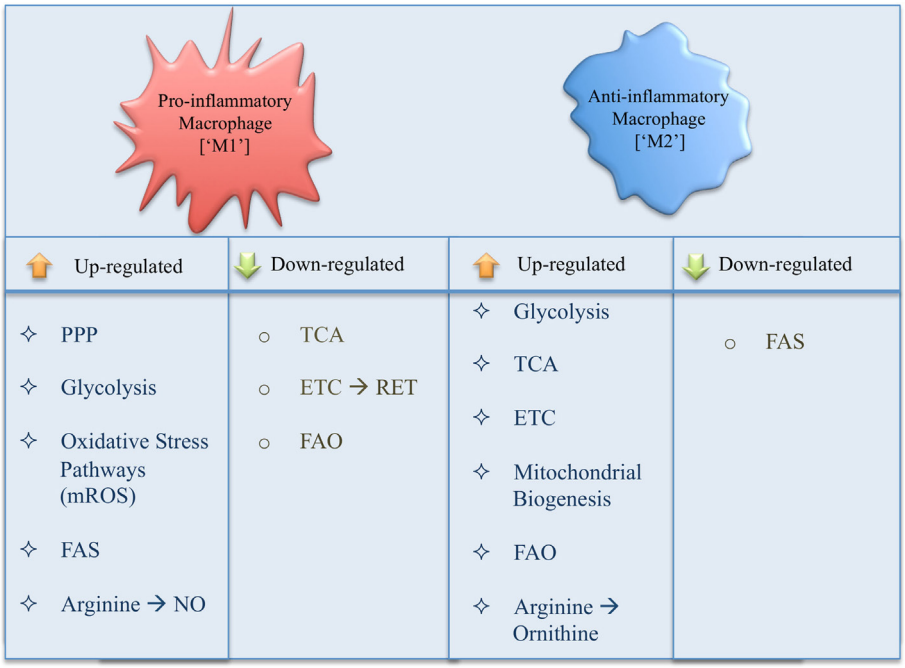

FIGURE 3 | Summary of changes in metabolic pathways occurring in a pro- versus anti-inflammatory macrophage.

The first evidence that amino-acid metabolism can regulate macrophage effector function came with the discovery that macrophages block tumor growth through the consumption of arginine leading to the production of NO (134–138). Since then we have learned that availability and metabolism of several other amino acids such as glutamine and tryptophan also regulate macrophage immune function. The serine/threonine kinase mTOR forms two complexes, mTORC1 and mTORC2, and has been shown to be an important regulator in both innate and adaptive immune cells (139). mTOR plays a key role in macrophages, providing a link between amino-acid availability, coupling this to growth, proliferation, and protein synthesis. Branched-chain ketoacids (BCKAs), a product of BCAA catabolism, has been shown to directly regulate macrophage function by reducing the phagocytic ability of TAMs (140). Further evidence for a role for BCAA comes from data demonstrating that BCAT1, the enzyme responsible for the first step in BCAA catabolism, regulates the metabolic reprogramming in human macrophages. Inhibition of BCAT1 results in decreased glycolysis, oxygen consumption, IRG1 expression, as well as itaconate levels (141). In the interest of space, we will here briefly discuss the role of arginine in macrophage function [amino-acid metabolism in immunity has been reviewed in Ref. (142, 143)].

Arginine

L-arginine has several key roles in macrophage function. During inflammation, macrophages are responsible for the majority of ROS and nitrogen species produced including NO. This NO production in macrophages, in response to LPS and IFN-γ as well as ILs such as IL-13, requires extracellular L-arginine which enters the cells through specific transmembrane transporters (144–152). Arginine uptake is regulated by pro-inflammatory signals such as IL-1β (149). Once inside the cell, apart from being a precursor in protein synthesis, arginine also acts as a substrate for multiple enzymes including inducible nitric-oxide synthase (iNOS) and arginase, resulting in the production of NO and citrulline, respectively. Both of these metabolic pathways are utilized in macrophages with great opposing effects on immune function, with M1 macrophages utilizing arginine through iNOS resulting in pro-inflammatory NO, and M2 macrophages fluxing arginine *via* arginase resulting in citrulline and a tolerant phenotype associated with wound healing (153). Furthermore, arginase expression in macrophages is linked to limiting inflammatory effector T cell function, as well as correlating with disease severity in visceral leishmaniasis and HIV infection (154, 155). Arginine supplies required for efficient NO output can be restricted by arginase activity, though macrophages can circumvent this by converting L-citrulline to L-arginine, thereby restoring intracellular availability of arginine. However, L-arginine generated in this manner is less effective as a substrate for arginase-derived L-ornithine production of the urea cycle compared with L-arginine originating from the extracellular milieu (156). Arginine metabolism in myeloid cells has been reviewed in depth by Rodriquez et al. (157).

CONCLUDING REMARKS

The ever-growing literature on immunometabolism demonstrates new roles for metabolic pathways as well as specific pathway intermediates in the metabolic reprogramming of macrophages, leading to profound changes in immune effector functions (**Figure 3**). In order to simplify and provide an overview of our

current understanding of immunometabolism in macrophages, we have here described each pathway as a separate entity; however, in reality these processes are intimately linked. In addition, using a simplified interpretation of macrophage activation gives us a general view of metabolism in inflammatory versus anti-inflammatory macrophages, while reality proves more complex with a spectrum of macrophage subsets occurring in disease. Many questions are still outstanding; however, by its very nature immunometabolism has already firmly established itself as a field, which will provide us with future therapeutic targets for the treatment of immune disorders.

AUTHOR CONTRIBUTIONS

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

FUNDING

This work was funded by Science Foundation Ireland, and Wellcome Trust, UK. Wellcome Trust (Award number(s): 205455/14107); Science Foundation Ireland (Award number(s): 12/IA/1531).

REFERENCES

1. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* (2014) 40(2):274–88. doi:10.1016/j.immuni.2014.01.006
2. Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, Lu W, et al. Glucose feeds the TCA cycle via circulating lactate. *Nature* (2017) 551 (7678):115–8. doi:10.1038/nature24057
3. Newsholme P, Curi R, Gordon S, Newsholme EA. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem J* (1986) 239(1):121–5. doi:10.1042/bj2390121
4. Alonso D, Nungester WJ. Comparative study of host resistance of guinea pigs and rats. V. The effect of pneumococcal products on glycolysis and oxygen uptake by polymorphonuclear leucocytes. *J Infect Dis* (1956) 99(2):174–81. doi:10.1093/infdis/99.2.174
5. Oren R, Farnham AE, Saito K, Milofsky E, Karnovsky ML. Metabolic patterns in three types of phagocytizing cells. *J Cell Biol* (1963) 17:487–501. doi:10.1083/jcb.17.3.487
6. Michl J, Ohlbaum DJ, Silverstein SC. 2-Deoxyglucose selectively inhibits Fc and complement receptor-mediated phagocytosis in mouse peritoneal macrophages II. Dissociation of the inhibitory effects of 2-deoxyglucose on phagocytosis and ATP generation. *J Exp Med* (1976) 144(6):1484–93. doi:10.1084/jem.144.6.1465
7. Pavlou S, Wang L, Xu H, Chen M. Higher phagocytic activity of thioglycollate-elicited peritoneal macrophages is related to metabolic status of the cells. *J Inflamm (Lond)* (2017) 14:4. doi:10.1186/s12950-017-0151-x
8. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, et al. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem* (2014) 289(11):7884–96. doi:10.1074/jbc.M113.522037
9. Fukuzumi M, Shinomiya H, Shimizu Y, Ohishi K, Utsumi S. Endotoxin-induced enhancement of glucose influx into murine peritoneal macrophages via GLUT1. *Infect Immun* (1996) 64(1):108–12.
10. Nagy C, Haschemi A. Time and demand are two critical dimensions of immunometabolism: the process of macrophage activation and the pentose phosphate pathway. *Front Immunol* (2015) 6:164. doi:10.3389/fimmu.2015.00164
11. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* (2016) 16(9):553–65. doi:10.1038/nri.2016.70
12. Rodriguez-Prados JC, Traves PG, Cuenca J, Rico D, Aragones J, Martin-Sanz P, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol* (2010) 185(1):605–14. doi:10.4049/jimmunol.0901698
13. Van den Bossche J, Baardman J, de Winther MP. Metabolic characterization of polarized M1 and M2 bone marrow-derived macrophages using real-time extracellular flux analysis. *J Vis Exp* (2015) (105):e53424. doi:10.3791/53424
14. Cheng SC, Scicluna BP, Arts RJ, Gresnigt MS, Lachmandas E, Giamarellos-Bourboulis EJ, et al. Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat Immunol* (2016) 17(4):406–13. doi:10.1038/ni.3398
15. Ip WKE, Hoshi N, Shouval DS, Snapper S, Medzhitov R. Anti-inflammatory effect of IL-10 mediated by metabolic reprogramming of macrophages. *Science* (2017) 356(6337):513–9. doi:10.1126/science.aal3535
16. Baseler WA, Davies LC, Quigley L, Ridnour LA, Weiss JM, Hussain SP, et al. Autocrine IL-10 functions as a rheostat for M1 macrophage glycolytic commitment by tuning nitric oxide production. *Redox Biol* (2016) 10:12–23. doi:10.1016/j.redox.2016.09.005
17. Huang LE, Arany Z, Livingston DM, Bunn HF. Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. *J Biol Chem* (1996) 271(50):32253–9. doi:10.1074/jbc.271.50.32253
18. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, et al. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* (1996) 271(51):32529–37. doi:10.1074/jbc.271.51.32529
19. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* (1999) 399(6733):271–5. doi:10.1038/20459
20. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science* (2001) 292(5516):468–72. doi:10.1126/science.1059796
21. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIFalpha targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. *Science* (2001) 292(5516):464–8. doi:10.1126/science.1059817
22. Bruick RK, McKnight SL. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* (2001) 294(5545):1337–40. doi:10.1126/science.1066373
23. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* (2006) 3(3):177–85. doi:10.1016/j.cmet.2006.02.002
24. Blouin CC, Page EL, Soucy GM, Richard DE. Hypoxic gene activation by lipopolysaccharide in macrophages: implication of hypoxia-inducible factor 1alpha. *Blood* (2004) 103(3):1124–30. doi:10.1182/blood-2003-07-2427
25. Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, et al. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. *Nature* (2008) 453(7196):807–11. doi:10.1038/nature06905
26. Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, et al. mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* (2014) 345(6204):1250684. doi:10.1126/science.1250684
27. Semba H, Takeda N, Isagawa T, Sugiura Y, Honda K, Wake M, et al. HIF-1alpha-PDK1 axis-induced active glycolysis plays an essential role in macrophage migratory capacity. *Nat Commun* (2016) 7:11635. doi:10.1038/ncomms11635
28. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, et al. Succinate is an inflammatory signal that induces IL-1beta

through HIF-1alpha. *Nature* (2013) 496(7444):238–42. doi:10.1038/nature11986
29. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, et al. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity* (2016) 45(4):817–30. doi:10.1016/j.immuni.2016.09.016
30. Tavakoli S, Short JD, Downs K, Nguyen HN, Lai Y, Zhang W, et al. Differential regulation of macrophage glucose metabolism by macrophage colony-stimulating factor and granulocyte-macrophage colony-stimulating factor: implications for 18F FDG PET imaging of vessel wall inflammation. *Radiology* (2017) 283(1):87–97. doi:10.1148/radiol.2016160839
31. Tan Z, Xie N, Cui H, Moellering DR, Abraham E, Thannickal VJ, et al. Pyruvate dehydrogenase kinase 1 participates in macrophage polarization via regulating glucose metabolism. *J Immunol* (2015) 194(12):6082–9. doi:10.4049/jimmunol.1402469
32. Moon JS, Hisata S, Park MA, DeNicola GM, Ryter SW, Nakahira K, et al. mTORC1-induced HK1-dependent glycolysis regulates NLRP3 inflammasome activation. *Cell Rep* (2015) 12(1):102–15. doi:10.1016/j.celrep.2015.05.046
33. Wolf AJ, Reyes CN, Liang W, Becker C, Shimada K, Wheeler ML, et al. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell* (2016) 166(3):624–36. doi:10.1016/j.cell.2016.05.076
34. Nagy E, Rigby WF. Glyceraldehyde-3-phosphate dehydrogenase selectively binds AU-rich RNA in the NAD(+)-binding region (Rossmann fold). *J Biol Chem* (1995) 270(6):2755–63. doi:10.1074/jbc.270.6.2755
35. Arif A, Chatterjee P, Moodt RA, Fox PL. Heterotrimeric GAIT complex drives transcript-selective translation inhibition in murine macrophages. *Mol Cell Biol* (2012) 32(24):5046–55. doi:10.1128/MCB.01168-12
36. Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* (2013) 153(6):1239–51. doi:10.1016/j.cell.2013.05.016
37. Millet P, Vachharajani V, McPhail L, Yoza B, McCall CE. GAPDH Binding to TNF-alpha mRNA contributes to posttranscriptional repression in monocytes: a novel mechanism of communication between inflammation and metabolism. *J Immunol* (2016) 196(6):2541–51. doi:10.4049/jimmunol.1501345
38. Bae S, Kim H, Lee N, Won C, Kim HR, Hwang YI, et al. alpha-Enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. *J Immunol* (2012) 189(1):365–72. doi:10.4049/jimmunol.1102073
39. Luo W, Semenza GL. Pyruvate kinase M2 regulates glucose metabolism by functioning as a coactivator for hypoxia-inducible factor 1 in cancer cells. *Oncotarget* (2011) 2(7):551–6. doi:10.18632/oncotarget.299
40. Yang L, Xie M, Yang M, Yu Y, Zhu S, Hou W, et al. PKM2 regulates the warburg effect and promotes HMGB1 release in sepsis. *Nat Commun* (2014) 5:4436. doi:10.1038/ncomms5436
41. Palsson-McDermott EM, Curtis AM, Goel G, Lauterbach MA, Sheedy FJ, Gleeson LE, et al. Pyruvate kinase M2 regulates Hif-1alpha activity and IL-1beta induction and is a critical determinant of the Warburg effect in LPS-activated macrophages. *Cell Metab* (2015) 21(1):65–80. doi:10.1016/j.cmet.2015.01.017
42. Xie M, Yu Y, Kang R, Zhu S, Yang L, Zeng L, et al. PKM2-dependent glycolysis promotes NLRP3 and AIM2 inflammasome activation. *Nat Commun* (2016) 7:13280. doi:10.1038/ncomms13280
43. Lu B, Nakamura T, Inouye K, Li J, Tang Y, Lundback P, et al. Novel role of PKR in inflammasome activation and HMGB1 release. *Nature* (2012) 488(7413):670–4. doi:10.1038/nature11290
44. Shirai T, Nazarewicz RR, Wallis BB, Yanes RE, Watanabe R, Hilhorst M, et al. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *J Exp Med* (2016) 213(3):337–54. doi:10.1084/jem.20150900
45. Palsson-McDermott EM, Dyck L, Zaslona Z, Menon D, McGettrick AF, Mills KHG, et al. Pyruvate Kinase M2 is required for the expression of the immune checkpoint PD-L1 in immune cells and tumors. *Front Immunol* (2017) 8:1300. doi:10.3389/fimmu.2017.01300
46. Callahan MK, Postow MA, Wolchok JD. Targeting T cell co-receptors for cancer therapy. *Immunity* (2016) 44(5):1069–78. doi:10.1016/j.immuni.2016.04.023
47. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* (2014) 513(7519):559–63. doi:10.1038/nature13490
48. Liu D, Chang C, Lu N, Wang X, Lu Q, Ren X, et al. Comprehensive proteomics analysis reveals metabolic reprogramming of tumor-associated macrophages stimulated by the tumor microenvironment. *J Proteome Res* (2017) 16(1):288–97. doi:10.1021/acs.jproteome.6b00604
49. Wenes M, Shang M, Di Matteo M, Goveia J, Martin-Perez R, Serneels J, et al. Macrophage Metabolism controls tumor blood vessel morphogenesis and metastasis. *Cell Metab* (2016) 24(5):701–15. doi:10.1016/j.cmet.2016.09.008
50. Penny HL, Sieow JL, Adriani G, Yeap WH, See Chi Ee P, San Luis B, et al. Warburg metabolism in tumor-conditioned macrophages promotes metastasis in human pancreatic ductal adenocarcinoma. *Oncoimmunology* (2016) 5(8):e1191731. doi:10.1080/2162402X.2016.1191731
51. Rabold K, Netea MG, Adema GJ, Netea-Maier RT. Cellular metabolism of tumor-associated macrophages – functional impact and consequences. *FEBS Lett* (2017) 591(19):3022–41. doi:10.1002/1873-3468.12771
52. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* (2015) 42(3):419–30. doi:10.1016/j.immuni.2015.02.005
53. Ray PD, Huang BW, Tsuji Y. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* (2012) 24(5):981–90. doi:10.1016/j.cellsig.2012.01.008
54. Haschemi A, Kosma P, Gille L, Evans CR, Burant CF, Starkl P, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab* (2012) 15(6):813–26. doi:10.1016/j.cmet.2012.04.023
55. Hard GC. Some biochemical aspects of the immune macrophage. *Br J Exp Pathol* (1970) 51(1):97–105.
56. Meiser J, Kramer L, Sapcariu SC, Battello N, Ghelfi J, D'Herouel AF, et al. Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. *J Biol Chem* (2016) 291(8):3932–46. doi:10.1074/jbc.M115.676817
57. Ryan DG, O'Neill LAJ. Krebs cycle rewired for macrophage and dendritic cell effector functions. *FEBS Lett* (2017) 591(19):2992–3006. doi:10.1002/1873-3468.12744
58. Infantino V, Convertini P, Cucci L, Panaro MA, Di Noia MA, Calvello R, et al. The mitochondrial citrate carrier: a new player in inflammation. *Biochem J* (2011) 438(3):433–6. doi:10.1042/BJ20111275
59. Infantino V, Iacobazzi V, Menga A, Avantaggiati ML, Palmieri F. A key role of the mitochondrial citrate carrier (SLC25A1) in TNFalpha- and IFNgamma-triggered inflammation. *Biochim Biophys Acta* (2014) 1839(11):1217–25. doi:10.1016/j.bbagrm.2014.07.013
60. Infantino V, Iacobazzi V, Palmieri F, Menga A. ATP-citrate lyase is essential for macrophage inflammatory response. *Biochem Biophys Res Commun* (2013) 440(1):105–11. doi:10.1016/j.bbrc.2013.09.037
61. Covarrubias AJ, Aksoylar HI, Yu J, Snyder NW, Worth AJ, Iyer SS, et al. Akt-mTORC1 signaling regulates Acly to integrate metabolic input to control of macrophage activation. *Elife* (2016) 9:5. doi:10.7554/eLife.11612
62. Zaslona Z, Palsson-McDermott EM, Menon D, Haneklaus M, Flis E, Prendeville H, et al. The Induction of Pro-IL-1beta by lipopolysaccharide requires endogenous prostaglandin E2 production. *J Immunol* (2017) 198(9):3558–64. doi:10.4049/jimmunol.1602072
63. Hu L, Yu Y, Huang H, Fan H, Hu L, Yin C, et al. Epigenetic regulation of Interleukin 6 by histone acetylation in macrophages and its role in paraquat-induced pulmonary fibrosis. *Front Immunol* (2016) 7:696. doi:10.3389/fimmu.2016.00696
64. Wang B, Rao YH, Inoue M, Hao R, Lai CH, Chen D, et al. Microtubule acetylation amplifies p38 kinase signalling and anti-inflammatory IL-10 production. *Nat Commun* (2014) 5:3479. doi:10.1038/ncomms4479
65. Koivunen P, Hirsila M, Remes AM, Hassinen IE, Kivirikko KI, Myllyharju J. Inhibition of hypoxia-inducible factor (HIF) hydroxylases by citric acid cycle intermediates: possible links between cell metabolism and stabilization of HIF. *J Biol Chem* (2007) 282(7):4524–32. doi:10.1074/jbc.M610415200
66. Peng C, Lu Z, Xie Z, Cheng Z, Chen Y, Tan M, et al. The first identification of lysine malonylation substrates and its regulatory enzyme. *Mol Cell Proteomics* (2011) 10(12):M111012658. doi:10.1074/mcp.M111.012658

67. Hirschey MD, Zhao Y. Metabolic regulation by lysine malonylation, succinylation, and glutarylation. *Mol Cell Proteomics* (2015) 14(9):2308–15. doi:10.1074/mcp.R114.046664
68. Du Y, Cai T, Li T, Xue P, Zhou B, He X, et al. Lysine malonylation is elevated in type 2 diabetic mouse models and enriched in metabolic associated proteins. *Mol Cell Proteomics* (2015) 14(1):227–36. doi:10.1074/mcp.M114.041947
69. Strelko CL, Lu W, Dufort FJ, Seyfried TN, Chiles TC, Rabinowitz JD, et al. Itaconic acid is a mammalian metabolite induced during macrophage activation. *J Am Chem Soc* (2011) 133(41):16386–9. doi:10.1021/ja2070889
70. McFadden BA, Purohit S. Itaconate, an isocitrate lyase-directed inhibitor in *Pseudomonas indigofera*. *J Bacteriol* (1977) 131(1):136–44.
71. Michelucci A, Cordes T, Ghelfi J, Pailot A, Reiling N, Goldmann O, et al. Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proc Natl Acad Sci U S A* (2013) 110(19):7820–5. doi:10.1073/pnas.1218599110
72. Naujoks J, Tabeling C, Dill BD, Hoffmann C, Brown AS, Kunze M, et al. IFNs modify the proteome of *Legionella*-containing vacuoles and restrict infection Via IRG1-derived itaconic acid. *PLoS Pathog* (2016) 12(2):e1005408. doi:10.1371/journal.ppat.1005408
73. Sasikaran J, Ziemski M, Zadora PK, Fleig A, Berg IA. Bacterial itaconate degradation promotes pathogenicity. *Nat Chem Biol* (2014) 10(5):371–7. doi:10.1038/nchembio.1482
74. Shin JH, Yang JY, Jeon BY, Yoon YJ, Cho SN, Kang YH, et al. (1)H NMR-based metabolomic profiling in mice infected with *Mycobacterium tuberculosis*. *J Proteome Res* (2011) 10(5):2238–47. doi:10.1021/pr101054m
75. Sugimoto M, Sakagami H, Yokote Y, Onuma H, Kaneko M, Mori M, et al. Non-targeted metabolite profiling in activated macrophage secretion. *Metabolomics* (2012) 8(4):624–33. doi:10.1007/s11306-011-0353-9
76. Lee CG, Jenkins NA, Gilbert DJ, Copeland NG, O'Brien WE. Cloning and analysis of gene regulation of a novel LPS-inducible cDNA. *Immunogenetics* (1995) 41(5):263–70. doi:10.1007/BF00172150
77. Li Y, Zhang P, Wang C, Han C, Meng J, Liu X, et al. Immune responsive gene 1 (IRG1) promotes endotoxin tolerance by increasing A20 expression in macrophages through reactive oxygen species. *J Biol Chem* (2013) 288(23):16225–34. doi:10.1074/jbc.M113.454538
78. Jamal Uddin M, Joe Y, Kim SK, Oh Jeong S, Ryter SW, Pae HO, et al. IRG1 induced by heme oxygenase-1/carbon monoxide inhibits LPS-mediated sepsis and pro-inflammatory cytokine production. *Cell Mol Immunol* (2016) 13(2):170–9. doi:10.1038/cmi.2015.02
79. Cheon YP, Xu X, Bagchi MK, Bagchi IC. Immune-responsive gene 1 is a novel target of progesterone receptor and plays a critical role during implantation in the mouse. *Endocrinology* (2003) 144(12):5623–30. doi:10.1210/en.2003-0585
80. Sherwin JR, Freeman TC, Stephens RJ, Kimber S, Smith AG, Chambers I, et al. Identification of genes regulated by leukemia-inhibitory factor in the mouse uterus at the time of implantation. *Mol Endocrinol* (2004) 18(9):2185–95. doi:10.1210/me.2004-0110
81. Degrandi D, Hoffmann R, Beuter-Gunia C, Pfeffer K. The proinflammatory cytokine-induced IRG1 protein associates with mitochondria. *J Interferon Cytokine Res* (2009) 29(1):55–67. doi:10.1089/jir.2008.0013
82. Nemeth B, Doczi J, Csete D, Kacso G, Ravasz D, Adams D, et al. Abolition of mitochondrial substrate-level phosphorylation by itaconic acid produced by LPS-induced Irg1 expression in cells of murine macrophage lineage. *FASEB J* (2016) 30(1):286–300. doi:10.1096/fj.15-279398
83. Cordes T, Wallace M, Michelucci A, Divakaruni AS, Sapcariu SC, Sousa C, et al. Immunoresponsive Gene 1 and itaconate inhibit succinate dehydrogenase to modulate intracellular succinate levels. *J Biol Chem* (2016) 291(27):14274–84. doi:10.1074/jbc.M115.685792
84. Lampropoulou V, Sergushichev A, Bambouskova M, Nair S, Vincent EE, Loginicheva E, et al. Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metab* (2016) 24(1):158–66. doi:10.1016/j.cmet.2016.06.004
85. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, et al. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. *Cancer Cell* (2005) 7(1):77–85. doi:10.1016/j.ccr.2004.11.022
86. He W, Miao FJ, Lin DC, Schwandner RT, Wang Z, Gao J, et al. Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature* (2004) 429(6988):188–93. doi:10.1038/nature02488
87. Littlewood-Evans A, Sarret S, Apfel V, Loesle P, Dawson J, Zhang J, et al. GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. *J Exp Med* (2016) 213(9):1655–62. doi:10.1084/jem.20160061
88. Rubic-Schneider T, Carballido-Perrig N, Regairaz C, Raad L, Jost S, Rauld C, et al. GPR91 deficiency exacerbates allergic contact dermatitis while reducing arthritic disease in mice. *Allergy* (2017) 72(3):444–52. doi:10.1111/all.13005
89. van Diepen JA, Robben JH, Hooiveld GJ, Carmone C, Alsady M, Boutens L, et al. SUCNR1-mediated chemotaxis of macrophages aggravates obesity-induced inflammation and diabetes. *Diabetologia* (2017) 60(7):1304–13. doi:10.1007/s00125-017-4261-z
90. Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. *Cell Metab* (2016) 24(6):807–19. doi:10.1016/j.cmet.2016.10.008
91. Liu PS, Wang H, Li X, Chao T, Teav T, Christen S, et al. alpha-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. *Nat Immunol* (2017) 18(9):985–94. doi:10.1038/ni.3796
92. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* (2006) 4(1):13–24. doi:10.1016/j.cmet.2006.08.006
93. Feng J, Li L, Ou Z, Li Q, Gong B, Zhao Z, et al. IL-25 stimulates M2 macrophage polarization and thereby promotes mitochondrial respiratory capacity and lipolysis in adipose tissues against obesity. *Cell Mol Immunol* (2017) 14:1–13. doi:10.1038/cmi.2016.71
94. Duroux-Richard I, Roubert C, Ammari M, Presumey J, Grun JR, Haupl T, et al. miR-125b controls monocyte adaptation to inflammation through mitochondrial metabolism and dynamics. *Blood* (2016) 128(26):3125–36. doi:10.1182/blood-2016-02-697003
95. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* (2011) 472(7344):476–80. doi:10.1038/nature09973
96. Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim KY, et al. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). *J Exp Med* (2011) 208(3):519–33. doi:10.1084/jem.20102049
97. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature* (2011) 469(7329):221–5. doi:10.1038/nature09663
98. Ricquier D, Bouillaud F. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem J* (2000) 345(Pt 2):161–79. doi:10.1042/0264-6021:3450161
99. Arsenijevic D, Onuma H, Pecqueur C, Raimbault S, Manning BS, Miroux B, et al. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat Genet* (2000) 26(4):435–9. doi:10.1038/82565
100. Lapuente-Brun E, Moreno-Loshuertos R, Acin-Perez R, Latorre-Pellicer A, Colas C, Balsa E, et al. Supercomplex assembly determines electron flux in the mitochondrial electron transport chain. *Science* (2013) 340(6140):1567–70. doi:10.1126/science.1230381
101. Maranzana E, Barbero G, Falasca AI, Lenaz G, Genova ML. Mitochondrial respiratory supercomplex association limits production of reactive oxygen species from complex I. *Antioxid Redox Signal* (2013) 19(13):1469–80. doi:10.1089/ars.2012.4845
102. Garaude J, Acin-Perez R, Martinez-Cano S, Enamorado M, Ugolini M, Nistal-Villan E, et al. Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense. *Nat Immunol* (2016) 17(9):1037–45. doi:10.1038/ni.3509
103. Acin-Perez R, Carrascoso I, Baixauli F, Roche-Molina M, Latorre-Pellicer A, Fernandez-Silva P, et al. ROS-triggered phosphorylation of complex II by Fgr kinase regulates cellular adaptation to fuel use. *Cell Metab* (2014) 19(6):1020–33. doi:10.1016/j.cmet.2014.04.015

104. Fernandez-Aguera MC, Gao L, Gonzalez-Rodriguez P, Pintado CO, Arias-Mayenco I, Garcia-Flores P, et al. Oxygen sensing by arterial chemoreceptors depends on mitochondrial complex I signaling. *Cell Metab* (2015) 22(5):825–37. doi:10.1016/j.cmet.2015.09.004
105. Scialo F, Sriram A, Fernandez-Ayala D, Gubina N, Lohmus M, Nelson G, et al. Mitochondrial ROS produced via reverse electron transport extend animal lifespan. *Cell Metab* (2016) 23(4):725–34. doi:10.1016/j.cmet.2016.03.009
106. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature* (2014) 515(7527):431–5. doi:10.1038/nature13909
107. Mills EL, Kelly B, Logan A, Costa AS, Varma M, Bryant CE, et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell* (2016) 167(2):457–70e13. doi:10.1016/j.cell.2016.08.064
108. Kelly B, Tannahill GM, Murphy MP, O'Neill LA. Metformin inhibits the production of reactive oxygen species from NADH:ubiquinone oxidoreductase to limit induction of interleukin-1beta (IL-1beta) and boosts interleukin-10 (IL-10) in lipopolysaccharide (LPS)-activated macrophages. *J Biol Chem* (2015) 290(33):20348–59. doi:10.1074/jbc.M115.662114
109. Gross CJ, Mishra R, Schneider KS, Medard G, Wettmarshausen J, Dittlein DC, et al. K+ Efflux-independent NLRP3 inflammasome activation by small molecules targeting mitochondria. *Immunity* (2016) 45(4):761–73. doi:10.1016/j.immuni.2016.08.010
110. Takeda N, O'Dea EL, Doedens A, Kim JW, Weidemann A, Stockmann C, et al. Differential activation and antagonistic function of HIF-{alpha} isoforms in macrophages are essential for NO homeostasis. *Genes Dev* (2010) 24(5):491–501. doi:10.1101/gad.1881410
111. Lorsbach RB, Murphy WJ, Lowenstein CJ, Snyder SH, Russell SW. Expression of the nitric oxide synthase gene in mouse macrophages activated for tumor cell killing. Molecular basis for the synergy between interferon-gamma and lipopolysaccharide. *J Biol Chem* (1993) 268(3):1908–13.
112. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett* (1994) 356(2–3):295–8. doi:10.1016/0014-5793(94)01290-3
113. Cleeter MW, Cooper JM, Darley-Usmar VM, Moncada S, Schapira AH. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. implications for neurodegenerative diseases. *FEBS lett* (1994) 345(1):50–4.
114. Clementi E, Brown GC, Feelisch M, Moncada S. Persistent inhibition of cell respiration by nitric oxide: crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proc Natl Acad Sci U S A* (1998) 95(13):7631–6. doi:10.1073/pnas.95.13.7631
115. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, et al. Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Rep* (2016) 17(3):684–96. doi:10.1016/j.celrep.2016.09.008
116. Feingold KR, Shigenaga JK, Kazemi MR, McDonald CM, Patzek SM, Cross AS, et al. Mechanisms of triglyceride accumulation in activated macrophages. *J Leukoc Biol* (2012) 92(4):829–39. doi:10.1189/jlb.1111537
117. Funk JL, Feingold KR, Moser AH, Grunfeld C. Lipopolysaccharide stimulation of RAW 264.7 macrophages induces lipid accumulation and foam cell formation. *Atherosclerosis* (1993) 98(1):67–82. doi:10.1016/0021-9150(93)90224-I
118. Lopes-Virella MF, Klein RL, Stevenson HC. Low density lipoprotein metabolism in human macrophages stimulated with microbial or microbial-related products. *Arteriosclerosis* (1987) 7(2):176–84. doi:10.1161/01.ATV.7.2.176
119. Oiknine J, Aviram M. Increased susceptibility to activation and increased uptake of low density lipoprotein by cholesterol-loaded macrophages. *Arterioscler Thromb* (1992) 12(6):745–53. doi:10.1161/01.ATV.12.6.745
120. Cader MZ, Boroviak K, Zhang Q, Assadi G, Kempster SL, Sewell GW, et al. C13orf31 (FAMIN) is a central regulator of immunometabolic function. *Nat Immunol* (2016) 17(9):1046–56. doi:10.1038/ni.3532
121. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol* (2014) 15(9):846–55. doi:10.1038/ni.2956
122. Malandrino MI, Fucho R, Weber M, Calderon-Dominguez M, Mir JF, Valcarcel L, et al. Enhanced fatty acid oxidation in adipocytes and macrophages reduces lipid-induced triglyceride accumulation and inflammation. *Am J Physiol Endocrinol and Metabolism* (2015) 308(9):E756–69. doi:10.1152/ajpendo.00362.2014
123. Thomas PG, Dash P, Aldridge JR Jr, Ellebedy AH, Reynolds C, Funk AJ, et al. The intracellular sensor NLRP3 mediates key innate and healing responses to influenza A virus via the regulation of caspase-1. *Immunity* (2009) 30(4):566–75. doi:10.1016/j.immuni.2009.02.006
124. Duncan JA, Gao X, Huang MT, O'Connor BP, Thomas CE, Willingham SB, et al. Neisseria gonorrhoeae activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J Immunol* (2009) 182(10):6460–9. doi:10.4049/jimmunol.0802696
125. Mariathasan S, Newton K, Monack DM, Vucic D, French DM, Lee WP, et al. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. *Nature* (2004) 430(6996):213–8. doi:10.1038/nature02664
126. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* (2010) 464(7293):1357–61. doi:10.1038/nature08938
127. Rajamaki K, Lappalainen J, Oorni K, Valimaki E, Matikainen S, Kovanen PT, et al. Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation. *PLoS One* (2010) 5(7):e11765. doi:10.1371/journal.pone.0011765
128. McGettrick AF, O'Neill LA. NLRP3 and IL-1beta in macrophages as critical regulators of metabolic diseases. *Diabetes Obes Metab* (2013) 15(Suppl 3):19–25. doi:10.1111/dom.12169
129. Moon JS, Nakahira K, Chung KP, DeNicola GM, Koo MJ, Pabon MA, et al. NOX4-dependent fatty acid oxidation promotes NLRP3 inflammasome activation in macrophages. *Nat Med* (2016) 22(9):1002–12. doi:10.1038/nm.4153
130. Hall CJ, Boyle RH, Astin JW, Flores MV, Oehlers SH, Sanderson LE, et al. Immunoresponsive gene 1 augments bactericidal activity of macrophage-lineage cells by regulating beta-oxidation-dependent mitochondrial ROS production. *Cell Metab* (2013) 18(2):265–78. doi:10.1016/j.cmet.2013.06.018
131. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat Immunol* (2011) 12(5):408–15. doi:10.1038/ni.2022
132. Moon JS, Lee S, Park MA, Siempos II, Haslip M, Lee PJ, et al. UCP2-induced fatty acid synthase promotes NLRP3 inflammasome activation during sepsis. *J Clin Invest* (2015) 125(2):665–80. doi:10.1172/JCI78253
133. Nomura M, Liu J, Rovira II, Gonzalez-Hurtado E, Lee J, Wolfgang MJ, et al. Fatty acid oxidation in macrophage polarization. *Nat Immunol* (2016) 17(3):216–7. doi:10.1038/ni.3366
134. Currie GA. Activated macrophages kill tumour cells by releasing arginase. *Nature* (1978) 273(5665):758–9. doi:10.1038/273758a0
135. Currie GA, Gyure L, Cifuentes L. Microenvironmental arginine depletion by macrophages in vivo. *Br J Cancer* (1979) 39(6):613–20. doi:10.1038/bjc.1979.112
136. Keller R, Geiges M, Keist R. L-arginine-dependent reactive nitrogen intermediates as mediators of tumor cell killing by activated macrophages. *Cancer Res* (1990) 50(5):1421–5.
137. Mills CD. Molecular basis of "suppressor" macrophages. Arginine metabolism via the nitric oxide synthetase pathway. *J Immunol* (1991) 146(8):2719–23.
138. MacMicking J, Xie QW, Nathan C. Nitric oxide and macrophage function. *Annu Rev Immunol* (1997) 15:323–50. doi:10.1146/annurev.immunol.15.1.323
139. Powell JD, Pollizzi KN, Heikamp EB, Horton MR. Regulation of immune responses by mTOR. *Annu Rev Immunol* (2012) 30:39–68. doi:10.1146/annurev-immunol-020711-075024
140. Silva LS, Poschet G, Nonnenmacher Y, Becker HM, Sapcariu S, Gaupel AC, et al. Branched-chain ketoacids secreted by glioblastoma cells via MCT1 modulate macrophage phenotype. *EMBO Rep* (2017) 18(12):2172–85. doi:10.15252/embr.201744154
141. Papathanassiu AE, Ko JH, Imprialou M, Bagnati M, Srivastava PK, Vu HA, et al. BCAT1 controls metabolic reprogramming in activated human macrophages and is associated with inflammatory diseases. *Nat Commun* (2017) 8:16040. doi:10.1038/ncomms16040

142. McGaha TL, Huang L, Lemos H, Metz R, Mautino M, Prendergast GC, et al. Amino acid catabolism: a pivotal regulator of innate and adaptive immunity. *Immunol Rev* (2012) 249(1):135–57. doi:10.1111/j.1600-065X.2012.01149.x
143. Murray PJ. Amino acid auxotrophy as a system of immunological control nodes. *Nat Immunol* (2016) 17(2):132–9. doi:10.1038/ni.3323
144. El-Gayar S, Thuring-Nahler H, Pfeilschifter J, Rollinghoff M, Bogdan C. Translational control of inducible nitric oxide synthase by IL-13 and arginine availability in inflammatory macrophages. *J Immunol* (2003) 171(9):4561–8. doi:10.4049/jimmunol.171.9.4561
145. Konig T, Bogdan C, Schleicher U. Translational repression of inducible NO synthase in macrophages by l-arginine depletion is not associated with an increased phosphorylation of eIF2alpha. *Immunobiology* (2009) 214(9–10):822–7. doi:10.1016/j.imbio.2009.06.008
146. Pekarova M, Kubala L, Martiskova H, Bino L, Twarogova M, Klinke A, et al. Asymmetric dimethylarginine regulates the lipopolysaccharide-induced nitric oxide production in macrophages by suppressing the activation of NF-kappaB and iNOS expression. *Eur J Pharmacol* (2013) 713(1–3):68–77. doi:10.1016/j.ejphar.2013.05.001
147. Pekarova M, Kubala L, Martiskova H, Papezikova I, Kralova S, Baldus S, et al. The unique role of dietary L-arginine in the acceleration of peritoneal macrophage sensitivity to bacterial endotoxin. *Immunol Res* (2013) 56(1):73–84. doi:10.1007/s12026-012-8379-2
148. Pekarova M, Lojek A, Martiskova H, Vasicek O, Bino L, Klinke A, et al. New role for L-arginine in regulation of inducible nitric-oxide-synthase-derived superoxide anion production in raw 264.7 macrophages. *ScientificWorldJournal* (2011) 11:2443–57. doi:10.1100/2011/321979
149. Yeramian A, Martin L, Arpa L, Bertran J, Soler C, McLeod C, et al. Macrophages require distinct arginine catabolism and transport systems for proliferation and for activation. *Eur J Immunol* (2006) 36(6):1516–26. doi:10.1002/eji.200535694
150. Barilli A, Rotoli BM, Visigalli R, Bussolati O, Gazzola GC, Dall'Asta V. Arginine transport in human monocytic leukemia THP-1 cells during macrophage differentiation. *J Leukoc Biol* (2011) 90(2):293–303. doi:10.1189/jlb.0910510
151. Nicholson B, Manner CK, Kleeman J, MacLeod CL. Sustained nitric oxide production in macrophages requires the arginine transporter CAT2. *J Biol Chem* (2001) 276(19):15881–5. doi:10.1074/jbc.M010030200
152. Niese KA, Chiaramonte MG, Ellies LG, Rothenberg ME, Zimmermann N. The cationic amino acid transporter 2 is induced in inflammatory lung models and regulates lung fibrosis. *Respir Res* (2010) 11:87. doi:10.1186/1465-9921-11-87
153. Albina JE, Mills CD, Barbul A, Thirkill CE, Henry WL Jr, Mastrofrancesco B, et al. Arginine metabolism in wounds. *Am J Physiol* (1988) 254(4 Pt 1):E459–67.
154. Pesce JT, Ramalingam TR, Mentink-Kane MM, Wilson MS, El Kasmi KC, Smith AM, et al. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog* (2009) 5(4):e1000371. doi:10.1371/journal.ppat.1000371
155. Takele Y, Abebe T, Weldegebreal T, Hailu A, Hailu W, Hurissa Z, et al. Arginase activity in the blood of patients with visceral leishmaniasis and HIV infection. *PLoS Negl Trop Dis* (2013) 7(1):e1977. doi:10.1371/journal.pntd.0001977
156. Rapovy SM, Zhao J, Bricker RL, Schmidt SM, Setchell KD, Qualls JE. Differential requirements for L-citrulline and L-arginine during anti-mycobacterial macrophage activity. *J Immunol* (2015) 195(7):3293–300. doi:10.4049/jimmunol.1500800
157. Rodriguez PC, Ochoa AC, Al-Khami AA. Arginine metabolism in myeloid cells shapes innate and adaptive immunity. *Front Immunol* (2017) 8:93. doi:10.3389/fimmu.2017.00093

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

MINI REVIEW
published: 09 February 2018
doi: 10.3389/fimmu.2018.00176

T-Cell Metabolism in Hematopoietic Cell Transplantation

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Metabolism, including catabolism and anabolism, is a basic cellular process necessary for cell survival. T lymphocytes have a distinct metabolism that can determine both fate and function. T-cell activation depends on glycolysis to obtain materials and energy for proliferation and effector function. Importantly, T cells utilize different metabolic processes under different conditions and diseases. Allogeneic hematopoietic cell transplantation (allo-HCT) is a classic immunotherapy for hematological malignancies; however, the development of graft-versus-host disease (GVHD) is a major factor limiting the success of allo-HCT. T cells in the donor graft drive GVHD by mounting a robust immunological attack against recipient normal tissues. Hence, understanding T-cell metabolism after allo-HCT would provide potential metabolic targets for the control of GVHD and primary tumor relapse. The purpose of the current review is to highlight the key metabolic pathways involved in alloantigen-activated T cells and to discuss how manipulating these pathways can serve as potential new therapeutic strategies to induce immune tolerance after allo-transplantation. We will also summarize the recent progress in regulating T-cell metabolism in bone marrow transplantation by targeting novel metabolic regulators or immune checkpoint molecules.

Keywords: T cell, metabolism, hematopoietic stem cell transplantation, graft-versus-host disease, glycolysis

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *13 November 2017*
Accepted: *19 January 2018*
Published: *09 February 2018*

Citation:
Nguyen HD, Kuril S, Bastian D and Yu X-Z (2018) T-Cell Metabolism in Hematopoietic Cell Transplantation. Front. Immunol. 9:176. doi: 10.3389/fimmu.2018.00176

INTRODUCTION

Allogeneic bone marrow transplantation [BMT; allogeneic hematopoietic stem-cell transplantation (allo-HCT)] is a curative option to treat hematological malignancies. However, graft-versus-host disease (GVHD) limits the success of allo-HCT (1). GVHD pathogenesis is characterized by a robust immunological attack by donor T cells against normal tissues of transplanted recipients (2). As donor T cells are the driving force in GVHD, suppressing T-cell responses is a standard therapeutic approach for the treatment of GVHD. However, these broadly immunosuppressive drugs, including corticosteroids and inhibitors of calcineurin or mammalian target of rapamycin (mTOR), leave patients highly susceptible to infections and induce remission in <50% of patients. The mortality rate of patients with steroid-refractory aGVHD is close to 90% (3). Hence, understanding T-cell pathobiology is critical to the development of effective therapies to prevent GVHD. Cell metabolism impacts the fate and function of T cells (4). Targeting T-cell metabolism is a viable therapeutic strategy in other immunological disorders, including systemic lupus erythematosus, rheumatoid arthritis, and experimental autoimmune encephalomyelitis (5–7). A growing body of evidence from multiple studies suggests T-cell metabolism is a promising target for controlling GVHD. Recently, our group and others attempted to characterize the metabolic profile of donor

T cells following allo-HCT, yet a consensus on the data has not been reached (2, 8, 9). In this review, we will detail the recent findings in the evolving field of immuno-metabolism with a focus on T-cell metabolism in the context of allo-HCT and discuss how this knowledge can help us reevaluate our current understanding of immune activation and suppression after allo-HCT, and promising immunotherapeutic strategies to archive long-term transplantation tolerance in transplanted recipients aiming to prevent allograft rejection and GVHD.

OVERVIEW OF T-CELL METABOLISM

Glycolysis and oxidative phosphorylation (OXPHOS) are fundamental cellular processes in generating energy, or adenosine triphosphate (ATP) (10, 11). Naïve T cells rely primarily on OXPHOS to meet their energy demands (12). Upon antigen recognition, naïve T cells clonally expand into T effector cells (Teffs). Upon antigen clearance, most of these effector T cells die, but a subset of long-lived memory T cells (Tm) persist with an enhanced mitochondrial capacity relying on fatty acid oxidation (FAO) to fuel OXPHOS (13). OXPHOS can generate up to 36 molecules of ATP. The transition from resting naïve T-cells into activated Teffs requires substantial metabolic reprogramming (12, 13). A Teff's metabolic profile is characterized by a shift to aerobic glycolysis as a main energy source (12, 14). Aerobic glycolysis involves the mitochondrion-independent metabolism of glucose into pyruvate and provides only two molecules of ATP per glucose (15). While glycolysis is less efficient than OXPHOS at yielding an abundance of ATP per molecule of glucose, aerobic glycolysis supplies metabolic intermediates for cell growth and proliferation as well as induces the pentose phosphate pathway (PPP), which produces nucleotides and amino acids that subsequently generate reducing power in the form of NADH to maintain cellular redox balance (NAD^+/NADH) (15). Teffs also use glutamine as a carbon source to fuel the tricarboxylic acid (TCA) cycle *via* α-ketoglutarate (α-KG) through the process of glutaminolysis (16, 17).

METABOLISM AND CD4$^+$T CELL DIFFERENTIATION

Depending on the nature of antigen and cytokine signal, $CD4^+$ T cells differentiate into Th1, Th2, Th9, Th17, T follicular helper cells (Tfh), Tr-1, or Treg. While Th1, Th2, and Th17 are pathogenic, Tr-1 and Treg are suppressive in acute GVHD (18–20). Metabolism plays a critical role in $CD4^+$ T-cell differentiation (12). While Th1, Th2, and Th17 lineages preferentially use glycolysis to meet energetic demand though activation of PI3K/Akt/mTOR pathway, $CD4^+$ Tregs use mitochondrial-dependent FAO (4). Therefore, enhanced FAO *via* inhibiting mTOR leads to increased Treg generation (21). Hypoxia-inducible factor 1 is the key regulator of anabolic metabolism in Th17 cells (22). Meanwhile, Tfh, a pathogenic T-cell subset in chronic GVHD, depend on glycolysis and lipogenesis to meet energy demands required for differentiation (23). The metabolic profiles of Th9 and Tr1 remain unclear.

METABOLISM OF ALLOGENEIC T CELLS

Glucose Metabolism

Using MHC-mismatched or haploidentical murine models of BMT, we uncovered that upon alloantigen activation, donor T cells increase both glycolysis and OXPHOS to obtain energetic materials necessary for activation and proliferation (2, 9). Albeit, they preferentially rely on glycolysis to maintain their capacity to induce GVHD (2, 9, 24). While OXPHOS of donor T cells isolated from syngeneic (no GVHD) and allogeneic (GVHD) recipients were similar, the glycolytic activity of donor T cells was significantly higher in allogeneic than syngeneic recipients, indicating an escalation of T-cell glucose metabolism correlated with GVHD development (2) (**Figure 1**). Furthermore, T cells isolated from livers of allogeneic recipients exhibited higher glycolytic activity compared to those of syngeneic recipients 14 days after allo-HCT, implying an enduring glycolytic response by allogeneic T cells in GVHD target organs. While *in vitro* activated T cells upregulate and maintain expression of Glut1 for sufficient glucose uptake (17), allo-activated T cells also increase Glut 3 to fulfill their extremely high demand for glucose (2). In addition, alloantigen-activated T cells upregulate both hexokinase 1 (HK1) and HK2 to facilitate induction of glycolysis (2). To maintain sufficient glycolytic activity, allogeneic $CD4^+$ T cells activate mTOR and increase differentiation into Th1 and Th17 (2, 25) while decreasing Treg generation (24). Inhibition of glycolysis by genetic depletion or pharmacological blockade of mTORC1 (2, 26) or glycolytic checkpoints, including glut-1 (24), HK-2, PFKB3 (2), or PKM2 (unpublished study), reduces alloreactive T-cell generation and subsequently ameliorates GVHD severity. Alternatively, enhancing FAO to inhibit mTOR using PI3K/AKT or AMPK inhibitors (27, 28) effectively prevents GVHD development.

OXPHOS and Oxidative Stress in Allogeneic T Cells

Allogeneic T cells in lymphoid or target organs of recipients significantly increase OXPHOS compared to resting T cells after allo-HCT (2, 9). Since OXPHOS activity was comparable in allogeneic and syngeneic T cells (2), increased OXPHOS may not be a direct mechanism by which pathogenic T cells are generated. However, due to increased non-mitochondrial oxygen consumption rate (OCR), allogeneic T cells had higher levels of oxidative stress yet lower levels of antioxidants (2, 9). As reactive oxygen species (ROS) are required for T-cell activation (30), this indicates chronic allo-activation of donor T cells after transplant. Increased ROS generation in allogeneic T cells may be the result of a hyperpolarized mitochondrial membrane potential ($\Delta\Psi m$), subsequently making alloreactive $CD4^+$ and $CD8^+$T cells highly susceptible to small-molecule inhibitors of mitochondrial F1F0 adenosine triphosphate synthase in haploidentical BMT model (9, 31).

The Pentose Phosphate Pathway

In murine models of GVHD, alloantigen-activated T cells have increased PPP activity (2, 31). Intracellular glucose metabolized by HK forms glucose 6-phospate (G-6P), which then enters the

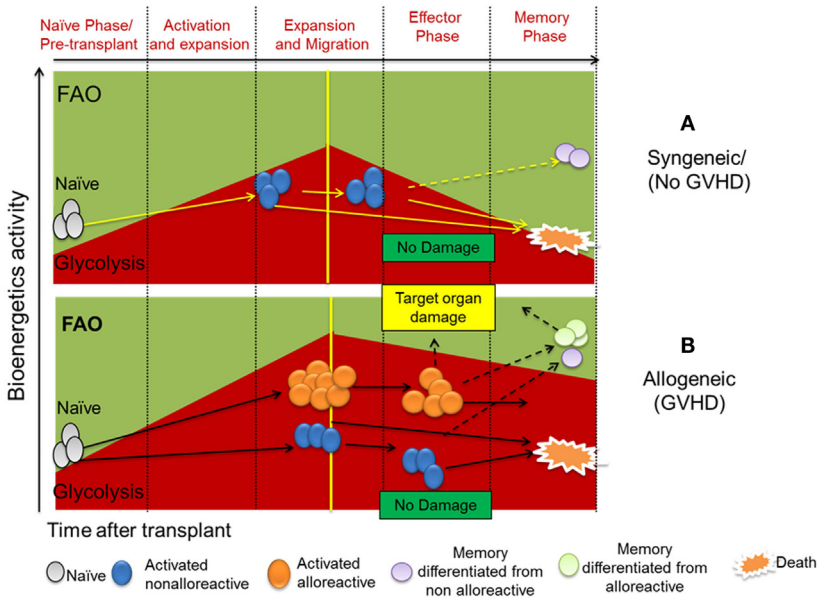

FIGURE 1 | **(A)** Naïve/resting T cells are dependent on oxidative phosphorylation with fatty acid oxidation (FAO) as a major material resource. Upon activation by self-antigens under homeostatic state, naïve/resting T cells reprogram their metabolic phenotype to become partially activated T cells (29), which possess glycolytic metabolic phenotype. Due to lack of specific TCR stimulation, a large proportion of non-alloreactive T cells gradually die. However, specific self-epitopes of T cells can become memory T cells (Tm) which depend upon FAO for their metabolism. **(B)** Upon activation by alloantigen in transplant recipients, naïve/resting T cells proliferate and their memory differentiate to activate T cells both alloreactive and non-alloreactive. Alloreactive T cells and their differentiated memory cells are capable of causing target organ damage. Alloreactive T cells have much higher glycolytic activity compared to non-alloreactive counterpart. Both alloreactive and non-alloreactive T cells can die or differentiate into Tms accordingly. Glucose retention and glycolytic activity decide survival and alloreactivity of alloreactive T cells to induce graft-versus-host disease (GVHD) after allogeneic hematopoietic cell transplantation.

PPP to generate ribose-5 phosphate (R-5P); the carbon donor during nucleotide biogenesis (32). The conversion of G-6P to R-5P is regulated by glucose-6-phosphate dehydrogenase in the oxidative arm of the PPP (33), which is significantly increased in allogeneic T cells (2, 31). The oxidative arm of the PPP is crucial for the formation of NADPH, which plays a critical role in reductive biosynthesis of antioxidant molecules, such as GSH (34). GSH promotes T-cell expansion by driving glycolysis and glutaminolysis, and supporting mTORC1 and c-Myc signaling in inflammation (35). Due to chronic stimulation by alloantigens, nucleotide biosynthesis is sustained to support anabolic growth of T cells during allogeneic responses; leading to a deficit in purine and pyrimidine catabolism (2) and exhaustion of GS and GSH (9).

Glutamine Metabolism

Glutamine uptake and metabolism are crucial for normal T-cell function (36). Donor T cells require the rapid synthesis of macromolecules for their growth, proliferation, and for energy after allo-HCT (11). Glutamine converted to glutamate can support the progression of the TCA cycle, ultimately leading to production of α-KG, a citrate precursor. To generate new lipids, citrate is secreted into the cytosol and metabolized to form acetyl-CoA, the backbone for lipid synthesis (34). In addition to the PPP, glutaminolysis can provide NADPH to support lipid and nucleotide biosynthesis as well as maintenance of GSH (37). *In vitro*-activated T cells utilize the transcription factor Myc to incorporate glutamine into metabolic pathways (17). Allogeneic T cells increase glutamine uptake by upregulating glutamine transport channels, such as glutamine-fructose-6-phosphate transaminase, phosphoribosyl pyrophosphate amidotransferase, and glutaminase 2 post allo-HCT (2). While the level of glutamine was increased in allogeneic T cells, the level of glutamate was lower. Moreover, the levels of aspartate and ornithine, products of glutamate conversion to α-KG by ornithine aminotransferase and glutamate oxaloacetate transaminase, respectively, were increased in allogenic T cells after allo-HCT (2, 31). These data suggest that alloantigen-activated T cells further increase glutaminolysis to replenish intermediate metabolites of the TCA cycle that are depleted in proliferating T cells after allo-HCT. Studies using radioactive tracers indicate that alloreactive $CD4^+$ and $CD8^+$ T cells preferentially use glutamine to provide substrates for ribose synthesis (31).

Fatty Acid Metabolism

Alloantigen-activated T cells accumulate various types of FAs and lysophospholipids after allo-HCT (2). In addition to glucose and glutamine, lipids are an effective energy source as well as biosynthetic intermediates (38). FAs can be generated through three different pathways: environmental uptake, synthesis, or hydrolysis of membrane or lipid droplets (39). FAs are classified according to (a) to their backbone lengths (short-, medium-, long-, and very long-chain), (b) saturation, i.e., the number of double bonds (unsaturated, mono-, poly-unsaturated), and (c)

position of the double bonds (37). During activation, *in vitro* activated T cells augment fatty acid synthase (FAS) while decreasing FAO, thus enhancing the accumulation of FA metabolites needed for the membrane (17). The effect of lipids on T-cell function seems to be mediated by a complex network dependent on the type of lipids (40).

Fatty Acid Synthesis

FAs have an important role in Teff function and differentiation. Acetyl-CoA carboxylases 1 (ACC1), ACC2, and FAS are recognized as key rate-limiting enzymes in this process (41). Inhibition of FAS limits development of Th1, Th2, and Th17 subsets (42, 43). Blockade of the enzyme ACC1 enhances the formation of Tregs during Th17 differentiation (43). *In vitro*, induction of FAS after TCR stimulation is regulated *via* the mTORC1–SREBP pathway (14, 44). Moreover, Myc is essential for activation of glucose-metabolizing genes and also for FA synthesis, linking glycolysis to *de novo* FAS (45). Recent studies showed that FAS is required for maintaining glycolytic activity in allogeneic T cells (46). Disruption of FAS at ACC1 effectively ameliorates GVHD development (46, 47). This study emphasizes the relationship between glycolysis and FAS in allogeneic T cells.

Fatty Acid Oxidation

Fatty acid oxidation is a multistep energetic process by which FAs are broken down in the mitochondria *via* sequential removal of 2-carbon units at the β-carbon position of a fatty acyl-CoA molecule (39, 48). A given long-chain acyl-CoA that enters the FAO yields one molecule of acetyl-CoA from each cycle of FAO. This acetyl-CoA can be directly shuttled into TCA cycle. The NADH and $FADH_2$ produced during FAO and the TCA cycle are then available to be used. While saturated short long-chain FA (SCFAs) and medium chain FA are almost exclusively oxidized in the mitochondria, long-chain FA and very long-chain fatty acids (>14 carbons) can also be oxidized in peroxisomes (49). Previous studies have indicated that alloreactive T cells increase FAO, and that targeting FAO could arrest GVHD in haploidentical allo-HCT (8, 9). Although they reported substantial increases in FA transport and intracellular acylcarnitines, suggesting changes in FA metabolism, it was not determined if FAO was directly responsible for the increase in OXPHOS (31, 34). Also, no improvement in survival of recipients treated with FAO inhibitors was shown. By contrast, our recent study showed intracellular carnitine-derived metabolites were diminished in alloantigen-activated T cells after MHC-mismatched or haploidentical allo-HCT (2). Allogeneic T cells dramatically decreased mitochondrial-dependent FAO and pyruvate oxidation through the TCA cycle. Therefore, it is possible that FAO is downregulated in allogeneic T cells after allo-HCT. These inconsistent observations likely result from the different controls used in these two studies. While studies from Ferrara's group compared bioenergetic parameters of allogeneic T cells to naïve/resting T cells (9), we used those isolated from syngeneic recipients as controls (2); intended to account for homeostatic proliferation of T cells under an inflammatory environment (29). In addition, we observed both Glut1 and Glut3 expression could serve as indicators of glycolytic activity (9), as alloreactive T cells increase Glut3 to an even larger extent than Glut1 in allogeneic recipients (2). Taken together, with study from by Rathmell's group (24), we speculate that FAO might not be the major material resource fueling the TCA cycle and OXPHOS in alloreactive T cells.

Sphingolipids (SLs) in Allogeneic T-Cell Metabolism

Sphingolipids represent a major class of lipids important for cell membrane formation (50). S1P is emerging as a key regulator of proliferation, inflammation, vasculogenesis, and resistance to apoptotic cell death (51). Recently, a report demonstrated that S1P1 regulates T cell metabolism through activation of mTOR-Akt, which suppressed Treg function (52). Blockade of the S-1P receptor effectively prevents GVHD by modulating the migration of allogeneic T cells. Ceramide plays a central role in the metabolism of SL (53, 54). Ceramide can be generated *via de novo* synthesis or by degradation of complex SLs, especially sphingomyelin (51). The key rate-limiting step in the biosynthesis of ceramide is the attachment of various acyl-CoA side chains to a sphingoid base by ceramide synthases (CerS) (55). The CerS show substrate preferences for specific chain lengths of fatty acyl CoAs. Briefly, CerS1 shows significant preference for C18-FA CoA, CerS4 for C18-/C20-FA CoA, CerS5 and CerS6 for C16- FA CoA, CerS2 for C22/C24- FA CoA, and CerS3 for ultra-long-chain FA CoA (51, 56). Recent work from our lab showed that CerS6 regulates SL metabolism in alloantigen-activated $CD4^+$ and $CD8^+$ T cells and required for alloreactive T cells to induce GVHD (57).

THE ROLE OF PD-1 AND CHECK POINT BLOCKADE ON ALLOGENEIC T CELL METABOLISM

The coinhibitory receptor programmed death 1 (PD-1; CD279) has key roles in modulating T-cell responses in both normal and antitumor immunity (58). PD-1 binds to PD-L1 (B7-H1; CD274), which is expressed by macrophages, DCs and non-hematopoietic cells, and PD-L2 (B7-DC; CD273), which is primarily expressed by monocytes and inflammatory macrophages in GVHD target organs (59, 60). Donor T cells significantly upregulate PD-1 expression, which can increase in response to FAO, superoxide, hyperpolarized mitochondrial membrane potential, and ROS formation which subsequently induces T-cell death following allo-HCT. In the absence of PD-1/PD-L1 ligation, donor T cells displayed higher glycolytic activity and OCR. Hence, PD-L1/PD-1 ligation, versus that of PD-L2/PD-1, plays a predominant role in downregulating GVHD (59).

MICROBIOTA REGULATES T CELL METABOLISM

The composition, or diversity, of intestinal microbiota shapes the innate and adaptive immune responses (61). The onset of GVHD is associated with a progressive reduction in microbiota diversity, with an increase in Lactobacillales and Blautia and a decrease in Clostridiales species (62–64). The microbiota metabolome, which consists of products generated by host metabolism, microbial

metabolism, and mammalian–microbial co-metabolism in the intestines, influences the development of GVHD (65, 66). SCFA-bacterial metabolites, derived from carbohydrate fermentation and include acetate, propionate, isobutyrate, and butyrate, increase histone H3 acetylation in the locus of Foxp3; thereby increasing the numbers of Tregs directly, yet also indirectly through increasing the production of TGFβ in the intestinal epithelium (67). The effect of SCFAs on T cells is also related to mTOR activation (68). SCFAs induce the expression of receptor GPR15, which is responsible for the recruitment of Tregs to the large intestine (69–71). Restoration of butyrate, which is diminished in intestinal epithelial cells (IECs) after allo-HCT, improved IEC junctional integrity, decreased apoptosis, and mitigated GVHD (66). Aryl hydrocarbon receptor (AhR) is a cellular metabolic sensor (72). AhR ligands are derived from intestinal microbiota metabolism. AhR ligand, indole-3-aldehyde, produced by Lactobacilli through tryptophan breakdown (73), modulates the development of GVHD through inducing Tregs and Tr1 cells (74).

TARGETING T-CELL METABOLISM TO SEPARATE GVHD AND THE GRAFT-VERSUS-TUMOR EFFECT

Given that tumors and alloreactive T cells share a glycolytic phenotype, pharmacological glycolysis inhibition could prevent both GVHD and tumor relapse, a primary complication after allo-HCT. Inhibition of glucose-metabolizing enzymes could reduce allogeneic T activation and function (2, 17) and, further, lower levels of glycolysis would support the generation of long-lived CD8 Tm (3) which are required for maintaining the graft-versus-tumor (GVT) effect. Moreover, *in vivo* activated $CD4^+$T cells are more dependent on glycolysis than $CD8^+$T cells (75), which are critically important for maintaining GVT activity in allo-HCT. Increasing evidence indicates that $CD8^+$ T cells with lower rates of glycolytic activity have better antitumor efficacy in eradicating established tumor in adoptive T cell transfer (ACT) models (76). Blocking glucose metabolism at HK2 by 2-deoxyglucose improves antitumor efficacy of ACT therapy (40). The aforementioned evidence suggests a valid possibility of targeting glycolysis to treat GVHD while preserving the GVT effect after allo-HCT.

IMPACT OF CURRENT IMMUNOSUPPRESSIVE DRUGS ON T-CELL METABOLISM IN ALLO-HCT

Corticosteroids inhibit glycolysis and endogenous respiration in donor lymphocytes and impair GVL activity (77). Inhibiting mTOR with rapamycin decreases glycolysis and enhances FAO in donor T cells; this is expected to reduce alloreactive T cells and enhance Treg function (27). However, attempts to conceptually translate this into patients have proven difficult. This challenge may be because rapamycin can promote CD8 memory T-cell responses by enhancing FAO and hence be detrimental in establishing tolerance (78). Alternatively, inhibition of calcineurin with cyclosporine diminishes glycolytic activity of donor T cells by decreasing glycolytic enzymes and the expression of glut1/3 (79); which support Treg expansion and GVHD attenuation (80).

CONCLUDING REMARKS

Current immunosuppressive regimens, including steroids and calcineurin inhibitors, help to prevent allograft rejection and GVHD. Consequently, patients are vulnerable to complications, such as opportunistic infections and tumor relapse. Therefore, bioenergetic signatures of immune cells at different stages of tolerance induction after transplant could serve as a promising clinical therapeutic strategy. Metabolism inhibitors, in concert with cancer immunotherapies, highlight an avenue by which to achieve better antitumor efficacy and functional tolerance to allografts. Hence, distinguishing metabolic signatures between allogeneic T cells and tumor cells is critical to truly fulfilling this goal.

AUTHOR CONTRIBUTIONS

HN and XZ-Y wrote manuscript and HN, SK, DB, and XZ-Y revised manuscript.

FUNDING

This work is partially supported by NIH grants R01 AI118305, CA118116, CA169116, and R21 CA192202 (to XZ-Y).

REFERENCES

1. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet* (2009) 373:1550–61. doi:10.1016/S0140-6736(09)60237-3
2. Nguyen HD, Chatterjee S, Haarberg KM, Wu Y, Bastian D, Heinrichs J, et al. Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. *J Clin Invest* (2016) 126:1337–52. doi:10.1172/JCI82587
3. Blazar BR, Murphy WJ, Abedi M. Advances in graft-versus-host disease biology and therapy. *Nat Rev Immunol* (2012) 12:443–58. doi:10.1038/nri3212
4. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic instruction of immunity. *Cell* (2017) 169:570–86. doi:10.1016/j.cell.2017.04.004
5. Tsokos GC. Metabolic control of arthritis: switch pathways to treat. *Sci Transl Med* (2016) 8:331fs8. doi:10.1126/scitranslmed.aaf4953
6. Yin Y, Choi SC, Xu Z, Perry DJ, Seay H, Croker BP, et al. Normalization of CD4+ T cell metabolism reverses lupus. *Sci Transl Med* (2015) 7:274ra18. doi:10.1126/scitranslmed.aaa0835
7. Chang CH, Pearce EL. Emerging concepts of T cell metabolism as a target of immunotherapy. *Nat Immunol* (2016) 17:364–8. doi:10.1038/ni.3415
8. Byersdorfer CA, Tkachev V, Opipari AW, Goodell S, Swanson J, Sandquist S, et al. Effector T cells require fatty acid metabolism during murine graft-versus-host disease. *Blood* (2013) 122:3230–7. doi:10.1182/blood-2013-04-495515
9. Gatza E, Wahl DR, Opipari AW, Sundberg TB, Reddy P, Liu C, et al. Manipulating the bioenergetics of alloreactive T cells causes their selective apoptosis and arrests graft-versus-host disease. *Sci Transl Med* (2011) 3:67ra8. doi:10.1126/scitranslmed.3001975
10. Cameron AM, Lawless SJ, Pearce EJ. Metabolism and acetylation in innate immune cell function and fate. *Semin Immunol* (2016) 28:408–16. doi:10.1016/j.smim.2016.10.003
11. Buck MD, O'Sullivan D, Pearce EL. T cell metabolism drives immunity. *J Exp Med* (2015) 212:1345–60. doi:10.1084/jem.20151159
12. Gerriets VA, Rathmell JC. Metabolic pathways in T cell fate and function. *Trends Immunol* (2012) 33:168–73. doi:10.1016/j.it.2012.01.010
13. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science* (2013) 342:1242454. doi:10.1126/science.1242454

14. Yang K, Shrestha S, Zeng H, Karmaus PW, Neale G, Vogel P, et al. T cell exit from quiescence and differentiation into Th2 cells depend on raptor-mTORC1-mediated metabolic reprogramming. *Immunity* (2013) 39:1043–56. doi:10.1016/j.immuni.2013.09.015
15. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* (2009) 324:1029–33. doi:10.1126/science.1160809
16. Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, et al. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. *Nature* (2011) 481:380–4. doi:10.1038/nature10602
17. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* (2011) 35:871–82. doi:10.1016/j.immuni.2011.09.021
18. Zeng H, Zhang R, Jin B, Chen L. Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. *Cell Mol Immunol* (2015) 12:566–71. doi:10.1038/cmi.2015.44
19. Zhang P, Lee JS, Gartlan KH, Schuster IS, Comerford I, Varelias A, et al. Eomesodermin promotes the development of type 1 regulatory T (TR1) cells. *Sci Immunol* (2017) 2:eaah7152. doi:10.1126/sciimmunol.aah7152
20. Coghill JM, Sarantopoulos S, Moran TP, Murphy WJ, Blazar BR, Serody JS. Effector CD4+ T cells, the cytokines they generate, and GVHD: something old and something new. *Blood* (2011) 117:3268–76. doi:10.1182/blood-2010-12-290403
21. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* (2013) 499:485–90. doi:10.1038/nature12297
22. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208:1367–76. doi:10.1084/jem.20110278
23. Zeng H, Cohen S, Guy C, Shrestha S, Neale G, Brown SA, et al. mTORC1 and mTORC2 kinase signaling and glucose metabolism drive follicular helper T cell differentiation. *Immunity* (2016) 45:540–54. doi:10.1016/j.immuni.2016.08.017
24. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20:61–72. doi:10.1016/j.cmet.2014.05.004
25. Park MJ, Lee SH, Lee SH, Lee EJ, Kim EK, Choi JY, et al. IL-1 receptor blockade alleviates graft-versus-host disease through downregulation of an interleukin-1beta-dependent glycolytic pathway in Th17 cells. *Mediators Inflamm* (2015) 2015:631384. doi:10.1155/2015/631384
26. Herrero-Sanchez MC, Rodriguez-Serrano C, Almeida J, San-Segundo L, Inoges S, Santos-Briz A, et al. Effect of mTORC1/mTORC2 inhibition on T cell function: potential role in graft-versus-host disease control. *Br J Haematol* (2016) 173:754–68. doi:10.1111/bjh.13984
27. Herrero-Sanchez MC, Rodriguez-Serrano C, Almeida J, San Segundo L, Inoges S, Santos-Briz A, et al. Targeting of PI3K/AKT/mTOR pathway to inhibit T cell activation and prevent graft-versus-host disease development. *J Hematol Oncol* (2016) 9:113. doi:10.1186/s13045-016-0343-5
28. Park MJ, Lee SY, Moon SJ, Son HJ, Lee SH, Kim EK, et al. Metformin attenuates graft-versus-host disease via restricting mammalian target of rapamycin/signal transducer and activator of transcription 3 and promoting adenosine monophosphate-activated protein kinase-autophagy for the balance between T helper 17 and Tregs. *Transl Res* (2016) 173:115–30. doi:10.1016/j.trsl.2016.03.006
29. Mirza N, Zierhut M, Korn A, Bornemann A, Vogel W, Schmid-Horch B, et al. Graft versus self (GvS) against T-cell autoantigens is a mechanism of graft-host interaction. *Proc Natl Acad Sci U S A* (2016) 113:13827–32. doi:10.1073/pnas.1609118113
30. Murphy MP, Siegel RM. Mitochondrial ROS fire up T cell activation. *Immunity* (2013) 38:201–2. doi:10.1016/j.immuni.2013.02.005
31. Glick GD, Rossignol R, Lyssiotis CA, Wahl D, Lesch C, Sanchez B, et al. Anaplerotic metabolism of alloreactive T cells provides a metabolic approach to treat graft-versus-host disease. *J Pharmacol Exp Ther* (2014) 351:298–307. doi:10.1124/jpet.114.218099
32. Weyand CM, Goronzy JJ. Immunometabolism in early and late stages of rheumatoid arthritis. *Nat Rev Rheumatol* (2017) 13:291–301. doi:10.1038/nrrheum.2017.49
33. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* (2012) 13:907–15. doi:10.1038/ni.2386
34. van der Windt GJ, Pearce EL. Metabolic switching and fuel choice during T-cell differentiation and memory development. *Immunol Rev* (2012) 249:27–42. doi:10.1111/j.1600-065X.2012.01150.x
35. Mak TW, Grusdat M, Duncan GS, Dostert C, Nonnenmacher Y, Cox M, et al. Glutathione primes T cell metabolism for inflammation. *Immunity* (2017) 46:1089–90. doi:10.1016/j.immuni.2017.03.019
36. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* (2002) 16:769–77. doi:10.1016/S1074-7613(02)00323-0
37. Pearce EL, Pearce EJ. Metabolic pathways in immune cell activation and quiescence. *Immunity* (2013) 38:633–43. doi:10.1016/j.immuni.2013.04.005
38. Angela M, Endo Y, Asou HK, Yamamoto T, Tumes DJ, Tokuyama H, et al. Fatty acid metabolic reprogramming via mTOR-mediated inductions of PPARgamma directs early activation of T cells. *Nat Commun* (2016) 7:13683. doi:10.1038/ncomms13683
39. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. *Trends Immunol* (2015) 36:81–91. doi:10.1016/j.it.2014.12.005
40. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, et al. Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. *J Clin Invest* (2013) 123:4479–88. doi:10.1172/JCI69589
41. Delgoffe GM, Kole TP, Zheng Y, Zarek PE, Matthews KL, Xiao B, et al. The mTOR kinase differentially regulates effector and regulatory T cell lineage commitment. *Immunity* (2009) 30:832–44. doi:10.1016/j.immuni.2009.04.014
42. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* (2009) 460:103–7. doi:10.1038/nature08097
43. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* (2014) 20:1327–33. doi:10.1038/nm.3704
44. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. *Nat Immunol* (2013) 14:489–99. doi:10.1038/ni.2570
45. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* (2010) 39:171–83. doi:10.1016/j.molcel.2010.06.022
46. Raha S, Raud B, Oberdorfer L, Castro CN, Schreder A, Freitag J, et al. Disruption of de novo fatty acid synthesis via acetyl-CoA carboxylase 1 inhibition prevents acute graft-versus-host disease. *Eur J Immunol* (2016) 46:2233–8. doi:10.1002/eji.201546152
47. Lee J, Walsh MC, Hoehn KL, James DE, Wherry EJ, Choi Y. Regulator of fatty acid metabolism, acetyl coenzyme a carboxylase 1, controls T cell immunity. *J Immunol* (2014) 192:3190–9. doi:10.4049/jimmunol.1302985
48. Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiol Rev* (2010) 90:207–58. doi:10.1152/physrev.00015.2009
49. Wanders RJ, Komen J, Kemp S. Fatty acid omega-oxidation as a rescue pathway for fatty acid oxidation disorders in humans. *FEBS J* (2011) 278:182–94. doi:10.1111/j.1742-4658.2010.07947.x
50. Simons K, Ikonen E. Functional rafts in cell membranes. *Nature* (1997) 387:569–72. doi:10.1038/42408
51. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. *Nat Rev Cancer* (2004) 4:604–16. doi:10.1038/nrc1411
52. Liu G, Burns S, Huang G, Boyd K, Proia RL, Flavell RA, et al. The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. *Nat Immunol* (2009) 10:769–77. doi:10.1038/ni.1743
53. Bartke N, Hannun YA. Bioactive sphingolipids: metabolism and function. *J Lipid Res* (2009) 50(Suppl):S91–6. doi:10.1194/jlr.R800080-JLR200
54. Hannun YA. Functions of ceramide in coordinating cellular responses to stress. *Science* (1996) 274:1855–9. doi:10.1126/science.274.5294.1855
55. Jiang W, Ogretmen B. Autophagy paradox and ceramide. *Biochim Biophys Acta* (2014) 1841:783–92. doi:10.1016/j.bbalip.2013.09.005
56. Pewzner-Jung Y, Ben-Dor S, Futerman AH. When do Lasses (longevity assurance genes) become CerS (ceramide synthases)? Insights into the regulation of ceramide synthesis. *J Biol Chem* (2006) 281:25001–5. doi:10.1074/jbc.R600010200

57. Sofi MH, Heinrichs J, Dany M, Nguyen H, Dai M, Bastian D, et al. Ceramide synthesis regulates T cell activity and GVHD development. *JCI Insight* (2017) 2:91701. doi:10.1172/jci.insight.91701
58. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. *Immunol Rev* (2010) 236:219–42. doi:10.1111/j.1600-065X.2010.00923.x
59. Saha A, Aoyama K, Taylor PA, Koehn BH, Veenstra RG, Panoskaltsis-Mortari A, et al. Host programmed death ligand 1 is dominant over programmed death ligand 2 expression in regulating graft-versus-host disease lethality. *Blood* (2013) 122:3062–73. doi:10.1182/blood-2013-05-500801
60. Al-Chaqmaqchi H, Sadeghi B, Abedi-Valugerdi M, Al-Hashmi S, Fares M, Kuiper R, et al. The role of programmed cell death ligand-1 (PD-L1/CD274) in the development of graft versus host disease. *PLoS One* (2013) 8:e60367. doi:10.1371/journal.pone.0060367
61. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol* (2009) 9:313–23. doi:10.1038/nri2515
62. Jenq RR, Ubeda C, Taur Y, Menezes CC, Khanin R, Dudakov JA, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. *J Exp Med* (2012) 209:903–11. doi:10.1084/jem.20112408
63. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* (2014) 124:1174–82. doi:10.1182/blood-2014-02-554725
64. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal blautia is associated with reduced death from graft-versus-host disease. *Biol Blood Marrow Transplant* (2015) 21:1373–83. doi:10.1016/j.bbmt.2015.04.016
65. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* (2009) 31:677–89. doi:10.1016/j.immuni.2009.08.020
66. Mathewson ND, Jenq R, Mathew AV, Koenigsknecht M, Hanash A, Toubai T, et al. Gut microbiome-derived metabolites modulate intestinal epithelial cell damage and mitigate graft-versus-host disease. *Nat Immunol* (2016) 17:505–13. doi:10.1038/ni.3400
67. Correa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MA. Regulation of immune cell function by short-chain fatty acids. *Clin Transl Immunol* (2016) 5:e73. doi:10.1038/cti.2016.17
68. Park J, Kim M, Kang SG, Jannasch AH, Cooper B, Patterson J, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. *Mucosal Immunol* (2015) 8(1):80–93. doi:10.1038/mi.2014
69. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* (2013) 341:569–73. doi:10.1126/science.1241165
70. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* (2013) 504:446–50. doi:10.1038/nature12721
71. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* (2013) 504:451–5. doi:10.1038/nature12726
72. Casado FL. The aryl hydrocarbon receptor relays metabolic signals to promote cellular regeneration. *Stem Cells Int* (2016) 2016:4389802. doi:10.1155/2016/4389802
73. Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity* (2013) 39:372–85. doi:10.1016/j.immuni.2013.08.003
74. Quintana FJ, Sherr DH. Aryl hydrocarbon receptor control of adaptive immunity. *Pharmacol Rev* (2013) 65:1148–61. doi:10.1124/pr.113.007823
75. Wahl DR, Petersen B, Warner R, Richardson BC, Glick GD, Opipari AW. Characterization of the metabolic phenotype of chronically activated lymphocytes. *Lupus* (2010) 19:1492–501. doi:10.1177/0961203310373109
76. Sukumar M, Kishton RJ, Restifo NP. Metabolic reprograming of anti-tumor immunity. *Curr Opin Immunol* (2017) 46:14–22. doi:10.1016/j.coi.2017.03.011
77. Blecher M, White A. Effects of various steroids and metabolic inhibitors on the incorporation of glycine-2-C 14 into total proteins and nucleic acids of normal and malignant lymphocytes in vitro. *J Biol Chem* (1958) 233:1161–8.
78. Araki K, Turner AP, Shaffer VO, Gangappa S, Keller SA, Bachmann MF, et al. mTOR regulates memory CD8 T-cell differentiation. *Nature* (2009) 460:108–12. doi:10.1038/nature08155
79. Vaeth M, Maus M, Klein-Hessling S, Freinkman E, Yang J, Eckstein M, et al. Store-operated Ca(2+) entry controls clonal expansion of T cells through metabolic reprogramming. *Immunity* (2017) 47:664–679e6. doi:10.1016/j.immuni.2017.09.003
80. Satake A, Schmidt AM, Nomura S, Kambayashi T. Inhibition of calcineurin abrogates while inhibition of mTOR promotes regulatory T cell expansion and graft-versus-host disease protection by IL-2 in allogeneic bone marrow transplantation. *PLoS One* (2014) 9:e92888. doi:10.1371/journal.pone.0092888

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 08 January 2018
doi: 10.3389/fimmu.2017.01889

Regulatory T Cell Metabolism in the Hepatic Microenvironment

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Thymic-derived naturally occurring regulatory T cells (tTreg) are crucial for maintaining peripheral immune homeostasis. They play a crucial role in preventing autoimmunity and maintaining organ transplant without requiring immunosuppression. Cellular metabolism has recently emerged as an important regulator of adaptive immune cell balance between Treg and effector T cells. While the metabolic requirements of conventional T cells are increasingly understood, the role of Treg cellular metabolism is less clear. The continuous exposure of metabolites and nutrients to the human liver *via* the portal blood flow influences the lineage fitness, function, proliferation, migration, and survival of Treg cells. As cellular metabolism has an impact on its function, it is crucial to understand the metabolic pathways wiring in regulatory T cells. Currently, there are ongoing early phase clinical trials with polyclonal and antigen-specific good manufacturing practice (GMP) Treg therapy to treat autoimmune diseases and organ transplantation. Thus, enhancing immunometabolic pathways of Treg by translational approach with existing or new drugs would utilize Treg cells to their full potential for effective cellular therapy.

Keywords: regulatory T cells, microenvironment, liver, Treg plasticity, function, immunometabolism, good manufacturing practice Treg, cell therapy

OPEN ACCESS

Edited by:
Claudio Mauro, Barts and The London School of Medicine and Dentistry, United Kingdom

Reviewed by:
Markus Feuerer, Deutsches Krebsforschungszentrum (DKFZ), Germany
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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *13 September 2017*
Accepted: *11 December 2017*
Published: *08 January 2018*

Citation:
Wawman RE, Bartlett H and Oo YH (2018) Regulatory T Cell Metabolism in the Hepatic Microenvironment. Front. Immunol. 8:1889. doi: 10.3389/fimmu.2017.01889

REGULATORY T CELLS AND PERIPHERAL SELF-TOLERANCE

Regulatory T (Treg) cells are a subset of $CD4^+$ T cells that maintain peripheral immune homeostasis by suppressing a range of untoward immune responses thus maintaining the balance between immune activation and tolerance (1). Sakaguchi and colleagues first reported Treg cells in 1995 *via* adoptive transfer studies, which demonstrated the subset of $CD4^+$ T cells expressing the interleukin-2 (IL-2) receptor alpha chain, CD25, preventing autoimmune diseases (2) (**Figure 1**). Around 5–10% of $CD4^+$ T cells are $CD25^+$, they are able to maintain peripheral immunologic self-tolerance by suppressing self-reactive lymphocytes (1, 2). Subsequently, Seddiki (3) and Liu (4) reported that low level expression of the IL-7 receptor, CD127, inversely correlated with FoxP3 expression and Treg cell's suppressive function due to the repressor function of FoxP3. FoxP3 is a master transcription factor and regulator of Treg phenotype and function (5). Mutations in the FoxP3 gene cause defective development of $CD4^+CD25^+$ Treg cells, leading to IPEX syndrome (immunodysregulation, polyendocrinopathy, enteropathy, X-linked genetic trait) (6). Lymphoproliferation and multiorgan autoimmunity in scurfy mutant mice is caused by the absence of FoxP3 (7). FoxP3 is regulated by conserved non-coding DNA sequences (CNS) 1–3. CNS2 is required for FoxP3 expression in the dividing Treg cell and CNS3 controls *de novo* Foxp3 expression and thymic Treg-cell

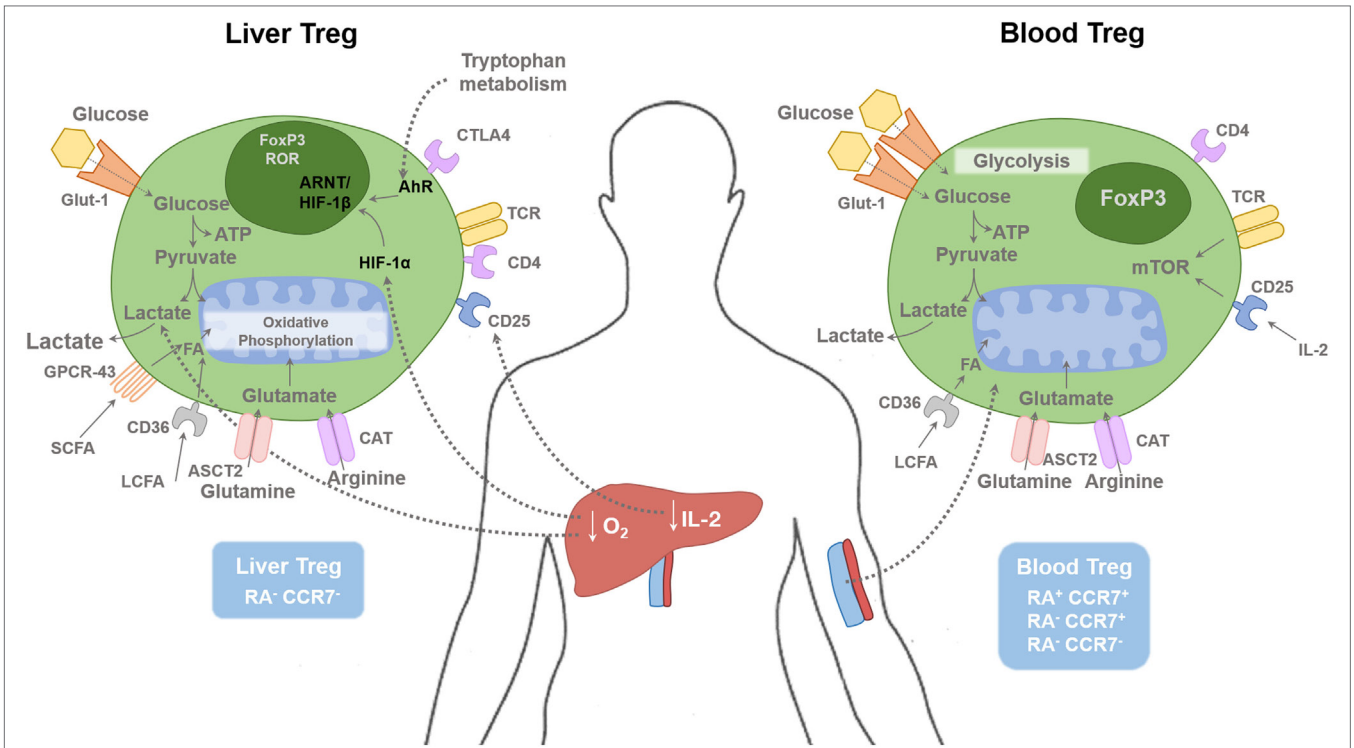

FIGURE 1 | Regulatory T cells in tissue and blood compartments. The human liver is a hypoxic environment as the majority of blood flow is from the portal venous system. This leads to hypoxic induced factor 1-α (HIF-1α) activation, which subsequently enhances FoxP3 expression along with Th17 differentiation. Hypoxia leads to anaerobic glycolysis and extracellular lactic acid accumulation. Short-chain fatty acids (SCFAs) bind to the receptor GPCR43; long-chain fatty acids (LCFAs) bind to CD36, glutamine binds to ASCT2, and arginine binds to CAT2. Glucose transporter-1 (Glut-1) is poorly expressed on Treg cells compared with effector T cells. Liver Treg cells are mainly of an effector memory phenotype (RA^-CCR7^-). There is only minimal level of IL-2 present in the human liver compared with the blood, which restrict hepatic Treg function. Blood Treg-cell subsets are composed of effector memory (RA^-CCR7^-), central memory (RA^-CCR7^+), and naive (RA^+CCR7^+) phenotype. HIF-1α, Hypoxia-inducible factor 1α; HIF-1β, Hypoxia-inducible factor-1β; AhR, aryl hydrocarbon receptor; FA, Fatty acids; ARNT, Aryl Hydrocarbon Receptor Nuclear Translocator; mTOR, mammalian target of rapamycin; SCFA, short chain fatty acid; LCFA, long chain fatty acid; ASCT2, Alanine, serine, cysteine-preferring transporter 2; CAT, Cationic amino acid transporter; GPCR, G protein–coupled receptor.

differentiation (8). Therefore, Treg cells are currently defined as $CD4^+CD25^{high}CD127^{low/-}FoxP3^+$ cells.

Treg cells are essential for maintaining peripheral tolerance by controlling autoreactive T cells, which escape negative selection in the thymus (9). They can be broadly divided into two types; thymic-derived Treg (tTreg) cells and peripheral Treg (pTreg) cells (10). Strong T cell receptor (TCR) signaling with CD28 co-stimulation, just below the threshold for negative selection, promotes tTreg lineage commitment in the thymus (11). pTreg cells are generated in the periphery from populations of mature T cells under certain antigenic stimulating conditions; persistent weak TCR stimulation along with IL-2, transforming growth factor-β (TGF-β) or retinoic acid (RA) (12, 13). The DNA in tTregs is demethylated in the Treg-specific demethylated region (TSDR) in the FoxP3 enhancer, whereas the TSDR of pTregs is only partially demethylated (14). Although both tTreg and pTreg are difficult to distinguish phenotypically, both are thought to play an essential role in immune regulation (15), with tTreg cells controlling reactivity toward self-antigens and pTreg cells controlling responses to antigen exposure in the periphery. Treg cells require IL-2 to maintain their function and survival. Because Treg cells do not make IL-2, they are dependent on IL-2 derived from other T cells (16). Treg cells are highly sensitive to IL-2, due to their constitutively high expression of CD25 and amplified intracellular signal transduction downstream of the IL-2 receptor, phosphorylation of STAT5 to upregualte essential Treg functional gene such as CD25, FoxP3, and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) (17). Treg cells can therefore compete with conventional T cells for IL-2 as a mechanism to prevent unwanted immune responses (17).

Treg conduct their suppressive function *via* multiple mechanisms throughout different compartments of the body. Treg are therefore also equipped with various functional markers. In the context of liver disease, they constitutively express CTLA-4 (16, 18, 19), ecto-nucleoside triphosphate diphosphohydrolase 1, CD39 (16, 20), and the intracellular immunosuppressive cytokine, IL-10 (16, 21).

Cytotoxic T lymphocyte-associated antigen 4 is a target gene of FoxP3 (22), and activation of Treg results in upregulation of CTLA-4; its deficiency in mice leads to fatal lymphoproliferation and multiorgan lymphocyte infiltration (23, 24). CTLA-4 binds the ligands CD80 and CD86 on antigen-presenting cells (APCs) such as dendritic cells (DCs). Its mechanism has been reported as removal of these ligands from APC cell surface by trans-endocytosis, which subsequently prevents the effective activation of naïve $CD4^+$ T cells by APCs (25). CTLA-4-CD80/CD86

binding leads to upregulation of indoleamine 2,3-dioxygenase (IDO), which catabolizes tryptophan (Trp) into immunosuppressive kynurenines (26). CD39 on both human and murine Treg exert their function *via* generation of adenosine by the breakdown of adenosine triphosphate (ATP) and other extracellular nucleotides, which then bind to adenosine 2A receptors expressed on effector T cells causing a rise in intracellular cyclic adenosine monophosphate, thus inhibiting proliferation of effector T cells (27).

INTRAHEPATIC MICROENVIRONMENT

The phenotype and function of Treg cells in circulatory and intrahepatic compartments is different as the intrahepatic microenvironment is hypoxic and enriched with cytokines and metabolic products (1) (**Figure 1**). Intrahepatic Treg cells respond to (i) engagement of the TCR with MHC Class II on APCs, (ii) the binding of CD28/CTLA-4 on cells with CD80/86 on APCs, and (iii) the influence of cytokines from APC for their activation, survival, and differentiation (28, 29). We reported that the intrahepatic microenvironment is highly enriched with the pro-inflammatory cytokines IL-1β, IL-6, and IL-12 (16) from hepatic DCs but lacks the crucial Treg cell survival cytokine; IL-2 (19). With the recent advances in research into the metabolism of individual immune cell including T cells, it is now realized that differentiation, survival, and function of Treg cells depends not only on TCR, co-stimulatory and cytokine signals but also on other signals in the environment, specifically the local milieu of oxygen, metabolites, and catabolites (30).

METABOLIC INFLUENCE ON PLASTICITY, FUNCTION, SURVIVAL, AND MIGRATION OF Treg CELLS

The human liver is uniquely situated to receive a blood supply from the portal venous system, which is enriched with metabolites and nutrients. Human liver has a dual blood supply, deriving 70–80% of its blood, rich in nutrients, from the portal vein and the other 20–30%, rich in oxygen, from the hepatic artery (31). Thus, Treg and T effector cells reside in the hepatic microenvironment with continuous exposure to metabolic signals (**Figure 2**). Resting T cells require little energy generation or expenditure; however, upon activation, their energy needs increase substantially, and they utilize glucose, amino acids, and fatty acids (FAs) to meet this demand. Metabolic effects on Treg cells could either be *via* direct binding of metabolites to Treg or *via* changes in cytokines profiles in DCs, which take up and process these metabolites.

Glucose

Glucose is a critical fuel for Treg cell ATP generation, cell activation, and function. Glucose transporter-1 (Glut-1) levels are low in Treg cells compared with effector cells because FoxP3 limits Glut-1 expression through inhibition of Akt (36). Treg cells exhibit low to modest glycolysis compared with effector T cells along with elevated mTOR activity (37–39). Kishore and colleagues recently demonstrated that enzyme glucokinase (GCK)-dependent glycolysis regulates Treg-cell migration as GCK promotes cytoskeletal rearrangements by associating with actin. Treg cells lacking this pathway were functionally suppressive but failed to migrate to skin allografts and inhibit rejection (40).

Fatty Acids

The colonic microbiota metabolizes complex carbohydrates and undigested dietary fibers to oligosaccharides and monosaccharaides, which are then fermented to short-chain fatty acids (SCFAs); acetate, propionate, and butyrate (41). Free FAs can diffuse across the plasma membrane into the cytosol. FAs are categorized into groups based on the length of their aliphatic chain. SCFAs have 2–6 carbons; medium chain fatty acids (MCFAs) have 7–12 carbons; and long-chain fatty acids (LCFAs) have more than 12 carbons. SCFAs and MCFAs are absorbed directly into the blood *via* intestinal capillaries and travel through the portal vein. However, LCFAs are absorbed into the intestinal villi and reassembled again into triglycerides. The triglycerides are coated with cholesterol and protein (protein coat), forming chylomicron, which is carried *via* the lymphatics to drain into the systemic circulation (**Figure 2**). MCFAs and LCFAs are considered one of the most abundant components of the "Western diet" (42). The concentration of SCFAs is highest in the proximal colon where fermentation mostly occurs.

Short-Chain Fatty Acids

Short-chain fatty acids exert metabolic regulation by signaling through metabolite- sensing G-protein-coupled receptors (GPCRs). GPCR43, or free fatty acid receptor 2, binds to SFCAs (43) (**Figure 1**). Immune cells such as Treg cells and DCs express GPR43, which bind SCFAs and promote their differentiation and function to maintain intestinal homeostasis (43). Regulation of colonic and pTreg-cell numbers also relies upon the expression of GPR103, a receptor for Niacin (vitamin B3), which is expressed on DCs, which promote Treg-cell differentiation (44, 45) (**Figure 2**). The SCFA, butyrate has been shown to inhibit histone deacetylase (HDAC) thereby enhancing histone acetylation in the FoxP3 promoter region to promote stable FoxP3 expression (45). Butyrate also promotes the extra-thymic induction of Treg cells *via* the intronic enhancer conserved non-coding sequence 1 (46).

GPCR84 recognizes MCFAs. LCFAs are transported across the membrane by fatty acid translocase (or CD36) (47) or GPCR40 (free FA receptor-1) (48) (**Figure 1**). The effect of LCFAs was studied recently by Haghikia and colleagues in EAE reporting that the addition of lauric acid (C12), to a culture of $CD4^+$ T cells, not only increases the differentiation of Th1 and Th17 cells but also leads to a reduction in Treg cells (49).

Data on immunometabolism on tissue resident T cells are limited. Recent data from Pan and colleagues described that mouse CD8 tissue resident memory cells generated by viral infection of the skin differentially express high levels of molecules that mediate lipid such as fatty acid-binding proteins 4 and 5. They then continued to link this finding with human psoriatic skin suggesting the important role of FAs and their oxidative metabolism for tissue resident cells to mediate protective immunity (50).

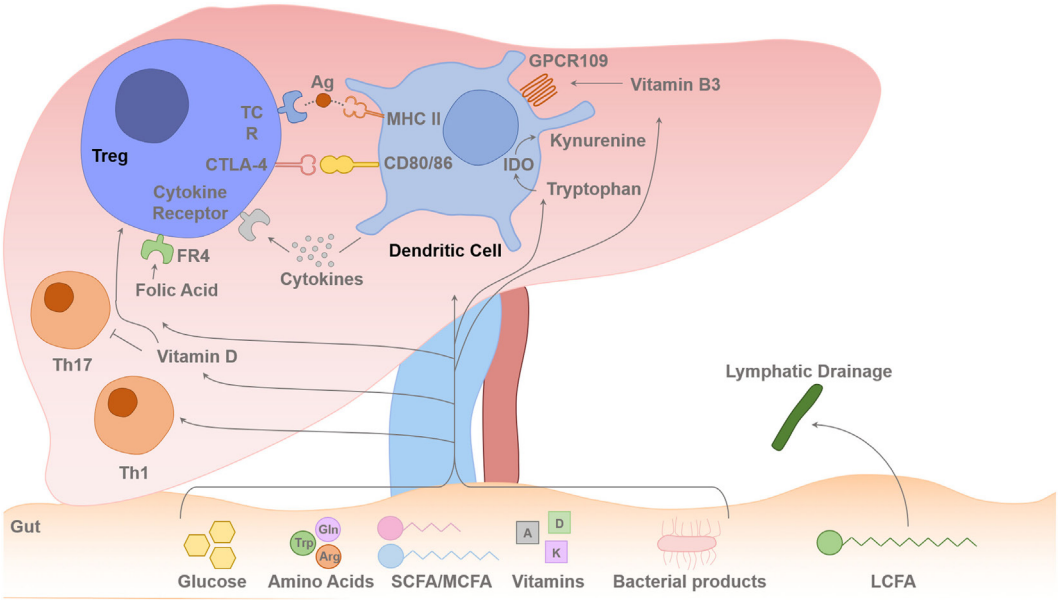

FIGURE 2 | Gut–liver axis and metabolites flux toward the liver. Intrahepatic T cell (including Treg cells) lineage fitness, function, survival, and proliferation are influenced by signals from the antigen presenting dendritic cells (DCs) and cytokines. The T cell receptor (TCR) binding to the foreign antigen–MHC II complex and co-stimulation provided by CD80 and CD86 binding to the T cells CD28/CTLA-4 induces T cell activation or inhibition of its function. Cytokines secreted by the DCs determine the differentiation pathway of the activated CD4 T cell to different Th1/Th2/Th17 lineages or Treg-cell survival with contact-dependent mechanism with DCs CD80/86. IL-2 is secreted in the liver by activated T effector cells, and this is required for Treg-cell survival and suppressive function. Dietary SCFAs (acetate, propionic acid, and butyrate) and MCFAs arrive to the liver *via* the portal vein, but LCFAs are absorbed *via* intestinal lymphatics and drain back into the systemic circulation *via* the thoracic duct. The amino acids, Glu and Arg, and glucose are also absorbed *via* the portal venous system towards the liver. There is always a certain degree of gut leakiness and bacterial product such as lipopolysaccharide reaches to the liver, and they rapidly undergo phagocytosis by hepatic sinusoidal Kupffer cells, which function as a sinusoidal firewall of the liver (32). The liver is enriched with fat-soluble vitamins such as vitamins A and D. DCs express immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO), which transform Trp into kynurenine, which is then metabolized into other catabolites through the action of enzymes within the kynurenine pathway (26, 33, 34). Trp-derived catabolites can mediate the tolerogenic effects of IDO by inducing apoptosis of activated but not resting T cells (35). Folic acid binds to folic acid receptor (FR4) on the Treg cell and Niacin (vitamin B3) interacts with GPCR109 on DCs. Glu, glutamine; Trp, tryptophan; Arg, arginine; SCFA, short-chain fatty acid; MCFA, median chain fatty acid; LCFA, long-chain fatty acid.

Investigating the role of SCFAs on human T cells is in its early stage. Peripheral blood mononuclear cells of healthy donors exposed to SCFAs, especially butyrate, reduced IL-6 levels and hence increased the differentiation of Treg cells over Th17 cells (51). Indeed, this is supported by the recent data from Schmidt et al. suggesting that exposure to butyrate along with TGF-β1 enhanced Foxp3 induction in human T cells to a greater extent than TGF-β1 alone (52).

Amino Acids

Amino acids and peptides are generated in the gut *via* the hydrolysis of endogenous and alimentary proteins by extracellular proteases and peptidases derived from the pancreatic and other digestive enzymes and commensal bacteria residing in the gut. Amino acids can serve as sources for metabolites that enter into the metabolic tricarboxylic acid (TCA) cycle. In the context of T cell biology, arginine (Arg), glutamine (Glu), and Trp are critical for efficient T cell function and proliferative responses.

Arg and Treg-Cell Proliferation

Metabolic activity is intimately linked to T cell function. Arg is transported to the cells by cationic transporters CAT1–4 (53) (**Figure 1**). BAZ1B, PSIP1, and Translin are also potential Arg sensors that promote T cell survival (54). Intracellular Arg is metabolized by arginase 1 and nitric-oxide synthase 2. The human liver contains myeloid-derived suppressor cells, which consume and deplete extracellular Arg (55). Arg-depleted environments impair T cell proliferation.

Glu and Treg Survival, Proliferation

The amino acids leucine and Glu enter into T cells *via* their transporter LAT1 (CD98 for leucine and ASCT2 for Glu) (**Figure 1**). Glu-deprived activated CD4 T cells differentiate into Treg cells rather than Th1 cells even in the presence of cytokines that would normally favor Th1 cell differentiation (56). Treg cells do not require LAT1 or ASCT2 for their differentiation *in vitro* (57). Treg-cell differentiation is favored during Glu deprivation (56) *via* mTOR signaling (58). Both leucine and Glu are positive regulators of CD4 T cell differentiation into Th1 and Th17 cells because absence of LAT1 expression impairs the differentiation of these lineages (59), and elevated Glu levels favor Th1 and Th17 cell differentiation (57). Glu-derived metabolite α-ketoglutarate promotes Th1 differentiation through enhancing mTORC1 signaling (56).

Trp and Treg-Cell Function

Dietary aryl hydrocarbon receptor (AhR) ligands absorbed *via* the gut have been shown to be involved in Th17 generation (60, 61). Trp, an essential amino acid derived from ingested proteins, is one of the AhR ligands. On the other hand, IDO in DCs catabolizes Trp, resulting in localized Trp depletion. Trp-derived kyurenins are a crucial mechanism of Treg-cell suppression (62) (**Figure 2**). Thus, different metabolites at different stages have their own roles in T cell lineage differentiation.

Vitamins and Treg Function

Treg-cell biology depends on vitamins A, D, B3 (Niacin), and B9. Fat-soluble vitamins, vitamin A, D, E, and K, are enriched in the human liver (**Figure 2**). Vitamin A, RA, has an important role in Treg-cell development and function in the gut *via* CD103 DCs in the mesenteric lymph nodes (63). RA can also generate gut homing Treg cells (64). Vitamin B3, nicotinic acid, signals through GPCR109a and leads to expression of retinal dehydrogenases in colonic DCs, which in turn induces Treg-cell differentiation thus vitamin B3 promotes colonic Treg-cell generation (65). It is likely that these DC subsets may be present in the inflamed human liver *via* the portal vein (**Figure 2**). 1,25$(OH)_2$ vitamin D3 inhibits T effector cell proliferation, induces Foxp3 expression, and enhances the suppressive activity of Treg cells (66). Folic acid, derived from vitamin B9, is required for DNA synthesis and repair. Human Treg cells express high level of folate receptor-4 (FR4) (67) and inhibit Treg-cell apoptosis (68) (**Figure 2**).

T Cell Migration and Metabolism

The majority of liver resident immune cells, including Treg cells, are normally observed within the hepatic portal tract and septum (interface hepatitis) and parenchyma (lobular hepatitis) depending on the local area of inflammatory response (69). We reported previously that the CXCR3–CXCL10 pathway is crucial for recruitment of blood Treg cells to the inflamed liver *via* the hepatic sinusoids (70) and the CCR6–CCL20 axis plays an essential role in the positioning of lymphocytes around the bile ducts (71). We have also shown that the survival of intrahepatic lymphocytes depends on VCAM1 expression on the bile ducts and VLA-4 expression on the lymphocytes (72). Once circulatory Treg cells are recruited *via* the sinusoids, their post-endothelial migration and positioning are influenced by integrin expression on the fibrous stromal framework in the liver and also the chemokines gradient (73). Some intrahepatic Treg cells may drain back to local draining portal lymph nodes, which drain the liver. In addition, the hepatic microenvironment is hypoxic, especially around the central vein region and there is a high level of lactate in the inflamed human liver. T effector cell migration is known to be highly dependent on aerobic glycolysis, and lactate seems to regulate their migration (74). Recent data suggested that glycolysis was instrumental for Treg migration and was initiated by pro-migratory stimuli *via* a PI3K–mTORC2-mediated pathway culminating in induction of the enzyme GCK. Subsequently, GCK promoted cytoskeletal rearrangements by associating with actin. Treg cells lacking this pathway were functionally suppressive but failed to migrate to skin allografts and inhibit rejection suggesting that GCK-dependent glycolysis regulates Treg-cell migration (40).

EPIGENETIC CONTROL OF T CELL METABOLISM

Epigenetic mechanisms, such as histone modification, DNA methylation of CpG residues, and nucleosome repositioning, alter the accessibility of transcription factors and RNA polymerase to regulatory regions of the genome are important regulators of the immune cells and their metabolism.

Methylation

The stable expression of Foxp3, which is important for Treg cell's suppressive function, is maintained *via* the demethylation of the TSDR (75, 76). However, pTreg cells show a lack of Treg-specific DNA hypomethylation, which correlates with Treg cell's genetic signature (75). Stable Foxp3 requires DNA hypomethylation at FOXP3 CNS2 (76, 77).

Acetylation, HDAC Inhibitor, and Metabolism

Epigenetic modifications influence the chromatin remodeling *via* acetylation; DNA methylation and histone modifications play a key role in the regulation of metabolic gene expression and cell differentiation, function, and recruitment. Histone acetylation by histone acetyl transferases allows gene expression and histone deacetylation by HDACs, which inhibits gene expression as well as regulating chromatin remodeling and functional transcription factors. Administration of an HDAC inhibitor (HDACi) *in vivo* increased Foxp3 gene expression, as well as the production and suppressive function of Treg cells. HDAC9 seems to be particularly important in regulating Foxp3-dependent suppression. HDACi therapy *in vivo* enhanced Treg cell-mediated suppression and decreased the degree of inflammatory bowel disease (78). Thus, pharmacological inhibitors of HDAC have potential therapeutic benefits in autoimmunity. Their action may also be mediated *via* immunometabolism; for example, HDAC inhibition leads to the induction Treg-cell generation by butyrate (78). HDACis, such as trichostatin A, SAHA, butyrate, and valproic acid, lead to immunomodulation by upregulating Treg-cell programming and suppressing Th1/Th17 programming (79) (**Figure 3**).

TRANSLATIONAL IMMUNOMETABOLISM OF REGULATORY T CELLS

Manipulation of metabolism to enhance Treg-cell function in autoimmune diseases is an exciting therapeutic avenue. Dysregulated effector T cell responses and failure of T regulatory cell suppression of these effector cells is a typical feature of autoimmunity. Repurposing of current metabolic drugs and exploring new targets to change immune balance is currently an attractive translational option for clinicians and scientists who aim to apply cell metabolism for patient benefit.

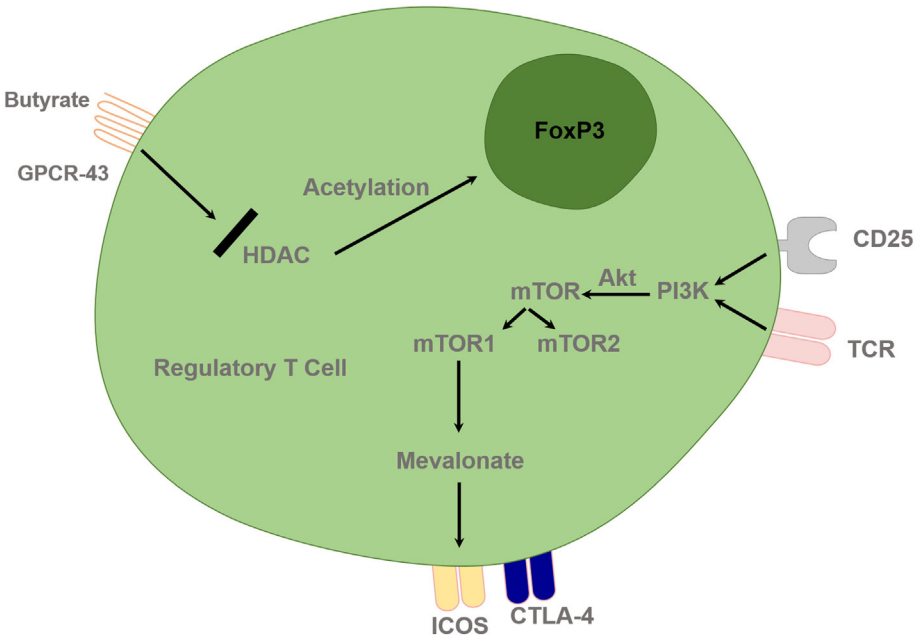

FIGURE 3 | Regulatory T cells are controlled by epigenetic mechanisms and T cell receptor (TCR) and interleukin-2 (IL-2) pathway. Histone acetylation facilitates FoxP3 gene expression and deacetylation inhibits gene expression. HDACi, such as butyrate, can lead to upregulation of the Treg-cell master regulatory gene, FoxP3, and enhance its function. TCR and IL-2 combined stimulation leads to PI3 kinase activation and subsequent downstream activation of mTOR signaling *via* Akt. Treg cells require lipogenic metabolism *via* the mevalonate pathway, which subsequently leads to upregulation of Treg-cell suppressive molecules cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and ICOS in mTORC1-dependent manner. HDAC, Histone deacetylase inhibitor; PI3K, phosphatidylinositol 3-kinase; Akt, Protein Kinase B; mTOR, mammalian target of rapamycin; GPCR, G protein–coupled receptor.

Targeting Carbohydrate Metabolism

Although it is accepted that repression of Akt/mTOR, hypoxic induced factor 1-α (HIF-1α), and aerobic glycolysis is important for the efficient generation of blood Treg cells *in vitro*, clear evidence how these pathways impact on blood and tissue resident Treg-cell development *in vivo* is limited. Understanding these pathways, not only in the circulation but also at the tissue level is necessary to enhance Treg-cell metabolism and its subsequent function. Although Glut-1 expression is a critical factor in driving glycolysis in circulatory T effector cells, Treg cells' function seems to be independently of Glut-1 (80).

HIF-1α Pathway

The hepatic environment in both inflammatory and autoimmune conditions is hypoxic as human liver receive only 25% of oxygen rich blood supply from hepatic artery, and the rest of the blood supply is from portal vein (1). Thus, targeting the transcription factor HIF-1α is a promising strategy as it exerts a crucial role in the balance between Th17 and Treg cells (81). HIF-1α predominantly affects effector T cell metabolism compared with Treg, thus there is a shift in immune cells balance under hypoxic conditions (82). HIF-1α and aryl hydrocarbon receptor (AhR) compete for limited amounts of aryl hydrocarbon receptor nuclear translocator (ARNT) also know as HIF-1β. This competition is the key to the mutual regulation of HIF-1α and AhR (83) (**Figure 1**). ARNT serves as a common binding partner for AhR as well as HIF-1α. HIF-1α proteins are regulated in an oxygen-dependent manner, whereas ARNT is constitutively expressed, as neither ARNT mRNA nor the protein level is influenced by hypoxia. In the context of transcription factor, HIF-1α induces FoxP3, which leads to Treg-cell abundance, and Treg-intrinsic HIF-1α is required for optimal Treg function (84). HIF-1α-deficient Treg cells fail to control T-cell-mediated colitis (84).

HIF-1α is selectively expressed in Th17 cells, and its induction requires signaling through mTOR, a central regulator of glycolytic metabolism. Therefore, blocking glycolysis inhibited Th17 cell development while it promotes Treg-cell generation (39). Lack of HIF-1α *in vivo* has been reported to diminish Th17 cell development but enhance Treg-cell differentiation and prevent autoimmune neuroinflammation (39).

Metformin and 2-Deoxy-D-Glucose (2DG)

We reported that human liver infiltrating Treg cells are of an effector memory phenotype (16). However, intrahepatic Treg metabolic phenotype is still unexplored. When resting naive T cells are activated, they differentiate toward an effector T cell lineage due to a shift in the catabolic state of metabolism, which is driven predominantly by the glycolytic–lipogenic pathway through the TCA cycle. This upregulation of aerobic glycolysis, "Warburg effect," is a feature of activated immune cells including T cells and is dependent on an mTOR–nutrient-sensing pathway with signaling *via* phosphoinositide 3-kinase (PI3K) and protein kinase B (Akt) (85). In addition, CD4 T cell differentiation into the effector T cell lineage toward Th1 or Th17 cell phenotypes is dependent on glucose and Glu for anabolic metabolism. Recent study demonstrated that CD4 effector

T cells from lupus-affected mice showed elevated glycolysis and mitochondrial oxidative metabolism and inhibition of these pathways with *mitochondrial metabolism inhibitor metformin and the glucose metabolism inhibitor 2DG* reduced IFNγ production (86). Furthermore, blocking glycolysis *via* 2DG can selectively impair effector T cells thereby by improving Treg-cell function in a mouse model of multiple sclerosis (87). Antidiabetic medication, Metformin not only reduce Th17 cell responses and attenuates disease severity in experimental autoimmune encephalomyelitis (88) but also increase numbers of Treg cells *via* suppressing the activation of mTOR and its downstream target, HIF-1α (89). Thus, priming the T cells with Metformin may be an attractive option to shift the immune cell balance to the regulatory arm.

Soraphen A

Th17 cells, but not Treg cells, depend on ACC1 (acetyl CoA carboxylase 1), a key enzyme that drives FA synthesis and the underlying glycolytic–lipogenic metabolic pathway for their development. Treatment with the ACC-specific inhibitor Soraphen A or T cell-specific deletion of ACC1 in mice attenuates Th17 cell-mediated autoimmune disease (90). Although Th17 cells use this pathway to produce phospholipids for cellular membranes, Treg cells readily take up exogenous FAs for this purpose. Pharmacologic inhibition or T cell-specific deletion of ACC1 not only blocks *de novo* FA synthesis but also interferes with the metabolic flux of glucose-derived carbon *via* glycolysis and the TCA cycle. Thus, the ACC1 pathway could be an attractive option to alter immune cell balance.

Phosphatase and Tensin Homolog (PTEN)

Phosphatase and tensin homolog lipid phosphatase is the main negative regulator of PI3K–Akt signaling and glycolysis in Treg cells. The activity of phosphoinositide-3-kinase (PI3K) is essential for Treg-cell lineage homeostasis and stability. Mechanistically, PTEN maintained Treg-cell stability and metabolic balance between glycolysis and mitochondrial fitness (91). Control of PI3K signaling by PTEN in Treg cells is critical for maintaining their homeostasis, function, and stability (92). PTEN deficiency upregulates activity of the metabolic checkpoint kinase complex mTORC2, and the serine–threonine kinase Akt, and loss of this activity restores functioning of PTEN-deficient Treg cells. Thus, PTEN–mTORC2 axis maintains Treg-cell stability and coordinates Treg cell-mediated control of effector responses (91), and PTEN inhibitor can lead to Treg destabilization (93).

Targeting Lipid Metabolism

Mevalonate and Statins

Cholesterol lowering medications, statins are inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase, which catalyzes the formation of mevalonate, the rate-limiting step for cholesterol synthesis. As a result, statins are widely used for cardiovascular disease prevention. Statins can differentiate T cells toward Treg cells instead of Th17 cells *via* a mechanism dependent on protein granulation (94). In general, Treg cells require lipid and cholesterol metabolism. The mevalonate pathway is particularly important for coordinating Treg proliferation and for upregulating the suppressive molecules CTLA-4 and ICOS to establish functional competency of Treg. Mevalonate can reverse the effects of statins involved in maintaining Treg functional fitness in an mTORC1-dependent manner (37) (**Figure 3**). Thus, mevalonate pathway could be manipulated to enhance Treg function in autoimmunity.

However, recent work from Hu and colleagues seems to contradict this finding as they suggested that cholesterol biosynthesis and uptake programs are induced during Th17 differentiation, resulting in the accumulation of the cholesterol precursor, desmosterol, which functions as a potent endogenous RORγ agonist (95, 96). Thus, blocking cholesterol synthesis with chemical inhibitors at steps before the formation of active precursors would reduce differentiation to Th17. In addition, Simvastatin has also been shown to improve disease activity and the inflammation factor in patients with multiple sclerosis (97) and SLE (98). As there are conflicting data, more studies are required to dissect the mechanistic immunomodulatory effects of statins on Treg and Th17 cells.

Rapamycin and mTOR

The activation of mTOR, which is the catalytic subunit of the mTORC1 and mTORC2 complexes, delivers signals for the activation and differentiation of effector CD4 T cells, whereas Akt–mTOR axis is a crucial negative regulator of Treg *de novo* differentiation and expansion (**Figure 3**). mTORC1 signals through the TCR and the co-receptor CD28, and Treg cells have elevated steady-state mTORC1 activity compared with naive T cells. Signals through the TCR and IL-2 provide major inputs for mTORC1 activation, which in turn programs the suppressive function of Treg (**Figure 3**). Disruption of mTORC1 through Treg-specific deletion of the essential component raptor results in profound loss of Treg-cell suppressive activity and the development of a fatal early onset inflammatory disorder (37). In addition, Raptor–mTORC1 signaling in Treg cells promotes cholesterol and lipid metabolism, with the mevalonate pathway particularly important for coordinating Treg-cell proliferation and upregulation of the suppressive molecules CTLA-4 and ICOS to establish Treg functional competency (**Figure 3**). Thus, mTORC1 acts as a "rheostat" in Treg cells to link immunological signals from TCR and IL-2 to lipogenic pathways and functional fitness, and highlights a central role of metabolic programming of Treg suppressive activity in immune homeostasis and tolerance. All these data may lead investigators to reconsider and dissect the current use of rapamycin in good manufacturing practice (GMP) Treg-cell culture media (to prevent effector Th17 cells outgrowth) in Treg-cell therapy setting.

Peroxisome Proliferator-Activated Receptor (PPAR) Agonist—Pioglitazone

Peroxisome proliferator-activated receptors are nuclear receptors that regulate gene transcription. PPARα is highly expressed in liver and skeletal muscle and controls genes involved in fatty-acid

oxidation (99). PPARγ is expressed in adipocytes, skeletal muscle, liver, and kidney and regulates expression of the genes that mediate metabolism (100). PPARγ agonists, thiazolidinedione drug pioglitazone, could potentially become an attractive drug candidate for anti-inflammatory therapies.

Targeting Protein Metabolism

Glu and α Ketoglutarate

Glutamine (Glu), a central anabolic nutrient in the TCA cycle, is critical for T cell survival, proliferation, and function. Glu is required for naive CD4 T cell differentiation toward Th1 and Th17 inflammatory T cells. In patients with multiple sclerosis, increased levels of both Glu and glutamate have been reported (101, 102). TCR engagement of naive CD4 T cells has been shown to trigger rapid uptake of Glu, *via* amino acid transporters. Glu deprivation has been shown to enhance the suppressive activity of Treg cells in an autoimmune colitis model (56). Thus, decline in Glu and α-ketoglutarate, Glu-derived TCA cycle metabolite could enhance Treg cells' function.

BENEFITS AND SHORTCOMINGS OF CURRENT TECHNOLOGY

Both glycolysis and mitochondrial respiration can be studied using a Seahorse machine for immune cell subsets including peripheral blood CD4 and CD8 T cells. Extracellular acidification rate (ECAR), a measure of lactate production by glycolysis, and mitochondrial oxygen consumption rate of both blood and tissue resident cells are necessary to analyze the metabolism and function of these cells. However, Seahorse technology is still not possible to apply for small frequency cell subset, such as regulatory T cells, to perform ECAR and OCAR experiments without cell expansion. However, expansion of the Treg will change their metabolic phenotype. Similarly, metabolic tracing with fluorescence uptake of glucose, Glu, lactate, or palmitate of Treg requires a significant number of cells. Thus, current available methodology to study tissue derived Treg is limited to Mitotracker and TMRE assays along with electron microscopy. In addition, comparing the metabolic activity of glycolysis and mitochondrial respiration in central memory ($CD45RA^{-}CCR7^{+}$), naïve ($CD45RA^{+}CCR7^{+}$), tissue resident ($CD45RA^{+}CCR7^{-}$), and effector memory ($CD45RA^{-}CCR7^{-}$) subsets of intrahepatic Treg cells is crucial to understand the metabolism and functional potential of each subset. However, investigators are limited to perform these analyses not by Seahorse technology but only by Mitotracker and TMRE assays along with electron microscopy due to the current requirement of high cell numbers. In addition, studies to assess tissue resident cell metabolism under hypoxic conditions and their migration would require a modified combined technology of hypoxic chambers or migration chambers in combination with Seahorse equipment.

CONCLUSION

T cell metabolism and immunology have recently been merged to form immunometabolism. Intrahepatic Treg cells can control local hepatic immune homeostasis. There is enormous potential to utilize Treg to restore tolerance in the treatment of human autoimmune diseases including autoimmune liver diseases. Modulation of immunometabolism of Treg represents a new avenue to enhance Treg-cell function and maintain a stable lineage. Immunometabolic manipulation may also have an impact on Treg cytoskeletal rearrangement and post-endothelial migration, positioning around hepatocytes and bile ducts and retention as intrahepatic tissue resident Treg. However, to date, there are no data on human liver tissue resident Treg-cell metabolism. In the future, improvement of technology may allow us to study the metabolic profile and associated function Treg. Manipulating the cell culture media to enhance the metabolism of Treg during GMP isolation and expansion and modulating the tissue and circulatory compartments with immunometabolic drugs in autoimmune patients before Treg infusion would enhance the potential of effective and successful Treg-cell therapy.

AUTHOR CONTRIBUTIONS

RW and HB wrote the manuscript. RW and YO constructed the mechanistic **Figures 1–3**. YO supervised and edited the final manuscript.

FUNDING

This study was supported by Medical Research Council, Queen Elizabeth Hospital Charity, National Institute of Health Research Birmingham Biomedical Research Centre, and Medical Research Council Clinician Scientist Award (Grant number—G1002552).

REFERENCES

1. Jeffery HC, Braitch MK, Brown S, Oo YH. Clinical potential of regulatory T cell therapy in liver diseases: an overview and current perspectives. *Front Immunol* (2016) 7:334. doi:10.3389/fimmu.2016.00334
2. Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* (2001) 182:18–32. doi:10.1034/j.1600-065X.2001.1820102.x
3. Seddiki N, Santner-Nanan B, Martinson J, Zaunders J, Sasson S, Landay A, et al. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *J Exp Med* (2006) 203(7):1693–700. doi:10.1084/jem.20060468
4. Liu W, Putnam AL, Xu-Yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ Treg cells. *J Exp Med* (2006) 203:1701–11. doi:10.1084/jem.20060772
5. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* (2003) 299:1057–61. doi:10.1126/science.1079490
6. Bennett CL, Ochs HD. IPEX is a unique X-linked syndrome characterized by immune dysfunction, polyendocrinopathy, enteropathy, and a variety of autoimmune phenomena. *Curr Opin Pediatr* (2001) 13:533–8. doi:10.1097/00008480-200112000-00007

7. Godfrey VL, Wilkinson JE, Russell LB. X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. *Am J Pathol* (1991) 138:1379–87.
8. Zheng Y, Josefowicz S, Chaudhry A, Peng XP, Forbush K, Rudensky AY. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T-cell fate. *Nature* (2010) 463:808–12. doi:10.1038/nature08750
9. Walker LS, Abbas AK. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat Rev Immunol* (2002) 2:11–9. doi:10.1038/nri701
10. Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, et al. Regulatory T cells: recommendations to simplify the nomenclature. *Nat Immunol* (2013) 14:307–8. doi:10.1038/ni.2554
11. Ohkura N, Kitagawa Y, Sakaguchi S. Development and maintenance of regulatory T cells. *Immunity* (2013) 38:414–23. doi:10.1016/j.immuni.2013.03.002
12. Liu ZM, Wang KP, Ma J, Guo Zheng S. The role of all-trans retinoic acid in the biology of Foxp3+ regulatory T cells. *Cell Mol Immunol* (2015) 12:553–7. doi:10.1038/cmi.2014.133
13. O'Garra A, Barrat FJ. In vitro generation of IL-10-producing regulatory CD4+ T cells is induced by immunosuppressive drugs and inhibited by Th1- and Th2-inducing cytokines. *Immunol Lett* (2003) 85:135–9. doi:10.1016/S0165-2478(02)00239-0
14. Haribhai D, Williams JB, Jia S, Nickerson D, Schmitt EG, Edwards B, et al. A requisite role for induced regulatory T cells in tolerance based on expanding antigen receptor diversity. *Immunity* (2011) 35:109–22. doi:10.1016/j.immuni.2011.03.029
15. Sakaguchi S. Regulatory T cells: key controllers of immunologic self-tolerance. *Cell* (2000) 101:455–8. doi:10.1016/S0092-8674(00)80856-9
16. Chen YY, Jeffery HC, Hunter S, Bhogal R, Birtwistle J, Braitch MK, et al. Human intrahepatic Tregs are functional, require IL-2 from effector cells for survival and are susceptible to Fas ligand mediated apoptosis. *Hepatology* (2016) 64(1):138–50. doi:10.1002/hep.28517
17. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. *Cell* (2008) 133:775–87. doi:10.1016/j.cell.2008.05.009
18. Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science* (2008) 322:271–5. doi:10.1126/science.1160062
19. Jeffery HC, Jeffery LE, Lutz P, Corrigan M, Webb GJ, Adams DH, et al. Low dose interleukin-2 promotes STAT5 phosphorylation, Treg survival and CTLA-4 dependent function in autoimmune liver diseases. *Clin Exp Immunol* (2017) 188(3):394–411. doi:10.1111/cei.12940
20. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J Exp Med* (2007) 204:1257–65. doi:10.1084/jem.20062512
21. Chaudhry A, Samstein RM, Treuting P, Liang Y, Pils MC, Heinrich JM, et al. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* (2011) 34:566–78. doi:10.1016/j.immuni.2011.03.018
22. Wu Y, Borde M, Heissmeyer V, Feuerer M, Lapan AD, Stroud JC, et al. FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell* (2006) 126:375–87. doi:10.1016/j.cell.2006.05.042
23. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* (1995) 3:541–7. doi:10.1016/1074-7613(95)90125-6
24. Chambers CA, Sullivan TJ, Allison JP. Lymphoproliferation in CTLA-4-deficient mice is mediated by costimulation-dependent activation of CD4+ T cells. *Immunity* (1997) 7:885–95. doi:10.1016/S1074-7613(00)80406-9
25. Qureshi OS, Zheng Y, Nakamura K, Attridge K, Manzotti C, Schmidt EM, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* (2011) 332:600–3. doi:10.1126/science.1202947
26. Pallotta MT, Orabona C, Volpi C, Vacca C, Belladonna ML, Bianchi R, et al. Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells. *Nat Immunol* (2011) 12:870–8. doi:10.1038/ni.2077
27. Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* (2007) 110:1225–32. doi:10.1182/blood-2006-12-064527
28. Lai WK, Curbishley SM, Goddard S, Alabraba E, Shaw J, Youster J, et al. Hepatitis C is associated with perturbation of intrahepatic myeloid and plasmacytoid dendritic cell function. *J Hepatol* (2007) 47:338–47. doi:10.1016/j.jhep.2007.03.024
29. Goddard S, Youster J, Morgan E, Adams DH. Interleukin-10 secretion differentiates dendritic cells from human liver and skin. *Am J Pathol* (2004) 164:511–9. doi:10.1016/S0002-9440(10)63141-0
30. Newton R, Priyadharshini B, Turka LA. Immunometabolism of regulatory T cells. *Nat Immunol* (2016) 17:618–25. doi:10.1038/ni.3466
31. Oo YH, Adams DH. The role of chemokines in the recruitment of lymphocytes to the liver. *J Autoimmun* (2010) 34:45–54. doi:10.1016/j.jaut.2009.07.011
32. Balmer ML, Slack E, de Gottardi A, Lawson MA, Hapfelmeier S, Miele L, et al. The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota. *Sci Transl Med* (2014) 6:237ra66. doi:10.1126/scitranslmed.3008618
33. Hwu P, et al. Indoleamine 2,3-dioxygenase production by human dendritic cells results in the inhibition of T cell proliferation. *J Immunol* (2000) 164:3596–9. doi:10.4049/jimmunol.164.7.3596
34. Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, et al. Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science* (2002) 297:1867–70. doi:10.1126/science.1073514
35. Terness P, Bauer TM, Röse L, Dufter C, Watzlik A, Simon H, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med* (2002) 196:447–57. doi:10.1084/jem.20020052
36. Basu S, Hubbard B, Shevach EM. Foxp3-mediated inhibition of Akt inhibits Glut1 (glucose transporter 1) expression in human T regulatory cells. *J Leukoc Biol* (2015) 97:279–83. doi:10.1189/jlb.2AB0514-273RR
37. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* (2013) 499:485–90. doi:10.1038/nature12297
38. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* (2011) 186:3299–303. doi:10.4049/jimmunol.1003613
39. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208:1367–76. doi:10.1084/jem.20110278
40. Kishore M, Cheung KCP, Fu H, Bonacina F, Wang G, Coe D, et al. Regulatory T cell migration is dependent on glucokinase-mediated glycolysis. *Immunity* (2017) 47:875–889.e10. doi:10.1016/j.immuni.2017.10.017
41. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* (1987) 28:1221–7. doi:10.1136/gut.28.10.1221
42. Montgomery MK, Osborne B, Brown SH, Small L, Mitchell TW, Cooney GJ, et al. Contrasting metabolic effects of medium- versus long-chain fatty acids in skeletal muscle. *J Lipid Res* (2013) 54:3322–33. doi:10.1194/jlr.M040451
43. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* (2009) 461:1282–6. doi:10.1038/nature08530
44. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* (2013) 341:569–73. doi:10.1126/science.1241165
45. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* (2013) 504:446–50. doi:10.1038/nature12721
46. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* (2013) 504:451–5. doi:10.1038/nature12726
47. Pepino MY, Kuda O, Samovski D, Abumrad NA. Structure-function of CD36 and importance of fatty acid signal transduction in fat metabolism. *Annu Rev Nutr* (2014) 34:281–303. doi:10.1146/annurev-nutr-071812-161220
48. Tomita T, Hosoda K, Fujikura J, Inagaki N, Nakao K. The G-protein-coupled long-chain fatty acid receptor GPR40 and glucose metabolism. *Front Endocrinol* (2014) 5:152. doi:10.3389/fendo.2014.00152
49. Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A, et al. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. *Immunity* (2015) 43:817–29. doi:10.1016/j.immuni.2015.09.007
50. Pan Y, Tian T, Park CO, Lofftus SY, Mei S, Liu X, et al. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. *Nature* (2017) 543:252–6. doi:10.1038/nature21379

51. Asarat M, Apostolopoulos V, Vasiljevic T, Donkor O. Short-chain fatty acids regulate cytokines and Th17/Treg cells in human peripheral blood mononuclear cells in vitro. *Immunol Invest* (2016) 45:205–22. doi:10.3109/08820139.2015.1122613
52. Schmidt A, Eriksson M, Shang MM, Weyd H, Tegnér J. Comparative analysis of protocols to induce human CD4+Foxp3+ regulatory T cells by combinations of IL-2, TGF-beta, retinoic acid, rapamycin and butyrate. *PLoS One* (2016) 11:e0148474. doi:10.1371/journal.pone.0148474
53. Bronte V, Zanovello P. Regulation of immune responses by L-arginine metabolism. *Nat Rev Immunol* (2005) 5:641–54. doi:10.1038/nri1668
54. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* (2016) 167:829–842.e13. doi:10.1016/j.cell.2016.09.031
55. Resheq YJ, Li KK, Ward ST, Wilhelm A, Garg A, Curbishley SM, et al. Contact-dependent depletion of hydrogen peroxide by catalase is a novel mechanism of myeloid-derived suppressor cell induction operating in human hepatic stellate cells. *J Immunol* (2015) 194:2578–86. doi:10.4049/jimmunol.1401046
56. Klysz D, et al. Glutamine-dependent alpha-ketoglutarate production regulates the balance between T helper 1 cell and regulatory T cell generation. *Sci Signal* (2015) 8:ra97. doi:10.1126/scisignal.aab2610
57. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* (2014) 40:692–705. doi:10.1016/j.immuni.2014.04.007
58. Chapman NM, Chi H. mTOR links environmental signals to T cell fate decisions. *Front Immunol* (2014) 5:686. doi:10.3389/fimmu.2014.00686
59. Sinclair LV, Rolf J, Emslie E, Shi YB, Taylor PM, Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat Immunol* (2013) 14:500–8. doi:10.1038/ni.2556
60. Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. *Nature* (2008) 453:106–9. doi:10.1038/nature06881
61. Veldhoen M, Hirota K, Christensen J, O'Garra A, Stockinger B. Natural agonists for aryl hydrocarbon receptor in culture medium are essential for optimal differentiation of Th17 T cells. *J ExpMed* (2009) 206:43–9. doi:10.1084/jem.20081438
62. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. *J Immunol* (2008) 181:5396–404. doi:10.4049/jimmunol.181.8.5396
63. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* (2007) 317:256–60. doi:10.1126/science.1145697
64. Sun CM, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 Treg cells via retinoic acid. *J Exp Med* (2007) 204(8):1775–85. doi:10.1084/jem.20070602
65. Singh N, Gurav A, Sivaprakasam S, Brady E, Padia R, Shi H, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity* (2014) 40:128–39. doi:10.1016/j.immuni.2013.12.007
66. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M, et al. 1,25-dihydroxyvitamin D(3) and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol* (2009) 183:5458–67. doi:10.4049/jimmunol.0803217
67. Yamaguchi T, Hirota K, Nagahama K, Ohkawa K, Takahashi T, Nomura T, et al. Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. *Immunity* (2007) 27:145–59. doi:10.1016/j.immuni.2007.04.017
68. Kinoshita M, Kayama H, Kusu T, Yamaguchi T, Kunisawa J, Kiyono H, et al. Dietary folic acid promotes survival of Foxp3+ regulatory T cells in the colon. *J Immunol* (2012) 189:2869–78. doi:10.4049/jimmunol.1200420
69. Protzer U, Maini MK, Knolle PA. Living in the liver: hepatic infections. *Nat Rev Immunol* (2012) 12:201–13. doi:10.1038/nri3169
70. Oo YH, Weston CJ, Lalor PF, Curbishley SM, Withers DR, Reynolds GM, et al. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. *J Immunol* (2010) 184:2886–98. doi:10.4049/jimmunol.0901216
71. Oo YH, Banz V, Kavanagh D, Liaskou E, Withers DR, Humphreys E, et al. CXCR3-dependent recruitment and CCR6-mediated positioning of Th-17 cells in the inflamed liver. *J Hepatol* (2012) 57:1044–51. doi:10.1016/j.jhep.2012.07.008
72. Afford SC, Humphreys EH, Reid DT, Russell CL, Banz VM, Oo Y, et al. Vascular cell adhesion molecule 1 expression by biliary epithelium promotes persistence of inflammation by inhibiting effector T-cell apoptosis. *Hepatology* (2014) 59:1932–43. doi:10.1002/hep.26965
73. Oo YH, Shetty S, Adams DH. The role of chemokines in the recruitment of lymphocytes to the liver. *Dig Dis* (2010) 28:31–44. doi:10.1159/000282062
74. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, et al. Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions. *PLoS Biol* (2015) 13:e1002202. doi:10.1371/journal.pbio.1002202
75. Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. *Immunity* (2012) 37:785–99. doi:10.1016/j.immuni.2012.09.010
76. Kim HP, Leonard WJ. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. *J Exp Med* (2007) 204:1543–51. doi:10.1084/jem.20070109
77. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3+ regulatory T cells in the human immune system. *Nat Rev Immunol* (2010) 10:490–500. doi:10.1038/nri2785
78. Tao R, de Zoeten EF, Ozkaynak E, Chen C, Wang L, Porrett PM, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nat Med* (2007) 13:1299–307. doi:10.1038/nm1652
79. Lu J, Chatain GP, Bugarini A, Wang X, Maric D, Walbridge S, et al. Histone deacetylase inhibitor SAHA is a promising treatment of cushing disease. *J Clin Endocrinol Metab* (2017) 102:2825–35. doi:10.1210/jc.2017-00464
80. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20:61–72. doi:10.1016/j.cmet.2014.05.004
81. Guan SY, Leng RX, Tao JH, Li XP, Ye DQ, Olsen N, et al. Hypoxia-inducible factor-1alpha: a promising therapeutic target for autoimmune diseases. *Expert Opin Ther Targets* (2017) 21:715–23. doi:10.1080/14728222.2017.1336539
82. Westendorf AM, Skibbe K, Adamczyk A, Buer J, Geffers R, Hansen W, et al. Hypoxia enhances immunosuppression by inhibiting CD4+ effector T cell function and promoting Treg activity. *Cell Physiol Biochem* (2017) 41:1271–84. doi:10.1159/000464429
83. Mandl M, Depping R. Hypoxia-inducible aryl hydrocarbon receptor nuclear translocator (ARNT) (HIF-1beta): is it a rare exception? *Mol Med* (2014) 20:215–20. doi:10.2119/molmed.2014.00032
84. Clambey ET, McNamee EN, Westrich JA, Glover LE, Campbell EL, Jedlicka P, et al. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. *Proc Natl Acad Sci U S A* (2012) 109:E2784–93. doi:10.1073/pnas.1202366109
85. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. *Science* (2013) 342:1242454. doi:10.1126/science.1242454
86. Yin Y, Choi SC, Xu Z, Perry DJ, Seay H, Croker BP, et al. Normalization of CD4+ T cell metabolism reverses lupus. *Sci Transl Med* (2015) 7:274ra218. doi:10.1126/scitranslmed.aaa0835
87. Gerriets VA, Kishton RJ, Nichols AG, Macintyre AN, Inoue M, Ilkayeva O, et al. Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. *J Clin Invest* (2015) 125:194–207. doi:10.1172/JCI76012
88. Nath N, Khan M, Paintlia MK, Singh I, Hoda MN, Giri S. Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. *J Immunol* (2009) 182:8005–14. doi:10.4049/jimmunol.0803563
89. Sun Y, Tian T, Gao J, Liu X, Hou H, Cao R, et al. Metformin ameliorates the development of experimental autoimmune encephalomyelitis by regulating T helper 17 and regulatory T cells in mice. *J Neuroimmunol* (2016) 292:58–67. doi:10.1016/j.jneuroim.2016.01.014
90. Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. *Nat Med* (2014) 20:1327–33. doi:10.1038/nm.3704
91. Shrestha S, Yang K, Guy C, Vogel P, Neale G, Chi H. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. *Nat Immunol* (2015) 16:178–87. doi:10.1038/ni.3076

92. Huynh A, DuPage M, Priyadharshini B, Sage PT, Quiros J, Borges CM, et al. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. *Nat Immunol* (2015) 16:188–96. doi:10.1038/ni.3077
93. Sharma MD, Shinde R, McGaha TL, Huang L, Holmgaard RB, Wolchok JD, et al. The PTEN pathway in Tregs is a critical driver of the suppressive tumor microenvironment. *Sci Adv* (2015) 1:e1500845. doi:10.1126/sciadv.1500845
94. Kagami S, Owada T, Kanari H, Saito Y, Suto A, Ikeda K, et al. Protein geranylgeranylation regulates the balance between Th17 cells and Foxp3+ regulatory T cells. *Int Immunol* (2009) 21:679–89. doi:10.1093/intimm/dxp037
95. Hu X, Wang Y, Hao LY, Liu X, Lesch CA, Sanchez BM, et al. Sterol metabolism controls T(H)17 differentiation by generating endogenous RORgamma agonists. *Nat Chem Biol* (2015) 11:141–7. doi:10.1038/nchembio0915-741b
96. Santori FR. Nuclear hormone receptors put immunity on sterols. *Eur J Immunol* (2015) 45:2730–41. doi:10.1002/eji.201545712
97. Chataway J, Schuerer N, Alsanousi A, Chan D, MacManus D, Hunter K, et al. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. *Lancet* (2014) 383:2213–21. doi:10.1016/S0140-6736(13)62242-4
98. Ulivieri C, Baldari CT. Statins: from cholesterol-lowering drugs to novel immunomodulators for the treatment of Th17-mediated autoimmune diseases. *Pharmacol Res* (2014) 88:41–52. doi:10.1016/j.phrs.2014.03.001
99. Duval C, Fruchart JC, Staels B. PPAR alpha, fibrates, lipid metabolism and inflammation. *Arch Mal Coeur Vaiss* (2004) 97:665–72.
100. Lefterova MI, Haakonsson AK, Lazar MA, Mandrup S. PPARgamma and the global map of adipogenesis and beyond. *Trends Endocrinol Metab* (2014) 25:293–302. doi:10.1016/j.tem.2014.04.001
101. Sarchielli P, Greco L, Floridi A, Floridi A, Gallai V. Excitatory amino acids and multiple sclerosis: evidence from cerebrospinal fluid. *Arch Neurol* (2003) 60:1082–8. doi:10.1001/archneur.60.8.1082
102. Tisell A, Leinhard OD, Warntjes JB, Aalto A, Smedby Ö, Landtblom AM, et al. Increased concentrations of glutamate and glutamine in normal-appearing white matter of patients with multiple sclerosis and normal MR imaging brain scans. *PLoS One* (2013) 8:e61817. doi:10.1371/journal.pone.0061817

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 11 December 2017
doi: 10.3389/fimmu.2017.01719

Amino Acid Sensing *via* General Control Nonderepressible-2 Kinase and Immunological Programming

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Metabolic adaptation to the changing nutrient levels in the cellular microenvironment plays a decisive role in the maintenance of homeostasis. Eukaryotic cells are equipped with nutrient sensors, which sense the fluctuating nutrients levels and accordingly program the cellular machinery to mount an appropriate response. Nutrients including amino acids play a vital role in maintaining cellular homeostasis. Therefore, over the evolution, different species have developed diverse mechanisms to detect amino acids abundance or scarcity. Immune responses have been known to be closely associated with the cellular metabolism especially amino acid sensing pathway, which influences innate as well as adaptive immune-effector functions. Thus, exploring the cross-talk between amino acid sensing mechanisms and immune responses in disease as well as in normal physiological conditions might open up avenues to explore how this association can be exploited to tailor immunological functions toward the design of better therapeutics for controlling metabolic diseases. In this review, we discuss the advances in the knowledge of various amino acid sensing pathways including general control nonderepressible-2 kinase in the control of inflammation and metabolic diseases.

Keywords: amino acid sensing, general control nonderepressible-2 kinase, mammalian target of rapamycin, inflammation, immune response, metabolic diseases

OPEN ACCESS

Edited by:
Duncan Howie, University of Oxford, United Kingdom

Reviewed by:
Thomas Weichhart, Medical University of Vienna, Austria
Natalio Garbi, University of Bonn, Germany

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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *15 September 2017*
Accepted: *21 November 2017*
Published: *11 December 2017*

Citation:
Battu S, Minhas G, Mishra A and Khan N (2017) Amino Acid Sensing via General Control Nonderepressible-2 Kinase and Immunological Programming. Front. Immunol. 8:1719. doi: 10.3389/fimmu.2017.01719

INTRODUCTION

The association between inflammation and metabolism has been known since 1930s when Kempner and Peschel published their work on the subject and used the term "immunometabolism" for the first time (1, 2). However, growing evidence suggests that the nutrient availability and cellular metabolism plays a central role in physiological processes including cell proliferation, differentiation, and cell death (3). Accordingly, all organisms have developed diverse sensing mechanisms to detect and respond to scarcity and abundance of different nutrients. Unlike unicellular organisms (which sense the availability of nutrients in the environment directly), multicellular organisms consist of various nutrient sensing mechanisms to sense and respond to both extracellular and intracellular nutrient fluctuations (4, 5). Eukaryotic cells detect changes in nutrients levels through sensors that could be a transporter, receptor, signaling proteins or an enzyme. This is also pertinent to the immune cells, as they highly rely on energy supplies for expansion, differentiation, and the synthesis of immune effector molecules required for clearance of the infected or altered cells (3). The close association of immune system with the metabolic pathways involved in the sensing, management of the metabolites plays a crucial role in the maintenance of immune homeostasis, and therefore, dysregulation in the functioning of these pathways results in chronic inflammatory

conditions (3). Accumulating evidence suggests that caloric restriction (CR) without malnutrition increases lifespan and protects from age-associated inflammatory diseases, such as diabetes, cardiovascular diseases, cancer, and brain atrophy (6, 7). It has been postulated that regulatory genes, which are fundamental to energy metabolism play a vital role in CR-induced physiological benefits (8). Although CR-associated benefits have often been related to reduced energy intake, growing shreds of evidence implicate dietary amino acid limitations to CR benefits (9). Amino acids are the building blocks of proteins and the predominant macromolecules in the cell. Also, amino acids are vital nutrients for cellular homeostasis, not only as the energy source or constituents of proteins but also as the signaling modules, which is evident from the evolutionarily conserved pathways that play a fundamental role in amino acid sensing (10). Recent studies have unveiled that amino acid restriction alone enhances insulin sensitivity in mice (11). Also, it has been shown that administration of reduced amino acid diet protects mice from DSS induced intestinal inflammation by activating the cellular homeostatic process, such as autophagy (12). Therefore, understanding the mechanisms through which cells sense and mount an appropriate response to the bioavailability of amino acids has been an area of active research. It has been well established that amino acids presence or absence in mammals are sensed by predestined distinct signaling pathway involving mammalian target of rapamycin (mTOR) or general control nonderepressible-2 kinase (GCN2), respectively (13). mTOR acts as one of the primary metabolic switches, which integrate amino acid, growth factor, and energy availability to promote anabolic processes such as protein synthesis, while at the same time it inhibits catabolic functions such as autophagy (14). In contrary, depletion of even single amino is directly sensed by GCN2, which in turn program cellular machinery to promote catabolic processes such as autophagy. Recent studies implicate the centrality of GCN2 in the maintenance of immune homeostasis (15), and the dysfunction underlies several chronic metabolic diseases (12, 16). Here, we present a survey of the current research advances made toward understanding the link between GCN2-mediated amino acid sensing mechanisms and immunological regulation and further discuss the recent findings of their implications in the pathogenesis of acute or chronic inflammatory and metabolic diseases.

AMINO ACID SENSING MECHANISMS

Amino acids are the vital macronutrients that not only serve as the primary building blocks of proteins but also serve as an alternate energy source (17). The process of protein synthesis is one of the most energy-requiring processes in the cell, and therefore, mechanisms to efficiently sense the availability of amino acids and trigger appropriate responses become pertinent for the maintenance of cellular homeostasis (18). Across the species, various mechanisms have been evolved to detect the scarcity or abundance of different extracellular and intracellular amino acids in the microenvironment. Bacterial cells sense amino acid bioavailability through programming its transcriptional control, while eukaryotic cells sense footprints of amino acids scarcity by diverse mechanisms, including accumulated uncharged cognate tRNAs sensing (9). Eukaryotic cells are well equipped with sensors such as mTOR, which gets activated during the amino acid sufficiency and programs various anabolic processes required for the growth (19, 20). In contrast, intracellular depletion of even single essential amino acid (EAA) or non-essential amino acid is directly sensed by the GCN2 *via* binding of uncharged cognate tRNAs (21). GCN2 and mTOR pathways have evolved together in eukaryotes to serve as a major regulatory switch dictating protein synthesis in response to the fluctuating levels of amino acids (22). mTOR, an evolutionarily conserved serine/threonine kinase initially identified in yeast as TOR (23), is activated in the presence of specific amino acids especially leucine, arginine, and methionine (19, 20) and links amino acids availability with the cell growth, proliferation, and differentiation (24–26). Accumulating evidence suggests that mTOR localizes to lysosomes as a function of amino acids (27). During amino acid sufficiency, vacular H^+ATPase (v-ATPase, the first downstream target known so far) triggers the guanine exchange factor activity of Ragulator complex, which results in the nucleotide exchange and activation of RAG GTPase (28). Further, activated RAG GTPase binds and recruits mTORC1 to the lysosomal membrane in close proximity to mTORC1 activator RHEB (27, 29). Together, these stimuli lead to the mTORC1 activation. Activated mTORC1 translocates to the lysosome and phosphorylates 4EBP1, to release the translation initiation factor, eIF4E, which recruits mRNA to the ribosomes to initiate protein synthesis (24, 30) (**Figure 1**). Also, mTOR has central control over various transcription factors, like NF-κB, STAT3, and HIF1α (31). Conversely, in the absence of amino acids, mTOR is inactivated and diffused in the cytosol (32), which increases the 4EBP1 de-phosphorylation and halts protein translation (20). Albeit the precise amino acid sensor in the cytosol or at the lysosome is unknown, recent cell-based biochemical studies have shown the proteins responsible for Rag GTPases tethering to the lysosomal surfaces (27), and other regulatory proteins functioning upstream of Rag GTPases (28, 33, 34).

Mammalian target of rapamycin integrates various cellular functions including protein synthesis, cell proliferation, autophagy, and metabolism. It gets activated by virtue of signaling events initiated by receptors for particular antigens, cytokines, and growth factors (35). Several studies report that antigen engagement of T-cell receptor (TCR) and CD28 (costimulatory receptor) leads to the activation of phosphatidyl inositol 3 kinase (PI3K) and Akt, which eventually leads to mTOR activation (36, 37). Albeit Akt plays a central regulatory role in signaling pathways implicated in T cell proliferation, metabolism, migration, and activation (38, 39), a study has demonstrated that phosphorylation of S6 downstream of the TCR and CD28 stimulation is not majorly dependent on Akt (40). Furthermore, a recent study by Hamilton et al., established that adaptor protein Carma1 and one of its associated proteins, MALT1 are essential for optimal activation of mTOR in T cells (41). Also, Akt and mTOR pathways play a key role B cell proliferation and differentiation (42).

Recent studies suggest that mTOR gets activated in immune cells through numerous factors including growth factors,

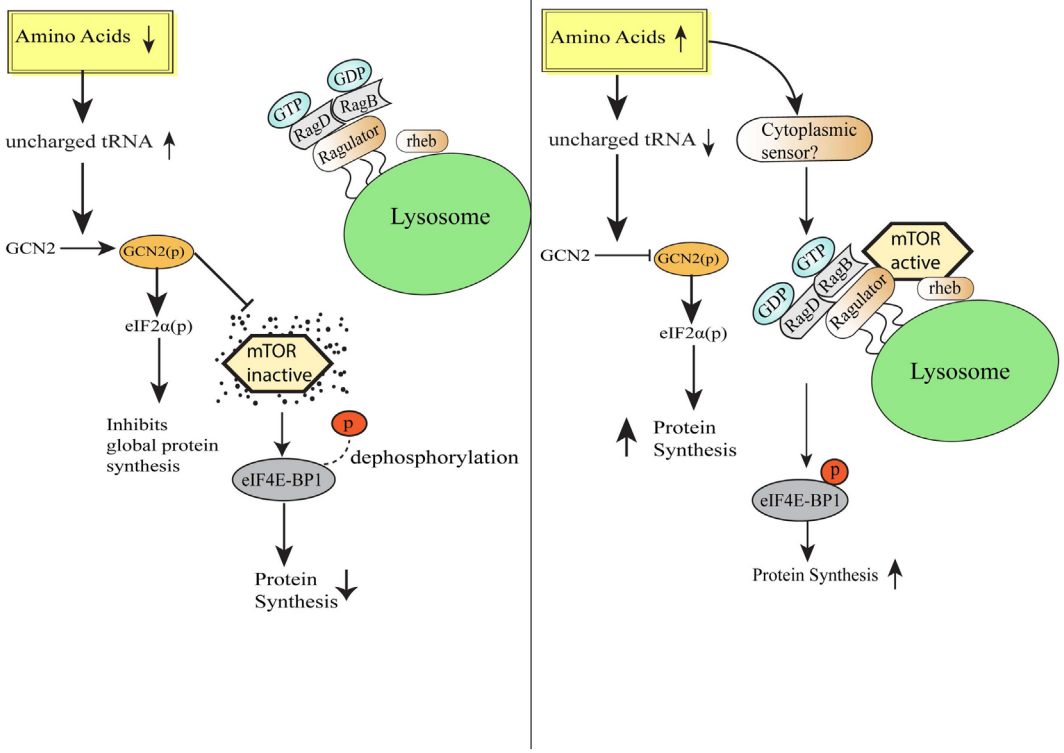

FIGURE 1 | Amino acid sensing and integration of downstream pathways. Schematic representation of the cellular events during amino acid-deficient and amino acid sufficient conditions. General control nonderepressible-2 kinase senses amino acid insufficiency and orchestrate various homeostatic processes *via* eIF2 phosphorylation followed by downregulation of global protein synthesis and simultaneously also inhibits mammalian target of rapamycin (mTOR) activation. On the other hand, under the condition of amino acid sufficiency, mTORC1 complex translocates to lysosomal surfaces by virtue of Rag GTPase activation and further initiates protein translation by the release of translation initiation factor eIF4E.

cytokines, and TLR ligands association with its cognate receptor. Activation of the receptor leads to the recruitment of PI3k to the receptor complex *via* various adaptor molecules including the GTPase RAB8A. Further PI3K induced secondary messenger phosphatidylinositol-3,4,5-trisphosphate recruits and activates Akt, which consists of two key effectors such as FOXO1 and TSC2. In unstimulated cells, TSC2 heterodimerizes with TS1 and causes mTOR inactivation. Conversely, stimulation of cells results in TSC2 phosphorylation at threonine 1462 by Akt, which further leads to mTOR activation eventually (43).

On the other hand, general control nonderepressible 2 kinase (GCN2), a serine/threonine kinase, which detects the scarcity of any amino acids and constitutes the evolutionarily conserved amino acid starvation response (AAR) pathway. Under normal physiology, during protein translation, amino acid-loaded tRNAs assemble at ribosome and provide amino acids for the elongation of nascent peptide. During amino acid deficiency, uncharged or unloaded tRNAs accumulate in the cell and initiate signaling pathways to reserve cellular energy and resources by repressing global protein translation, at the same time derepressing the translation of particular mRNAs required for the restoration of cell homeostasis (44). During amino acid deficiency, accumulated tRNAs bind to the GCN2 kinase, which undergoes a conformational change to initiate a downstream pathway. Basically, binding of GCN2 kinase with uncharged tRNAs initiates autophosphorylation (due to the conformational change) and simultaneous phosphorylation of translation initiation factor, eIF2α at serine 51, which results in the attenuation of general protein translation initiation due to decrease in eIF2/tRNAiMet/GTP ternary complex, required for polysome formation and translation (45) (**Figure 1**). Thus, translationally stalled mRNAs along with initiation factors assemble into stress granules (SGs) through the recruitment of RNA-binding proteins (RBPs), such as TIA-1/TIAR, which in turn determines the fate of mRNAs translatability or decay (46). Simultaneously, translation of specific stress-responsive mRNAs, such as the transcription factors ATF4 and CHOP that influence the genes involved in the amino acid synthesis, homeostasis, and cell survival are upregulated (47).

General control nonderepressible-2 kinase is among the four vital kinases along with PKR, PERK, HRI which form the core of integrated stress response (ISR) pathway (46). While GCN2 is a metabolic sensor, HRI senses heme deprivation within the cell, PERK senses ER stress, and PKR gets activated as a part of the antiviral response of mammalian cell by detecting viral dsRNA, interferons (IFNs), and growth factors (26). All these four kinases

upon activation phosphorylate eIF2α, which in turn decreases the global proteome of the cell as an artistry to economize the energy of the cell for the maintenance of cellular homeostasis (48, 49). Earlier studies report a cross-talk between the GCN2 and mTOR signaling pathways in both yeast and mammals (50, 51). Further, through mixed lymphocyte reaction in human $CD4^+$ T cells, Eleftheriadis et al., demonstrated that both these pathways elicit immunosuppression, influence cell proliferation, and differentiation, but through different mechanisms (52). Moreover, in a study by Xiao et al., the authors reported that dietary leucine deprivation enhances the insulin sensitivity in mice (11). Similarly, pharmacological or genetic reduction in mTOR signaling has been shown to increase the lifespan in model mammalian organisms. Consequently, it becomes inevitable to gain the better understanding of the role of amino acid sensing pathways in immunomodulation and how it can be used to design potential therapeutic targets for metabolic diseases.

AMINO ACID SENSING BY GCN2 AND INFLAMMATION

The initiation of inflammation during any infection/injury is characterized by the recruitment of neutrophils and other immune cells at the site of pathogen invasion (53) followed by the release of a storm of cytokines, chemokines, and other immune effector molecules. As the pathogen is cleared, resolution phase of inflammation sets in, that is marked by tissue homeostasis and regeneration (54). All these processes require enormous energy and nutrients especially amino acids.

In recent times, burgeoning information about the amino acid sensing pathways and their link to innate as well as adaptive immunity has emerged (55). Immune cells are known to be auxotrophs for amino acids, and the condition of inflammation or infection influences cellular amino acid requirement for protein synthesis (56). During an immune response, a redistribution of amino acids is observed, with a shift from cellular growth to the synthesis of immunological proteins (57). Lately, it has also become evident that local deprivation of amino acids could modulate immune responses (58). For instance, during pathogen invasion, such as *Shigella* infection, the bacteria damage the host cell membrane and create an environment of amino acid scarcity, which induces the GCN2 kinase-mediated activation of ISR pathway, and simultaneously reduction in mTOR activity (59). Thus, host senses the amino acid starvation induced by pathogens and triggers protective immune responses. It has also been shown that immune response activated under the conditions of starvation severely affects the survival of insects (60). Further insights into the mechanisms implicated in amino acid sensing driven protective responses identified a crucial role for the amino acid sensor, GCN2 in the metabolic reprogramming in response to cellular stress (61). In *Drosophila*, ingestion of *Pseudomonas entomophila* damages the gut and inhibits translation *via* GCN2 kinase activation and TOR inhibition. The inhibition of protein translation not only leads to the shaping of immune response but also in pathogenesis, by inhibiting repair processes (62). Concomitantly, mice deficient in GCN2 are highly prone to intestinal inflammation, while activation of the GCN2 pathway by reduced amino acid diet dramatically reduced inflammation-associated intestinal pathologies (12). During amino acid insufficiency, uncharged tRNAs accumulate and bind to GCN2 which results in a conformational change in GCN2 and its kinase activation (4). Activated GCN2 phosphorylates translation initiation factor eIF2α which further leads to decrease in ternary complex, such as eIF2/tRNAiMet/GTP, essential for translational initiation (45). Thus, 48S preinitiation complex assembly at 5′ region of capped mRNA is interrupted, which leads to polysome disassembly and SG formation through recruitment of RBPs, such as TIA-1/TIAR (45) (**Figure 2**). These RBPs specifically bind to adenine- and uridine-rich elements present at 3′ untranslated regions of mRNAs through RNA recognition motif and dictate their stability/degradation (63). In immune cells, RBPs play a central role in the posttranscriptional regulation of immune effector molecules. It has been shown that RBPs, HUR, and TIA-1 act together and inhibit the translation of proinflammatory cytokines such as TNF and IL-1β thereby offer anti-inflammatory effect (63). In addition to SGs formation, amino acid deprivation activates autophagy (a cellular stress response that targets long-lived proteins, damaged cell organelles to lysosomes for degradation and is known to affect inflammation negatively) (64, 65) to maintain cellular homeostasis. In a recent study Ravindran et al. show that mice deficient of GCN2 produced substantially high level of reactive oxygen species (ROS) and subsequently proinflammatory mediator, IL-1β, in response to cellular stress, where they suggest that this effect is due to lack of autophagy in GCN2-deficient mice, whereas reduced amino acid diet fed mice show significantly low level of oxidative stress and inflammatory responses to cellular stress (12). Further mechanistic insights depict that GCN2 induced autophagy interferes with oxidative stress and inflammasome activation thereby controls inflammation (12) (**Figure 2**). Furthermore, Liu et al., demonstrate that GCN2 kinase signaling significantly affect macrophage cytokine production in response to LPS stimulation of macrophages expressing indolamine 2,3-dioxygenase (IDO), and further suggest that although GCN2 mediates the enhanced IL-6 mRNA expression, its translation is blocked by virtue of eIF2 phosphorylation (61, 66).

GCN2-MEDIATED AMINO ACID SENSING AND T-CELL IMMUNE RESPONSE

T cells constitute the major component of adaptive immunity and are prevalent during inflammation, infection, and autoimmune diseases. αβT cells are classified majorly based on the coreceptor as $CD8^+$ and $CD4^+$ (67). Upon activation of naive or resting T cell by cognate antigen-MHC signals, they undergo rapid expansion, differentiation followed by contraction (68). $CD8^+$ T cells expand and differentiate into effector cytotoxic cells, which help in clearing tumor, virally infected cells (69), while $CD4^+$ cells undergo proliferation and functional specialization, depending upon the cytokine milieu, as Th1, Th2, Th17, or regulatory T cell (Treg) response, each with its effector function (70). T cell population undergoes different stages of development in the thymus to

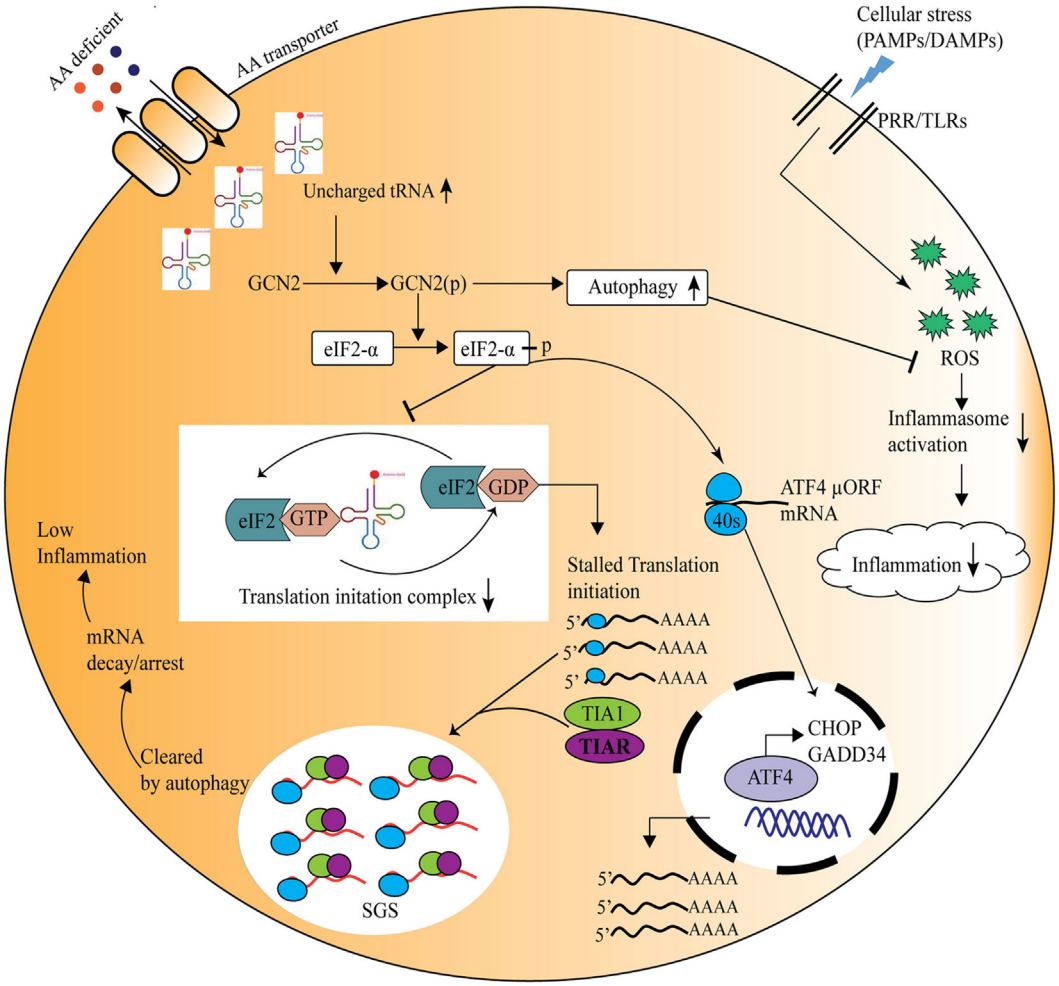

FIGURE 2 | General control nonderepressible-2 kinase (GCN2)-mediated amino acid sensing in the control of inflammation during cellular stress. Amino acid deficiency leads to accumulation of uncharged tRNAs, which are recognized by the nutrient sensor GCN2. Upon its activation, the phospho-GCN2 inhibits the translation initiation ternary complex through the phosphorylation of eIF2α, thus stalls the protein translation. As a result, the RNA-binding proteins are recruited to the translationally stalled mRNAs, forming stress granules that either arrest or decay the stalled mRNAs, and ultimately decreasing the inflammation. At the same time eIF2 phosphorylation leads to translation of specific mRNA, such as ATF4, which translates stress-response genes, GADD34, CHOP, etc. On the other hand, GCN2 pathway induces autophagy which reduces inflammation through inhibition of the reactive oxygen species and inflammasome activation.

activation, expansion, differentiation, and migration at the site of requirement, driven by synthesis or degradation of multiple new proteins. Therefore, to achieve continuous supply of new proteins they are heavily dependent on nutrient availability, including amino acids, and associated metabolic pathways (71). Although T cell activation in response to antigenic exposure is driven by a multitude of factors ranging from the immune receptor, signaling proteins, cytokines, transcription factors, growing evidence suggests that the dynamic regulation of cellular metabolome controls T cell proliferation, expansion, differentiation, and contraction (71). For instance, mTOR has been shown to integrate multiple cues (cytokines, environmental signals) and governs the outcome of TCR activation or anergy. Recent studies suggest mTOR as a major metabolic switch activated during TCR and CD28 signaling, which triggers the surface expression of glucose, nutrients particularly amino acid transporters (72, 73).

The amino acid uptake in T cells is predominantly controlled by the SLC7 family of transporters (74). The foremost amino acid transporters expressed by T cells include LAT1 (SLC7A5), ASCT2, and GAT1 that play an important role in T cell metabolism and hence the inflammatory responses (75). Also, it has been reported by Sinclair et al. that the leucine transporter, SLC7A5 null T cells are unable to either evoke a robust response to antigen exposure or differentiate into effector cells (76). Moreover, the expression of amino acid transporters is increased upon any antigen exposure and it has been established that the type of amino acid transporter could decide the fate of T cell differentiation into different T cell populations (76). Expression of transporters, LAT1 and ASCT2, and subsequent activation of mTOR pathway tilts the T cell differentiation toward a Th1/Th17-mediated response, which was further confirmed by ASCT2 KO mice that show

defective mTORc1 activation and hence, impaired $CD4^+$ T cell responses (77–79).

In a separate investigation, GCN2-mediated pathway involvement in T cells proliferation and differentiation has also been reported, where it has been shown that the GCN2 activation has a negative impact on T cells proliferative capacity and affects the Treg cells differentiation (80). Further, a link has also been reported between indoleamine 2,3-dioxygenase (IDO), a tryptophan catabolizing enzyme, regulated T cell responses and immune tolerance with subsequent activation of GCN2 kinase-mediated ISR pathway (80). A recent study reported that topical exposure of phorbol myristate acetate causes plasmacytoid dendritic cell (pDCs) induction in local draining lymph nodes to express IDO, which confers T cell suppressive activity, thereby favors tumor development after carcinogen exposure. Further mechanistic insights depict that IDO expression in pDCs is dependent on inflammatory signaling including interferon-I and II (IFN-I/II) receptors, IL-1/TLR signaling (81). Although depletion of tryptophan locally is known to create an immunosuppressive environment for tumors, GCN2 activation in T cells has no role to play toward tumor immunity (82). Tryptophan depletion and GCN2 activation associated induction of autophagy has also been observed in case of kidney transplantation, where it is known that IFNγ is the central regulator of rejection upon transplantation. The study by Fougeray et al. demonstrates that IFNγ causes tryptophan depletion and thus, controls the immune response toward transplantation, by activating GCN2 and the downstream autophagy (83). Moreover, as the IDO-dependent tryptophan catabolism mimics the condition of amino acid deficiency (80, 84), it has been reported in inflammatory macrophages that the IDO increases IL-10 expression and simultaneously decreases IL-12 levels *via* translation inhibition in a GCN2 dependent manner, which has also been confirmed by the absence of immune-tolerance on GCN2 deletion (46). Macrophages expressing IDO reduce tryptophan in microenvironment, and thus, restrict the $CD8^+$ T cell proliferation (65). IDO has also been shown to induce GCN2-dependent immune tolerance to apoptosis antigens. During apoptosis or apoptosis antigen exposure macrophages decrease the proinflammatory cytokine expression posttranscriptionally by inhibiting the IL-12 mRNA association with polysomes while increasing IL-10 transcripts translation (61). Thus, IDO-mediated T cell regulation has been implicated in different inflammatory conditions, but it requires further investigation to elucidate the upstream and downstream pathways involved.

In a separate study, the GCN2 arbitrated T cell response has been associated with antigen presentation and subsequent $CD8^+$ immune response to yellow fever vaccine (YF-17D) (85). Yellow fever vaccine is one of the most potent vaccines ever developed in humans. Despite its efficiency and extensive use, the mechanisms implicated in YF-17D-induced protective immune responses remain poorly defined. In an earlier investigation using systems biology approach, it was highlighted that there is an association between the $CD8^+$ immune response in blood upon YF17D vaccination and the activation of GCN2 (86). Consequently, in the study by Ravindran et al., the authors investigated the role of GCN2 in adaptive immunity upon YF17D vaccination, where they report that the stimulation of dendritic cells (DCs) with YF17D induces SGs formation, and activates autophagy in a GCN2 dependent manner. The study also confirmed that induction of autophagy enhanced antigen cross-presentation in the YF17D vaccinated mice (85).

Th17 immune responses among the varied immune effector functions have been concomitant with autoimmune diseases. The naïve $CD4^+$ T cells upon stimulation with cytokines IL6, IL1β, TGFβ, result in expression of RORγt and STAT3, the transcription factors, which induce IL17a production and elicits the Th17 response. Carlson et al., in their study investigated the role of AAR pathway on the immune response, where it was observed that AAR pathway activator Halofuginone (HF) dampens the Th17 response, influences STAT3 posttranscriptionally and hence, blocks its expression (87) (**Figure 3**).

It becomes evident that the amino acid regulation is crucial during T cell immune responses. Therefore, targeting nutrient sensors and amino acid transporters could be an approach to regulate T cell activation and immune response during diseases and thus, to design translational therapeutics.

REGULATION OF AMINO ACID SIGNALING DURING METABOLIC AND AUTOIMMUNE DISEASES

Growing evidence categorically suggest a close association between the cellular metabolism and immune response, therefore, it becomes imperative to tightly control nutrient sensing mechanisms to maintain cellular homeostasis. The malfunction of nutrient sensing pathways has been linked with different metabolic diseases in recent times, including obesity, diabetes, atherosclerosis, inflammatory bowel diseases (IBDs), autoimmune diseases, and others, which pose a huge burden on global health (3, 88–90).

The prevalence of obesity and its associated metabolic diseases, such as diabetes, increases enormously worldwide (91–93). In addition to metabolic alterations, both obesity and diabetes are associated with inflammation as well, which led to the notion of the term "meta-inflammation" or metabolically triggered inflammation (94). With nutrient signaling pathways being all interconnected and associated with insulin signaling, it has been reported that the levels of branched-chain amino acids (BCAA) affect the nutrient signaling and metabolism (95, 96). mTOR being a central regulator of amino acid and insulin signaling (97, 98), under amino acid abundance, it phosphorylates IRS1 (at serine 307) and inhibits the downstream insulin signaling (99, 100). Whereas the amino acid deprivation can increase insulin sensitivity, where Xiao et al. reported the activation of GCN2 and simultaneous inhibition of mTOR upon leucine deprivation *in vivo* as well as *in vitro*, which improved the insulin activity under insulin-resistant conditions typically observed in diabetes (11). Furthermore, a recent study unveiled that increased insulin sensitivity is associated with the rise in serum BCCA and decreased BCAA catabolism concomitantly with enhanced proinflammatory gene expression in adipose tissue (101).

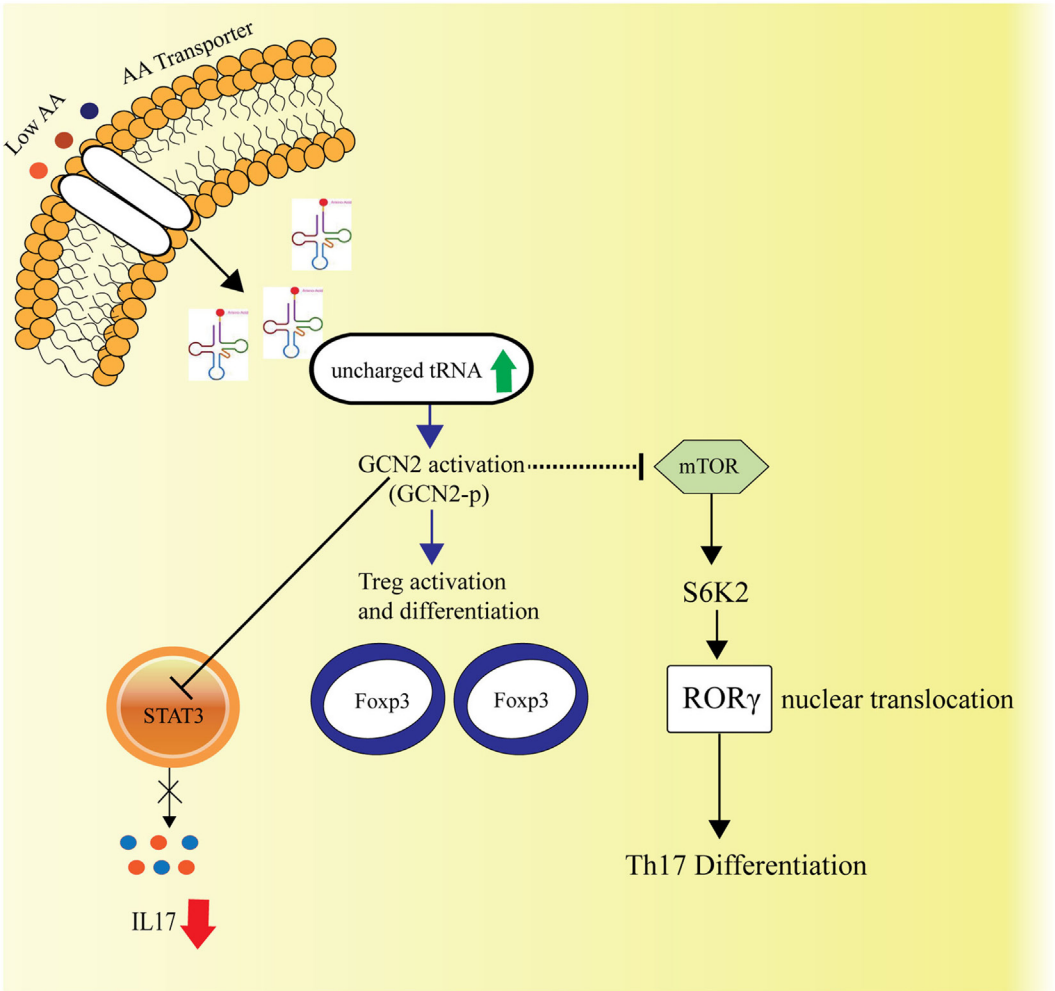

FIGURE 3 | Amino acid sensing and cellular Th17-immune responses. General control nonderepressible-2 kinase (GCN2)-mediated amino acid sensing is also known to dampen the Th17 immune responses by inhibiting the STAT3 transcription factor, essential for Th17 response and simultaneously by hindering the mTORC1 activity, which is known to facilitate Th17 differentiation *via* S6K2 activation followed by nuclear translocation of RORγ. The GCN2 activation also leads to activation and differentiation of the regulatory T cells.

Autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis (MS), psoriasis, have been associated with immune responses, particularly with the Th17-mediated responses. Under normal physiology, cells have their own mechanisms to control immune responses and hence, prevent autoimmunity, by induction of apoptosis or through immune tolerance toward self-antigens. The apoptotic cells initiate an anti-inflammatory response to prevent any further development of autoimmunity. In a study on lupus mice, Ravishankar et al. have reported an increase in immune responses and overall mortality post-GCN2 depletion (61). Systemic Lupus Erythematosus is an autoimmune disease where apoptotic cell components act as the antigens to increase cellular immune responses. The authors demonstrated that the IDO in macrophages influences the cytokine levels in a GCN2-dependent manner. It was observed that the anti-inflammatory IL-10 responses are elevated while the IL-12 levels are reduced (61). The role of GCN2 in T cell response has also been identified with the neuronal chronic inflammatory condition, MS (82). MS is characterized by infiltration of Th1 and Th17 cells in CNS, leading to demyelination. It has been reported that during the recovery phase of the disease, the Tregs migrate to the affected area in the CNS and play an important role in keeping MS and inflammation under check. However, the GCN2-depleted Tregs are unable to migrate to the concerned area (82). In another study by Orsini et al., the authors investigated in the GCN2-KO mice subjected to myelin oligodendrocyte glycoprotein peptide and reported higher inflammation and Th1/Th17 cells in the CNS (102). Psoriasis is an autoimmune inflammatory skin disease, associated with $CD4^+$ T cell responses (103). In a preliminary study on keratinocyte differentiation, Collier et al. have reported the role of GCN2 phosphorylation-mediated translational control in the normal differentiation and formation of the epidermis. It was observed that the absence of GCN2 disturbed this control

over differentiation and therefore could be a cause of various skin diseases, including psoriasis (104).

Amino acid sensing pathways also have control over intestinal inflammation, where the study by Ravindran et al. has demonstrated the role of GCN2 pathway in IBDs (12). Since intestine is the constant environment of fluctuations in nutrient levels, the amino acid sensing becomes crucial to maintain the homeostasis. The authors revealed that induction of DSS-mediated colitis in the absence of GCN2 increased inflammation and subsequent production of inflammatory mediators, such as IL-1β and IL-17. GCN2 activation in macrophages and DCs during IBD results in autophagy activation, which is known to decrease the proinflammatory cytokine production (12, 105, 106). Different studies indicate that the amino acid signaling pathways could be exploited for health benefits. Dietary or calorie restriction, since a long time, is known to have a positive influence on health, stress resistance, and longevity, as it is evident from different studies across species from yeast to rodents (107, 108). The study has shown that proteins demonstrate utmost impact upon dietary restriction (109). It has also been established in humans that the low-protein diet is associated with protection from cancer and diabetes (110). In another study, it has been demonstrated in *Drosophila* that the withdrawal of EAAs contributes more toward the beneficial effect of dietary restriction as compared to the carbohydrates (111, 112). The study by Peng et al. demonstrates dietary tryptophan depletion decreases inflammation and extends protective role in hepatic ischemic injury (14). The evolutionarily conserved GCN2-mediated ISR pathway responds to dietary starvation and is known to modulate immune responses (58). These different studies highlight the health benefits of dietary or amino acid restriction. Concurrently, pharmacological activators like HF can also mimic amino acid starvation, and various studies have reported their immunomodulating anti-inflammatory impact (87). HF is an analog of plant-derived compound febrifugine, which is a known GCN2 activator and has shown to activate amino acid starvation response pathway. It acts by imitating the amino acid proline restriction by competitively binding to and inhibiting the prolyl-tRNA synthetase, leading to accumulation of uncharged prolyl-tRNA and further initiation of AAR pathway (47). In a separate study by Sundrud et al., the authors have also demonstrated in a mouse model of experimental autoimmune encephalomyelitis (EAE) or MS that HF targets precisely the proinflammatory Th17 response by disrupting the STAT3 phosphorylation (113). HF has been tested in different disease conditions, including malaria, cancer, and autoimmune diseases, with a few clinical trials in the field (114). Consequently, more studies with an emphasis on the mechanism of action of HF in amino acid sensing and the downstream responses could help us in designing therapeutics that could be tested in the different conditions discussed earlier.

CONCLUSION

Although the association between nutrient sensing with immune response and the concept of "immunometabolism" is not new, yet there has been a lack of mechanistic understanding, which requires further investigations. Several studies illustrate the importance of amino acid sensing in evoking the innate as well as adaptive effector immune functions. For instance, recent studies have established the link between amino acid starvation sensor GCN2 and its control over inflammation by highlighting the posttranscriptional mechanisms, and autophagy. These studies not only raise some important questions with respect to metabolic control of Th17/Treg ratios and immune response but also point out that it might be interesting to test these hypotheses in the case of obesity as well as during inflammation observed in various diseases including neurological disorders. Therefore, all these reports and preliminary investigations open up avenues for future research in cross-talk between the nutrient sensing and immune responses, which can be extrapolated to clinical trials for inflammatory and metabolic diseases.

AUTHOR CONTRIBUTIONS

NK conceptualized the idea. SB, GM, and NK wrote the manuscript. AM made the figures.

FUNDING

This work has been supported by the Department of Biotechnology, Government of India [BT/PR8624/MED/29/798/2013], the Nano Mission Council, Department of Science and Technology [DST No.: SB/YS/LS-163/2013], and the University Grants Commission, Government of India [MRP-MAJOR-BIOT-2013-40689]. SB acknowledges the Council of Scientific and Industrial Research (CSIR), Government of India for providing fellowship and GM acknowledges Science and Engineering Research Board (SERB) for National Post-Doctoral Fellowship [PDF/2016/003500].

REFERENCES

1. Nagy C, Haschemi A. Time and demand are two critical dimensions of immunometabolism: the process of macrophage activation and the pentose phosphate pathway. *Front Immunol* (2015) 6:164. doi:10.3389/fimmu.2015.00164
2. Kempner W, Peschel E. Stoffwechsel der entzuendung. *Ztschr f klin Med* (1930) 114:439–55.
3. Hotamisligil GS, Erbay E. Nutrient sensing and inflammation in metabolic diseases. *Nat Rev Immunol* (2008) 8:923–34. doi:10.1038/nri2449
4. Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. *Nature* (2015) 517:302–10. doi:10.1038/nature14190
5. Chantranupong L, Wolfson RL, Sabatini DM. Nutrient-sensing mechanisms across evolution. *Cell* (2015) 161:67–83. doi:10.1016/j.cell.2015.02.041
6. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* (2009) 325:201–4. doi:10.1126/science.1173635
7. Fontana L, Partridge L, Longo VD. Extending healthy life span – from yeast to humans. *Science* (2010) 328:321–6. doi:10.1126/science.1172539

8. Anderson RM, Weindruch R. Metabolic reprogramming in dietary restriction. *Interdiscip Top Gerontol* (2007) 35:18–38. doi:10.1159/000096554
9. Gallinetti J, Harputlugil E, Mitchell JR. Amino acid sensing in dietary-restriction-mediated longevity: roles of signal-transducing kinases GCN2 and TOR. *Biochem J* (2013) 449:1–10. doi:10.1042/BJ20121098
10. Wu G. Amino acids: metabolism, functions, and nutrition. *Amino Acids* (2009) 37:1–17. doi:10.1007/s00726-009-0269-0
11. Xiao F, Huang Z, Li H, Yu J, Wang C, Chen S, et al. Leucine deprivation increases hepatic insulin sensitivity via GCN2/mTOR/S6K1 and AMPK pathways. *Diabetes* (2011) 60:746–56. doi:10.2337/db10-1246
12. Ravindran R, Loebbermann J, Nakaya HI, Khan N, Ma H, Gama L, et al. The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. *Nature* (2016) 531:1–17. doi:10.1038/nature17186
13. González A, Hall MN. Nutrient sensing and TOR signaling in yeast and mammals. *EMBO J* (2017) 36:397–408. doi:10.15252/embj.201696010
14. Peng W, Robertson L, Gallinetti J, Mejia P, Vose S, Charlip A, et al. Surgical stress resistance induced by single amino acid deprivation requires GCN2 in mice. *Sci Transl Med* (2012) 4:118ra11. doi:10.1126/scitranslmed.3002629
15. Bunpo P, Cundiff JK, Reinert RB, Wek RC, Aldrich CJ, Anthony TG. The eIF2 kinase GCN2 is essential for the murine immune system to adapt to amino acid deprivation by asparaginase. *J Nutr* (2010) 140:2020–7. doi:10.3945/jn.110.129197
16. Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN, et al. The GCN2-ATF4 pathway is critical for tumour cell survival and proliferation in response to nutrient deprivation. *EMBO J* (2010) 29:2082–96. doi:10.1038/emboj.2010.81
17. Wu G. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. *J Anim Sci Biotechnol* (2014) 5:34. doi:10.1186/2049-1891-5-34
18. Jewett MC, Miller ML, Chen Y, Swartz JR. Continued protein synthesis at low [ATP] and [GTP] enables cell adaptation during energy limitation. *J Bacteriol* (2009) 191:1083–91. doi:10.1128/JB.00852-08
19. Nakajo T, Yamatsuji T, Ban H, Shigemitsu K, Haisa M, Motoki T, et al. Glutamine is a key regulator for amino acid-controlled cell growth through the mTOR signaling pathway in rat intestinal epithelial cells. *Biochem Biophys Res Commun* (2005) 326:174–80. doi:10.1016/j.bbrc.2004.11.015
20. Hara K, Yonezawa K, Weng Q-PP, Kozlowski MT, Belham C, Avruch J. Amino acid sufficiency and mTOR regulate p70 S6 kinase and eIF-4E BP1 through a common effector mechanism. *J Biol Chem* (1998) 273:14484–94. doi:10.1074/jbc.273.23.14484
21. Wek RC, Jiang H-YY, Anthony TG. Coping with stress: eIF2 kinases and translational control. *Biochem Soc Trans* (2006) 34:7–11. doi:10.1042/BST20060007
22. Harper AE, Benevenga NJ, Wohlhueter RM. Effects of ingestion of disproportionate amounts of amino acids. *Physiol Rev* (1970) 50:428–558.
23. Heitman J, Movva NR, Hall MN. Targets for cell cycle arrest by the immunosuppressant rapamycin in yeast. *Science* (1991) 253:905–9. doi:10.1126/science.1715094
24. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. *Nat Rev Mol Cell Biol* (2011) 12:21–35. doi:10.1038/nrm3025
25. Kim E, Goraksha-Hicks P, Li L, Neufeld TP, Guan K-L. Regulation of TORC1 by Rag GTPases in nutrient response. *Nat Cell Biol* (2008) 10:935–45. doi:10.1038/ncb1753
26. Tokunaga C, Yoshino KI, Yonezawa K. mTOR integrates amino acid- and energy-sensing pathways. *Biochem Biophys Res Commun* (2004) 313:443–6. doi:10.1016/j.bbrc.2003.07.019
27. Sancak Y, Bar-Peled L, Zoncu R, Markhard AL, Nada S, Sabatini DM. Ragulator-Rag complex targets mTORC1 to the lysosomal surface and is necessary for its activation by amino acids. *Cell* (2010) 141:290–303. doi:10.1016/j.cell.2010.02.024
28. Bar-Peled L, Schweitzer LD, Zoncu R, Sabatini DM. Ragulator is a GEF for the Rag GTPases that signal amino acid levels to mTORC1. *Cell* (2012) 150:1196–208. doi:10.1016/j.cell.2012.07.032
29. Sancak Y, Peterson TR, Shaul YD, Lindquist RA, Thoreen CC, Bar-Peled L, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. *Science* (2008) 320:1496–501. doi:10.1126/science.1157535
30. Jewell JL, Guan KL. Nutrient signaling to mTOR and cell growth. *Trends Biochem Sci* (2013) 38:233–42. doi:10.1016/j.tibs.2013.01.004
31. Soliman GA. The role of mechanistic target of rapamycin (mTOR) complexes signaling in the immune responses. *Nutrients* (2013) 5:2231–57. doi:10.3390/nu5062231
32. Jewell JL, Russell RC, Guan K-L. Amino acid signaling upstream of mTOR. *Nat Rev Mol Cell Biol* (2013) 14:133–9. doi:10.1038/nrm3522
33. Panchaud N, Péli-Gulli M-P, De Virgilio C. Amino acid deprivation inhibits TORC1 through a GTPase-activating protein complex for the Rag family GTPase Gtr1. *Sci Signal* (2013) 6:ra42. doi:10.1126/scisignal.2004112
34. Petit CS, Roczniak-Ferguson A, Ferguson SM. Recruitment of folliculin to lysosomes supports the amino acid-dependent activation of Rag GTPases. *J Cell Biol* (2013) 202:1107–22. doi:10.1083/jcb.201307084
35. Sarbassov DD, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. *Curr Opin Cell Biol* (2005) 17:596–603. doi:10.1016/j.ceb.2005.09.009
36. Ward SG. Phosphoinositide 3-kinases and leukocyte migration. *Curr Immunol Rev* (2012) 8:154–60. doi:10.2174/157339512800099684
37. Sinclair LV, Finlay D, Feijoo C, Cornish GH, Gray A, Ager A, et al. Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. *Nat Immunol* (2008) 9:513–21. doi:10.1038/ni.1603
38. Cantrell DA. Regulation and function of serine kinase networks in lymphocytes. *Curr Opin Immunol* (2003) 15:294–8. doi:10.1016/S0952-7915(03)00052-9
39. Juntilla MM, Koretzky GA. Critical roles of the PI3K/Akt signaling pathway in T cell development. *Immunol Lett* (2008) 116:104–10. doi:10.1016/j.imlet.2007.12.008
40. Macintyre AN, Finlay D, Preston G, Sinclair LV, Waugh CM, Tamas P, et al. Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism. *Immunity* (2011) 34:224–36. doi:10.1016/j.immuni.2011.01.012
41. Hamilton KS, Phong B, Corey C, Cheng J, Gorentla B, Zhong X, et al. T cell receptor-dependent activation of mTOR signaling in T cells is mediated by Carma1 and MALT1, but not Bcl10. *Sci Signal* (2014) 7:ra55. doi:10.1126/scisignal.2005169
42. Limon JJ, Fruman DA. Akt and mTOR in B cell activation and differentiation. *Front Immunol* (2012) 3:228. doi:10.3389/fimmu.2012.00228
43. Weichhart T, Hengstschlager M, Linke M. Regulation of innate immune cell function by mTOR. *Nat Rev Immunol* (2015) 15:599–614. doi:10.1038/nri3901
44. Kilberg MS, Shan J, Su N. ATF4-dependent transcription mediates signaling of amino acid limitation. *Trends Endocrinol Metab* (2009) 20:436–43. doi:10.1016/j.tem.2009.05.008
45. Kedersha N, Chen S, Gilks N, Li W, Miller IJ, Stahl J, et al. Evidence that ternary complex (eIF2-GTP-tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules. *Mol Biol Cell* (2002) 13:195–210. doi:10.1091/mbc.01-05-0221
46. Ganguly K, Giddaluru J, August A, Khan N. Post-transcriptional regulation of immunological responses through riboclustering. *Front Immunol* (2016) 7:161. doi:10.3389/fimmu.2016.00161
47. Keller TL, Zocco D, Sundrud MS, Hendrick M, Edenius M, Yum J, et al. Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase. *Nat Chem Biol* (2012) 8:311–7. doi:10.1038/nchembio.790
48. Taniuchi S, Miyake M, Tsugawa K, Oyadomari M, Oyadomari S. Integrated stress response of vertebrates is regulated by four eIF2α kinases. *Sci Rep* (2016) 6:32886. doi:10.1038/srep32886
49. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress response. *EMBO Rep* (2016) 17:1374–95. doi:10.15252/embr.201642195
50. Anthony TG, McDaniel BJ, Byerley RL, McGrath BC, Cavener DRB, McNurlan MA, et al. Preservation of liver protein synthesis during dietary leucine deprivation occurs at the expense of skeletal muscle mass in mice deleted for eIF2 kinase GCN2. *J Biol Chem* (2004) 279:36553–61. doi:10.1074/jbc.M404559200
51. Bunpo P, Dudley A, Cundiff JK, Cavener DR, Wek RC, Anthony TG. GCN2 protein kinase is required to activate amino acid deprivation responses in mice treated with the anti-cancer agent L-asparaginase. *J Biol Chem* (2009) 284:32742–9. doi:10.1074/jbc.M109.047910

52. Eleftheriadis T, Pissas G, Antoniadi G, Liakopoulos V, Tsogka K, Sounidaki M, et al. Differential effects of the two amino acid sensing systems, the GCN2 kinase and the mTOR complex 1, on primary human alloreactive CD4+ T-cells. *Int J Mol Med* (2016) 37:1412–20. doi:10.3892/ijmm.2016.2547
53. Ashley NT, Weil ZM, Nelson RJ. Inflammation: mechanisms, costs, and natural variation. *Annu Rev Ecol Evol Syst* (2012) 43:385–406. doi:10.1146/annurev-ecolsys-040212-092530
54. Mountziaris PM, Mikos AG. Modulation of the inflammatory response for enhanced bone tissue regeneration. *Tissue Eng Part B Rev* (2008) 14:179–86. doi:10.1089/ten.teb.2008.0038
55. McGettrick AF, O'Neill LAJ. How metabolism generates signals during innate immunity and inflammation. *J Biol Chem* (2013) 288:22893–8. doi:10.1074/jbc.R113.486464
56. Murray PJ. Amino acid auxotrophy as a system of immunological control nodes. *Nat Immunol* (2015) 17:132–9. doi:10.1038/ni.3323
57. Le Floc'h N, Melchior D, Obled C. Modifications of protein and amino acid metabolism during inflammation and immune system activation. *Livest Prod Sci* (2004) 87:37–45. doi:10.1016/j.livprodsci.2003.09.005
58. Mcgaha TL, Huang L, Lemos H, Metz R, Mautino M, Prendergast GC, et al. Amino acid catabolism: a pivotal regulator of innate and adaptive immunity. *Immunol Rev* (2012) 249:135–57. doi:10.1111/j.1600-065X.2012.01149.x
59. Tattoli I, Sorbara MT, Vuckovic D, Ling A, Soares F, Carneiro LA, et al. Amino acid starvation induced by invasive bacterial pathogens triggers an innate host defense program. *Cell Host Microbe* (2012) 11:563–75. doi:10.1016/j.chom.2012.04.012
60. Moret Y, Schmid-Hempel P. Survival for immunity: the price of immune system activation for bumblebee workers. *Science* (2000) 290:1166–8. doi:10.1126/science.290.5494.1166
61. Ravishankar B, Liu H, Shinde R, Chaudhary K, Xiao W, Bradley J, et al. The amino acid sensor GCN2 inhibits inflammatory responses to apoptotic cells promoting tolerance and suppressing systemic autoimmunity. *Proc Natl Acad Sci U S A* (2015) 112:10774–9. doi:10.1073/pnas.1504276112
62. Chakrabarti S, Liehl P, Buchon N, Lemaitre B. Infection-induced host translational blockage inhibits immune responses and epithelial renewal in the *Drosophila* gut. *Cell Host Microbe* (2012) 12:60–70. doi:10.1016/j.chom.2012.06.001
63. Anderson P. Post-transcriptional control of cytokine production. *Nat Immunol* (2008) 9:353–9. doi:10.1038/ni1584
64. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang B-G, Satoh T, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 beta production. *Nature* (2008) 456:264–8. doi:10.1038/nature07383
65. Shi C-SS, Shenderov K, Huang N-NN, Kabat J, Abu-Asab M, Fitzgerald KA, et al. Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. *Nat Immunol* (2012) 13:255–63. doi:10.1038/ni.2215
66. Liu H, Huang L, Bradley J, Liu K, Bardhan K, Ron D, et al. GCN2-dependent metabolic stress is essential for endotoxemic cytokine induction and pathology. *Mol Cell Biol* (2014) 34:428–38. doi:10.1128/MCB.00946-13
67. Ellmeier W, Sawada S, Littman DR. The regulation of CD4 and CD8 coreceptor gene expression during T cell development. *Annu Rev Immunol* (1999) 17:523–54. doi:10.1146/annurev.immunol.17.1.523
68. Pennock ND, White JT, Cross EW, Cheney EE, Tamburini BA, Kedl RM. T cell responses: naïve to memory and everything in between. *Adv Physiol Educ* (2013) 37:273–83. doi:10.1152/advan.00066.2013
69. Kalia V, Sarkar S, Ahmed R. CD8 T-cell memory differentiation during acute and chronic viral infections. *Adv Exp Med Biol* (2010) 684:79–95. doi:10.1007/978-1-4419-6451-9_7
70. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) 31:259–83. doi:10.1146/annurev-immunol-032712-095956
71. Walls J, Sinclair L, Finlay D. Nutrient sensing, signal transduction and immune responses. *Semin Immunol* (2016) 28:396–407. doi:10.1016/j.smim.2016.09.001
72. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. *Mol Biol Cell* (2007) 18:1437–46. doi:10.1091/mbc.E06-07-0593
73. Zheng Y, Collins SL, Lutz MA, Allen AN, Kole TP, Zarek PE, et al. A role for mammalian target of rapamycin in regulating T cell activation versus anergy. *J Immunol* (2007) 178:2163–70. doi:10.4049/jimmunol.178.4.2163
74. Fotiadis D, Kanai Y, Palacín M. The SLC3 and SLC7 families of amino acid transporters. *Mol Aspects Med* (2013) 34:139–58. doi:10.1016/j.mam.2012.10.007
75. Ren W, Liu G, Yin J, Tan B, Wu G, Bazer FW, et al. Amino-acid transporters in T-cell activation and differentiation. *Cell Death Dis* (2017) 8:e2655. doi:10.1038/cddis.2016.222
76. Sinclair LV, Rolf J, Emslie E, Shi Y-B, Taylor PM, Cantrell DA. Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. *Nat Immunol* (2013) 14:500–8. doi:10.1038/ni.2556
77. Kopf H, de la Rosa GM, Howard OMZ, Chen X. Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells. *Int Immunopharmacol* (2007) 7:1819–24. doi:10.1016/j.intimp.2007.08.027
78. Nakaya M, Xiao Y, Zhou X, Chang JH, Chang M, Cheng X, et al. Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. *Immunity* (2014) 40:692–705. doi:10.1016/j.immuni.2014.04.007
79. Delgoffe GM, Pollizzi KN, Waickman AT, Heikamp E, Meyers DJ, Horton MR, et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat Immunol* (2011) 12:295–303. doi:10.1038/ni.2005
80. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity* (2005) 22:633–42. doi:10.1016/j.immuni.2005.03.013
81. Muller AJ, Sharma MD, Chandler PR, Duhadaway JB, Everhart ME, Johnson BA III, et al. Chronic inflammation that facilitates tumor progression creates local immune suppression by inducing indoleamine 2,3 dioxygenase. *Proc Natl Acad Sci U S A* (2008) 105:17073–8. doi:10.1073/pnas.0806173105
82. Keil M, Sonner JK, Lanz TV, Oezen I, Bunse T, Bittner S, et al. General control non-derepressible 2 (GCN2) in T cells controls disease progression of autoimmune neuroinflammation. *J Neuroimmunol* (2016) 297:117–26. doi:10.1016/j.jneuroim.2016.05.014
83. Fougeray S, Mami I, Bertho G, Beaune P, Thervet E, Pallet N. Tryptophan depletion and the kinase GCN2 mediate IFN-γ-induced autophagy. *J Immunol* (2012) 189:2954–64. doi:10.4049/jimmunol.1201214
84. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* (1999) 189:1363–72. doi:10.1084/jem.189.9.1363
85. Ravindran R, Khan N, Nakaya HI, Li S, Loebbermann J, Maddur MS, et al. Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation. *Science* (2014) 343:313–7. doi:10.1126/science.1246829
86. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nat Immunol* (2009) 10:116–25. doi:10.1038/ni.1688
87. Carlson TJ, Pellerin A, Djuretic IM, Trivigno C, Koralov SB, Rao A, et al. Halofuginone-induced amino acid starvation regulates STAT3-dependent Th17 effector function and reduces established autoimmune inflammation. *J Immunol* (2014) 192:2167–76. doi:10.4049/jimmunol.1302316
88. Oldham S. High fat diet induced obesity and nutrient sensing TOR signaling. *Trends Endocrinol Metab* (2012) 22:45–52. doi:10.1016/j.tem.2010.11.002.High
89. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* (2006) 124:471–84. doi:10.1016/j.cell.2006.01.016
90. Tsang CK, Qi H, Liu LF, Zheng XFS. Targeting mammalian target of rapamycin (mTOR) for health and diseases. *Drug Discov Today* (2007) 12:112–24. doi:10.1016/j.drudis.2006.12.008
91. World Health Organization. *Global Report on Diabetes*. Geneva: World Health Organization (2016). 88 p.
92. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* (2010) 87:4–14. doi:10.1016/j.diabres.2009.10.007

93. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med* (2006) 3:2011–30. doi:10.1371/journal.pmed.0030442
94. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* (2006) 444:860–7. doi:10.1038/nature05485
95. Monirujjaman M, Ferdouse A. Metabolic and physiological roles of branched-chain amino acids. *Adv Mol Biol* (2014) 2014:1–6. doi:10.1155/2014/364976
96. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signaling and insulin resistance. *Nat Rev Endocrinol* (2014) 10:723–36. doi:10.1038/nrendo.2014.171
97. Whites MF, Kahn CR. The insulin signaling system*. *J Biol Chem* (1994) 269:1–4.
98. White MF. Insulin signaling in health and disease. *Science* (2003) 302:1710–1. doi:10.1126/science.1092952
99. Yu Y, Yoon S-O, Poulogiannis G, Yang Q, Ma XM, Villén J, et al. Phosphoproteomic analysis identifies Grb10 as an mTORC1 substrate that negatively regulates insulin signaling. *Science* (2011) 332:1322–6. doi:10.1126/science.1199484
100. Manning BD. Balancing Akt with S6K: implications for both metabolic diseases and tumorigenesis. *J Cell Biol* (2004) 167:399–403. doi:10.1083/jcb.200408161
101. Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y, et al. Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. *Sci Rep* (2016) 6:24540. doi:10.1038/srep24540
102. Orsini H, Araujo LP, Maricato JT, Guereschi MG, Mariano M, Castilho BA, et al. GCN2 kinase plays an important role triggering the remission phase of experimental autoimmune encephalomyelitis (EAE) in mice. *Brain Behav Immun* (2014) 37:177–86. doi:10.1016/j.bbi.2013.12.012
103. Sticherling M. Psoriasis and autoimmunity. *Autoimmun Rev* (2016) 15:1167–70. doi:10.1016/j.autrev.2016.09.004
104. Collier AE, Wek RC, Spandau DF. Human keratinocyte differentiation requires translational control by the eIF2α kinase GCN2. *J Invest Dermatol* (2017) 137:1924–34. doi:10.1016/j.jid.2017.04.029
105. Levine B, Mizushima N, Virgin HW. Autophagy in immunity and inflammation. *Nature* (2011) 469:323–35. doi:10.1038/nature09782
106. Kroemer G, Mariño G, Levine B. Autophagy and the integrated stress response. *Mol Cell* (2010) 40:280–93. doi:10.1016/j.molcel.2010.09.023
107. Zimmerman JA, Malloy V, Krajcik R, Orentreich N. Nutritional control of aging. *Exp Gerontol* (2003) 38:47–52. doi:10.1016/S0531-5565(02)00149-3
108. Fontana L, Partridge L, Longo VD. Dietary restriction, growth factors and aging: from yeast to humans. *Science* (2010) 328:321–6. doi:10.1126/science.1172539
109. Couzin-Frankel J. Nutrition. Diet studies challenge thinking on proteins versus carbs. *Science* (2014) 343:1068. doi:10.1126/science.343.6175.1068
110. Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, et al. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab* (2014) 19:407–17. doi:10.1016/j.cmet.2014.02.006
111. Tatar M, Post S, Yu K. Nutrient control of *Drosophila* longevity. *Trends Endocrinol Metab* (2014) 25:509–17. doi:10.1016/j.tem.2014.02.006
112. Partridge L, Piper MDW, Mair W. Dietary restriction in *Drosophila*. *Mech Ageing Dev* (2005) 126:938–50. doi:10.1016/j.mad.2005.03.023
113. Sundrud MS, Koralov SB, Feuerer M, Calado DP, Kozhaya AE, Rhule-Smith A, et al. Halofuginone inhibits TH17 cell differentiation by activating the amino acid starvation response. *Science* (2009) 324:1334–8. doi:10.1126/science.1172638
114. Pines M, Spector I. Halofuginone – the multifaceted molecule. *Molecules* (2015) 20:573–94. doi:10.3390/molecules20010573

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

ORIGINAL RESEARCH
published: 09 November 2017
doi: 10.3389/fimmu.2017.01516

Metabolic Adaptation of Human CD4+ and CD8+ T-Cells to T-Cell Receptor-Mediated Stimulation

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Linking immunometabolic adaptation to T-cell function provides insight for the development of new therapeutic approaches in multiple disease settings. T-cell activation and downstream effector functions of CD4+ and CD8+ T-cells are controlled by the strength of interaction between the T-cell receptor (TCR) and peptides presented by human leukocyte antigens (pHLA). The role of TCR–pHLA interactions in modulating T-cell metabolism is unknown. Here, for the first time, we explore the relative contributions of the main metabolic pathways to functional responses in human CD4+ and CD8+ T-cells. Increased expression of hexokinase II accompanied by higher basal glycolysis is demonstrated in CD4+ T-cells; cytokine production in CD8+ T-cells is more reliant on oxidative phosphorylation. Using antigen-specific CD4+ and CD8+ T-cell clones and altered peptide ligands, we demonstrate that binding affinity tunes the underlying metabolic shift. Overall, this study provides important new insight into how metabolic pathways are controlled during antigen-specific activation of human T-cells.

Keywords: T-cell, immunometabolism, metabolism, T-cell receptor, glycolysis, GLUT1

OPEN ACCESS

Edited by:
Claudio Mauro, Barts and The London School of Medicine and Dentistry, United Kingdom

Reviewed by:
Laurence Morel, University of Florida, United States
Wilfried Ellmeier, Medical University of Vienna, Austria

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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *22 June 2017*
Accepted: *26 October 2017*
Published: *09 November 2017*

Citation:
Jones N, Cronin JG, Dolton G, Panetti S, Schauenburg AJ, Galloway SAE, Sewell AK, Cole DK, Thornton CA and Francis NJ (2017) Metabolic Adaptation of Human CD4+ and CD8+ T-Cells to T-Cell Receptor-Mediated Stimulation. Front. Immunol. 8:1516. doi: 10.3389/fimmu.2017.01516

INTRODUCTION

T-cells can be sub-divided into two main types by their expression of an accessory glycoprotein co-receptor, either CD4 or CD8, which facilitates their preferential interaction with MHC Class II or Class I molecules (HLA), respectively (1). CD4+ and CD8+ T-cells have divergent yet interacting roles related to immune homeostasis and pathogenesis of both communicable and non-communicable diseases. Effective functioning of CD4+ and CD8+ T-cells is energy demanding. The universal energy carrier adenosine 5′-triphosphate (ATP) in addition to assimilation and generation of biosynthetic precursors are required to initiate and sustain an immune response (2, 3). Immunometabolism describes how immune cells obtain ATP *via* differing rates of the energy-producing pathways and generate biosynthetic intermediates under quiescence and activation (4, 5). T-cell quiescence is associated with energy utilization *via* high-yield, slow burning metabolic processes dependent on fueling mitochondria for oxidative phosphorylation (6).

There is a burgeoning literature regarding T-cell metabolism, but with the exception of CD8+ T-cells (7–10), most data on T-cell metabolism are derived from mouse models and direct comparisons of human CD4+ and CD8+ T-cells have not been made. Murine CD4+ and CD8+ T-cells are bioenergetically similar when quiescent and are metabolically reprogrammed to a highly glycolytic metabolic state upon activation with CD8+ T-cells the more bioenergetic (11). Constitutive glycolytic metabolism results in long-lived effector T-cells in viral specific murine CD8+ T-cells (12). Activation is also accompanied by increased expression of GLUT1 and glycolysis pathway enzymes

in both murine CD4+ and CD8+ T-cells (11, 13, 14). Surface levels of GLUT1 have been shown to identify human CD4+ and CD8+ T-cell with distinct characteristics. GLUT1^{Hi} T-cells produced elevated levels of IFNγ and had increased effector function (15). Naïve T-cell activation is linked to asymmetric division and the effector T-cell and memory T-cell that arise upon interaction with an antigen-presenting cell have metabolic differences. The effector T-cell is largely glycolytic, whereas the memory T-cell relies on oxidative metabolism governed by transcription factor c-myc (16). Post-infection, murine CD8+ memory T-cells retain a high spare respiratory capacity should re-infection occur (17). Increased glucose metabolism upon T-cell activation is critical for the rapid engagement of cellular proliferation, achieved *via* the generation of biosynthetic intermediate serine and downstream nucleotide production (2). Manipulating this pathway offers the potential to modulate regulatory T-cell differentiation and function (18, 19).

T-cell receptor (TCR) ligation to a peptide presenting HLA molecule (pHLA) is critical to the effective activation of T-cells (20, 21). The binding affinity between the TCR and core region of the peptide coupled with the half-life of peptide-TCR interaction collectively govern the downstream effector function (22, 23). The TCR-pHLA binding affinity confers underlying signaling cascades leading to an increased demand for the extracellular glucose needed to produce biosynthetic intermediates for proliferation in addition to cellular ATP (24, 25). Synthesis of metabolites, such as polyamines, cholesterol *via* fatty acids synthase, and pentose phosphate intermediates, has been shown to enhance T-cell activation (26, 27). To initiate and sustain this demand, hematopoietic cells generally exhibit a "Warburg-like" switch to glycolysis (28). The reliance of human CD8+ T-cells on glycolysis when stimulated with natural ligands (Epstein–Barr Viral peptides) has been reported (7); how TCR-pHLA binding affinity might control the corresponding metabolic response in human T-cells is unknown. Murine CD8+ T-cells show TCR binding affinity-dependent induction of IRF4 and downstream metabolic control (29).

This is the first study to investigate the metabolic tuning that occurs in human T-cells upon activation *via* the TCR and includes consideration of the role of TCR-pHLA binding affinities. Stimulation with native peptide provides a more physiologically relevant mechanism of T-cell activation compared to anti-CD3/anti-CD28. Furthermore, cytokine production by both CD4+ and CD8+ T-cells is shown to depend on glycolysis with differential mitochondrial dependence between these T-cell subsets.

MATERIALS AND METHODS

Human CD4+ and CD8+ T-Cell Isolation

Human peripheral blood was collected between 0830 hours and 1000 hours from healthy, non-fasted individuals into heparinised Vacuettes™ (Greiner Bio-one, Frickenhausen, Germany) and processed within 10 min of collection. All samples were collected with informed written consent and ethical approval was obtained from Wales Research Ethics Committee 6 (13/WA/0190).

Mononuclear cells (MNCs) were isolated by layering whole blood (1:1) onto Histopaque (Sigma-Aldrich, Poole, UK) prior to centrifugation at 805*g* for 20 min at room temperature. MNCs were removed and washed with RPMI 1640 (Life Technologies, Paisley, UK) twice by centrifugation at 515*g*. The MNC pellet was resuspended in media specific for the downstream assay and cell density determined using the Countess® automated cell counter (Life Technologies).

CD4+ or CD8+ T-cells were isolated *via* a negative selection process using magnetic microbeads as described by the manufacturer (autoMACS; Miltenyi Biotec, Cologne, Germany). Purity of individual populations was monitored using flow cytometry and was typically >90%. For non-matched T-cell experiments, the mean ± SD donor age for CD4+ T-cell preparations was 39.2 ± 14.68 years ($n = 12$; 7 females and 5 males) and for CD8+ T-cells was 35.1 ± 13.21 years ($n = 16$; 7 females and 9 males).

T-Cells

T-cell clones, DCD10, and ILA1 were created (30, 31) and passaged as previously described (32). Briefly, clones were expanded with irradiated (3,100 Gy) PBMCs from three donors in R10 [RPMI 1640 supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 1× MEM non-essential amino acid, 1 mM sodium pyruvate, 10 mM HEPES buffer (Life Technology)] with 20 IU/ml of IL-2 (Aldesluekin, Proleukin, Prometheus, San Diego, CA, USA) and 1 µg/ml of phytohaemaglutinin (Alere, Cheshire, UK). Additionally, ILA1 was cultured with 25 ng/ml of IL-15 (PeproTech, Rocky Hill, NJ, USA) and IL-2 increased to 200 IU/ml 7 days post expansion. For this purpose of this study, clones were used spanning 3–4 passages. Prior to performing assays, clones were washed from culture media and rested in R5 (as for R10 with 5% FBS) for 24 h. Peptides (Peptide Protein Research Limited, Fareham, UK) were synthesized to greater than 95% purity, stored as 20 mM stocks at −80°C in DMSO and working aliquots made to 1 mM with R0 (as for R10 but with no FBS) and stored at −20°C or 4°C.

Metabolic Analysis

Metabolic analysis was carried out using an Extracellular Flux Analyzer XF^{e}24 (Seahorse Bioscience). Briefly, 0.25×10^6 cells were seeded onto a Cell-Tak (Corning)-coated microplate allowing the adhesion of T-cells. Mitochondrial stress and glycolytic parameters were measured *via* oxygen consumption rate (OCR) (pmoles/min) and extracellular acidification rate (ECAR) (mpH/min), respectively, with use of real-time injections. For mitochondrial stress, cells were resuspended in XF assay media supplemented with 5.5 mM glucose and 1 mM pyruvate and injections oligomycin (0.75 µM), carbonyl cyanide-*4*-(trifluoromethoxy)phenylhydrazone (FCCP; 1 µM) and rotenone and antimycin (both 1 µM) were used. For glycolysis, cells were resuspended in XF assay media with use of injections glucose (11.1 mM), oligomycin (0.75 µM) and 2-deoxy-D-glucose (100 mM). Respiratory parameters were calculated as previously described (33). All chemicals were purchased from Sigma unless stated otherwise. Calculations for individual metabolic parameters can be found as described previously (33) or per manufacturer's instructions (Seahorse Bioscience).

Activation

To monitor the glycolytic switch upon activation, $CD4^+$ and $CD8^+$ T-cells were resuspended in serum-free XF Assay media supplemented with 11.1 mM glucose and 2 mM L-glutamine (Sigma). ECAR and OCR were measured simultaneously throughout the experiment, i.e., 1 h before activation and 4 h after. T-cells were activated *via* the multi-injection port with anti-CD3 (0.2 μg/ml; HIT3a, BioLegend) and CD28 (20 μg/ml; CD28.2, BioLegend). A final injection of 2-DG (100 mM) was used to immediately arrest glycolysis. Isotype controls, mIgG2a κ (0.2 μg/ml; MOPC-173, BioLegend) and mIgG1 κ (20 μg/ml; MOPC-21; BioLegend) were used. The OCR/ECAR ratio was calculated by dividing the eight corresponding OCR and ECAR measurements pre- (dotted boxes) or post- (dashed boxes) antibody injection. Fold ECAR change was calculated by dividing the single point post antibody injection by the single point pre antibody injection. Peptide stimulation relied on the cross presentation of specific peptides by corresponding T-cell clones.

Inhibition

Baseline ECAR of $CD4^+$ and $CD8^+$ T-cells was determined for roughly 1 h prior to injection of GLUT1/4 inhibitor ritonavir (20 μM; Sigma). A 40-min period of incubation with the inhibitor occurred prior to injection of αCD3/28 as above. Corresponding ECAR was monitored for 4 h after αCD3/28 injection. A final injection of 2-DG (100 mM) arrested glycolysis. Fold ECAR change was calculated by dividing the 13 measurements post antibody injection (dashed box) by the 13 measurements pre antibody injection (dotted box).

Flow Cytometry

Purity of $CD4^+$, or $CD8^+$ T-cells was monitored using flow cytometry. Briefly, 2.5×10^5 cells were left unstained or incubated with anti-$CD4^+$ AlexaFluor®647 (mIgG2b, clone OKT4, eBioscience) or anti-$CD8^+$ PE (mIgG1, clone HIT8a, eBioscience) using standard techniques. Cells were acquired (FACSAria I, BD Biosciences) and downstream analysis was with FlowJo version 1.3 (Tree Star, OR, USA). To assess mitochondrial content, MNCs were stained with the mitochondrial probe MitoTracker Green (Life Technologies). MNCs (5×10^5 cells) were incubated with 20 nM MitoTracker Green for 30 min at 37°C then surface labeled with lineage markers as above before acquisition and analysis. T-cell activation was monitored by expression of CD69 (mIgG1, FN50, BioLegend), flow cytometry plots are representative of live cells with dead cell exclusion performed *via* DRAQ7 (1 μM; Biostatus, UK).

Effect of Respiratory Inhibitors on Cytokine Output

$CD4^+$ and $CD8^+$ T-cells were cultured at 0.5×10^6 cells/500 μl of phenol red free RPMI (Sigma) + 2 mM GlutaMAX (ThermoFisher). T-cells were cultured with 2-deoxy-D-glucose (25 mM) or oligomycin (1 μM) at 37°C in 5% CO_2-in-air for 24 h. All chemicals were purchased from Sigma. To prevent impaired T-cell activation, after 3 h 5% fetal bovine serum (FBS, HyClone, ThermoFisher Scientific) was added. Cells were analyzed *via* flow cytometry for cell death (DRAQ7) and activation (CD69); the supernatant was removed and stored at −20°C for downstream cytokine analysis. IFNγ and IL-2 were analyzed using ELISA as per manufacturer's instructions (DuoSets; R&D Systems).

Immunoblot

$CD4^+$ and $CD8^+$ T-cell lysate proteins were quantified using the DC Assay (Bio-Rad, Hemel Hempstead, UK) and separated (10 mg per lane) using 10% (vol/vol) SDS-polyacrylamide gel electrophoresis, with molecular weight markers in parallel lanes (Bio-Rad). After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad); non-specific binding was blocked using 5% (wt/vol) bovine serum albumin (BSA; Sigma) in Tris-buffered saline (Sigma) for 1 h at room temperature. Membranes were probed with rabbit monoclonal antibodies targeting glucose transporter 1 (GLUT1; ab115730; Abcam), hexokinase I (HKI; 2024), hexokinase II (HKII; 2867), phosphofructokinase (PFKP; 8164), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 5174), pyruvate kinase (PKM2; 4053) lactate dehydrogenase (LDH; 3582), total S6 ribosomal protein (2217), and phospho-S6 ribosomal protein (Ser235-236; 4858). Protein loading was evaluated and normalized using mouse monoclonal antibody targeting β-actin expression (3700). All antibodies were purchased from Cell Signaling unless otherwise stated (Danvers, MA, USA). Primary antibodies were used at 1:1,000 dilutions in Tris-buffered saline, 0.1% Tween 20 (pH 7.6; Sigma) overnight at 4°C. Membranes were washed and incubated in either anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibody (Cell Signaling) in 5% (wt/vol) BSA in Tris-buffered saline for 1.5 h, and then washed. Steady-state levels of immunoreactive proteins were visualized using enhanced chemiluminescence (Western C, Bio-Rad), and densitometry on non-saturated immunoblots was measured using ImageJ software (FIJI). Full immunoblots are shown in supplementary material.

Data Analysis

Statistical analysis was performed using GraphPad Prism version 6 (USA). Data are represented as the mean + SEM. A non-paired *t*-test was used for the different metabolic data, densitometry immunoblots, and metabolic inhibition comparisons. One-way ANOVA was used to compare 24 h activated T-cells samples and altered peptide ligand (APL) ECAR and OCR fold change. Statistical analysis was performed on the technical repeats when considering the clone data. Significant values were taken as $^*p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$.

RESULTS

$CD4^+$ T-Cells Have a Greater Glycolytic Potential than $CD8^+$ T-Cells

To investigate the glycolytic potential of human T-cells, we undertook bioenergetics analysis of total, non-matched $CD4^+$ versus $CD8^+$ T cells. ECAR was measured and showed that all glucose starved T-cells responded to glucose injection with increased ECAR but failed to show a further increase after

injection of the ATP synthase inhibitor, oligomycin (**Figure 1A**). There was no significant difference in the non-glycolytic acidification between CD4+ and CD8+ T-cells (Figure S1A in Supplementary Material). Most notably, CD4+ T-cells exhibited significantly higher levels of basal glycolysis compared to CD8+ T-cells (**Figure 1B**). Oxidative phosphorylation profiles of CD4+ and CD8+ T-cells were also determined using extracellular flux analysis for oxygen consumption rate (OCR; **Figure 1C**). CD4+ and CD8+ T-cells did not differ in rates of basal, maximal or ATP-linked respiration, spare respiratory capacity (**Figures 1D–G**) or non-mitochondrial respiration and proton leak (Figures S1B,C in Supplementary Material). The combined changes in OCR (basal respiration) and ECAR (basal glycolysis) give CD4+ T-cells a significantly lower OCR/ECAR ratio than CD8+ T-cells (**Figure 1H**). Analysis of mitochondrial content using flow cytometry with the mitochondrial stain MitoTracker Green, revealed that donor-matched CD4+ T-cells have a significantly higher mitochondrial content than CD8+ T-cells (**Figures 1I,J**) in agreement with findings in murine models (11).

CD4+ T-Cells Express High Levels of Hexokinase Isoforms

To investigate the underlying cause for increased glycolytic metabolism by quiescent CD4+ T-cells, key transporters and enzymes within the glycolysis pathway were analyzed (**Figure 2A**): GLUT1 as the predominant glucose transporter in human and murine CD4+ T-cells (13); hexokinase (HK) I and II that catalyze the transfer of phosphate from ATP to glucose thereby consuming one molecule of ATP and 'trapping" glucose in the cell (34); phosphofructokinase (PFKP) which catalyzes a rate-limiting reaction that consumes a second ATP molecule; GAPDH which is critical to the production of two ATP molecules and two nicotinamide adenine dinucleotide molecules (NADH + H^+) (35); pyruvate kinase (PKM2) that catalyzes the final rate-limiting step of glycolysis to produce two ATP molecules per glucose; and lactate dehydrogenase (LDH) that converts pyruvate into lactate yielding the protons measured as ECAR.

Immunoblotting of CD4+ and CD8+ T-cells from non-matched donors revealed that GLUT1 levels were consistent between CD4+ and CD8+ T-cells (**Figure 2B**). A double band observed in one of the CD4+ T-cell donors could be due to altered glycosylation status of the GLUT1 protein as observed in other human glucose transporters (36, 37). HKII (**Figure 2D**), PKM2 (**Figure 2G**), and LDH (**Figure 2H**) were all increased in CD4+ T-cells and likely underpin the increased glycolytic capacity of this population compared to CD8+ T-cells. There were no differences in HKI (**Figure 2C**), PFKP (**Figure 2E**), or GAPDH (**Figure 2F**). However, we cannot rule out differential kinetics of any of these glycolytic enzymes between the two T-cell populations and this should be considered in future experiments.

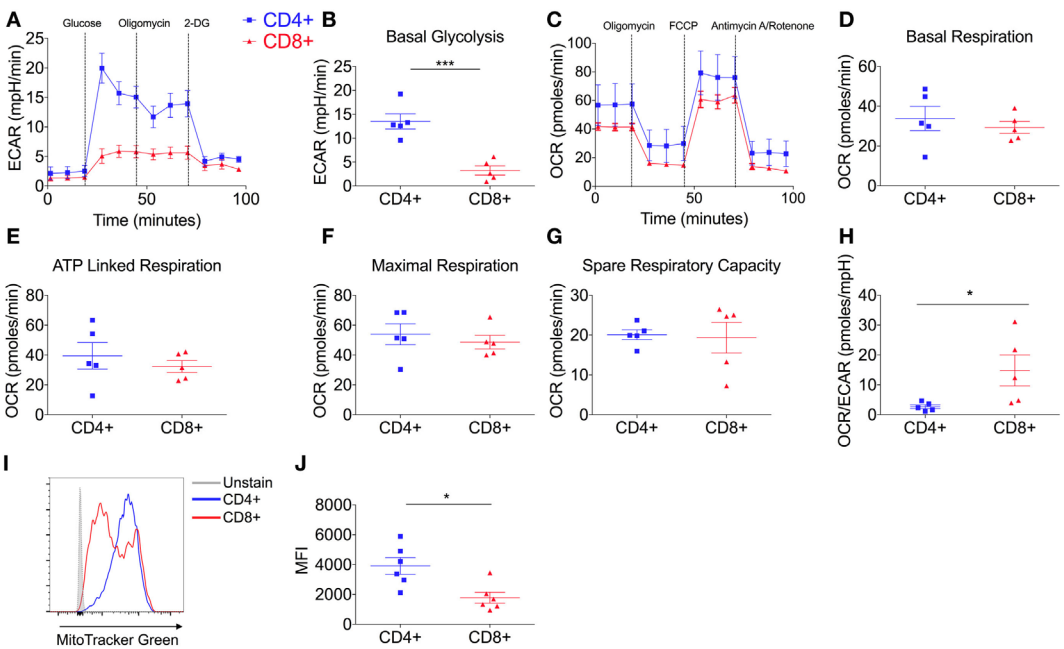

FIGURE 1 | CD4+ T-cells are more glycolytic than CD8+ T-cells. **(A)** Glycolysis by CD4+ and CD8+ T-cells isolated from non-matched donors was measured using extracellular acidification rate (ECAR) with injections glucose (11.1 mM), oligomycin (0.75 μM), and 2-DG (100 mM); including parameter **(B)** basal glycolysis calculated by subtracting the three averaged measurements after glucose injection from the non-glycolytic acidification. **(C)** Oxidative phosphorylation profiles of CD4+ and CD8+ T-cells measured by the oxygen consumption rate (OCR) with injections oligomycin (0.75 μM), FCCP (1 μM) and antimycin A/rotenone (both 1 μM); including OXPHOS parameters **(D)** basal respiration, **(E)** ATP-linked respiration, **(F)** maximal respiration and **(G)** spare respiratory capacity. **(H)** OCR/ECAR ratio of CD4+ and CD8+ T-cells (pmoles/mpH) as determined *via* the division of basal respiration and basal glycolysis parameters. Mitochondrial content of CD4+ and CD8+ T-cells was assessed by flow cytometry with MitoTracker Green and **(I)** a representative example is shown and **(J)** summary data for $n = 6$. Data are from five **(A–H)** non-matched donors, and six **(J)** matched independent experiments. Data expressed as mean ± SEM; $^*p \leq 0.05$, $^{***}p \leq 0.001$.

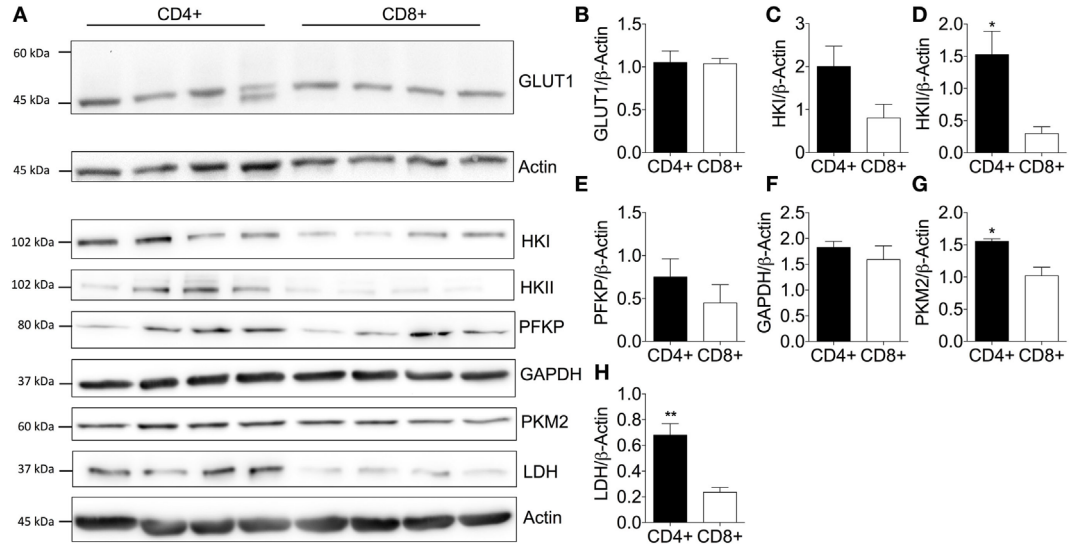

FIGURE 2 | Elevated expression of key glycolytic enzymes enhances basal glycolysis of CD4+ T-cells. **(A)** Protein immunoblot and respective densitometry showing **(B)** GLUT1, **(C)** hexokinase I (HK), **(D)** hexokinase II, **(E)** phosphofructokinase (PFKP), **(F)** glyceraldehyde-3-phosphate dehydrogenase (GAPDH), **(G)** pyruvate kinase (PKM2), and **(H)** lactate dehydrogenase (LDH) expression levels between basal CD4+ and CD8+ T-cells. Full scan blots are shown in Figure S9 in Supplementary Material. Data are from four independent experiments. Data expressed as mean + SEM; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

CD4+ and CD8+ T-Cells Increase Glycolytic Flux upon Stimulation

Having established that human CD4+ T-cells are more glycolytic than CD8+ T-cells during quiescence, we then investigated the metabolic plasticity of both subsets in response to stimulation. Donor-matched CD4+ and CD8+ T-cells were activated with anti-human CD3 and anti-human CD28 antibodies and both ECAR and OCR monitored for a period of 30 cycles using extracellular flux analysis (4.13 h; **Figures 3A,B**).

Immediately upon injection of anti-CD3/CD28, both CD4+ and CD8+ T-cells increase ECAR indicating heightened glycolysis (**Figure 3A**). This was accompanied by a slight increase in OCR (**Figure 3B**). Isotype controls had no effect on either glycolysis or oxygen consumption (Figures S2A,B in Supplementary Material). Elevated ECAR levels were maintained by CD4+ and CD8+ T-cells for the duration of the experiment. Calculation of the OCR/ECAR ratio revealed a significant immediate increase in glycolytic flux in both CD4+ and CD8+ T-cells upon activation (**Figure 3C**). The early engagement of glycolysis, represented as a fold ECAR change, was homologous in CD4+ and CD8+ T-cells (**Figure 3D**). There was a temporal decline in glycolysis by CD8+ T-cells, whereas this response was sustained in CD4+ T-cells (**Figure 3D**). To determine the role of glucose transporters in the activation-dependent glycolytic switch, ritonavir, an inhibitor of both GLUT1 and GLUT4 (38), was used. Ritonavir significantly dampened activation-induced glycolysis in both CD4+ and CD8+ T-cells (**Figures 3E–G**).

The potential contribution of key enzymes to an activation-dependent metabolic switch was then determined by comparing expression at 24 h with or without exposure to anti-CD3/CD28 (Figures S3A–G in Supplementary Material). Activation did not affect levels of HKI in donor-matched CD4+ or CD8+ T-cells (Figure S3B in Supplementary Material), whereas HKII was markedly increased in both CD4+ and CD8+ T-cells (Figure S3C in Supplementary Material). PFKP and GAPDH expression also increased upon activation of both cell types (Figures S3D,E in Supplementary Material). PKM2 levels were increased in CD4+ T-cells upon activation but remained constant in CD8+ T-cells (Figure S3F in Supplementary Material). Levels of LDH were increased in both subsets upon activation with the response by CD8+ T-cells significant (Figure S3G in Supplementary Material). These experiments indicate that the immediate metabolic switch upon activation is probably dependent on glucose transport *via* GLUT1. Subtle differences in the expression and probable activity of GLUT1 and different glycolytic enzymes contribute to altered kinetics of response by CD4+ versus CD8+ T-cells.

Increased GLUT1 Expression Facilitates CD4+ and CD8+ T-Cell Activation

To further explore the contribution of glucose transporters to enhanced glycolysis upon activation, GLUT1 expression before and after activation was analyzed by immunoblotting (**Figure 4A**). GLUT1 expression was significantly increased upon activation in donor-matched CD4+ and CD8+ T-cells (**Figure 4B**). The *de novo* synthesis of proteins such as cytokines requiring transcription and translation is a key feature of T-cell effector responses. Therefore, we next assessed the levels of downstream mTORC1 target, ribosomal S6, a protein involved in the translation of 5′TOP mRNAs (39, 40) (**Figure 4A**). There was an increase in phosphorylated ribosomal protein S6 (pS6Ser235/236), with ratio of pS6/S6 significantly different between unactivated and activated T-cell subsets (**Figure 4C**). A marked increase in translation *via* increased phosphorylated S6 would support the production of cytokines to mount

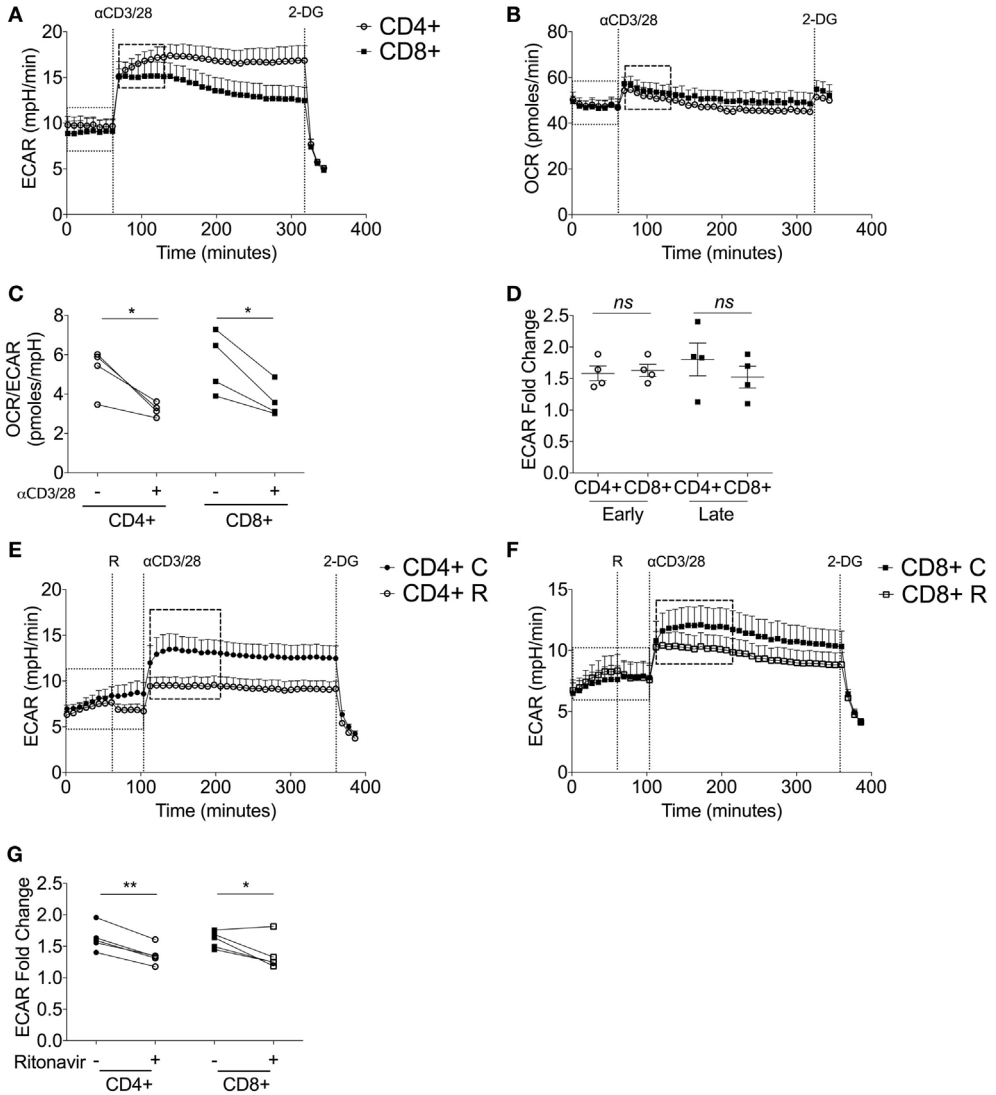

FIGURE 3 | Upon stimulation, both CD4+ and CD8+ T-cells switch to glycolytic metabolism. **(A)** Extracellular acidification rate (ECAR) and **(B)** oxygen consumption rate (OCR) of donor-matched CD4+ and CD8+ T-cells upon stimulation with antibodies, anti-CD3 (0.2 μg/ml), and anti-CD28 (20 μg/ml). 2-deoxy-D-glucose (100 mM) was added at the end of the experiment to immediately arrest glycolysis. **(C)** OCR/ECAR ratio pre- and post- activation calculated by dividing OCR by ECAR (dotted box; pre, dashed box; post). **(D)** ECAR fold change of early and late glycolytic changes comparing CD4+ and CD8+ T-cells. ECAR of **(E)** CD4+ T-cells and **(F)** CD8+ T-cells ± ritonavir (20 μM) prior to injection of activating antibodies as above with a final injection of 2-DG (100 mM). *C, control; R, ritonavir.* **(G)** Fold change calculated *via* division of the dotted boxes of CD4+ and CD8+ T-cells ± ritonavir. Data are from four donor-matched **(A–D)** or five donor-matched independent experiments **(E–G)**. Graphed points represent averaged data from the four/five independent experiments. Data expressed as mean + SEM; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, *ns*, not significant.

a successful immune response. This is feasible as a marked decrease in pS6 and mTORC1 activity upon triple therapy strategies reduces murine T-cell effector function thus decreasing allograft rejection (41, 42).

CD8+ T-Cells Are Dependent on Mitochondrial Metabolism for Cytokine Production

Currently, little is known about the metabolic pathways utilized by human CD4+ and CD8+ T-cells and whether they differ upon effector function. In mice, NK cells require both glycolysis and oxidative phosphorylation for IFNγ production (43). Understanding the metabolic pathways that contribute to downstream cytokine production might offer potential therapeutic targets. Therefore, the role of glycolysis and oxidative phosphorylation were considered by activating cells with anti-CD3/CD28 in the presence of the metabolic inhibitors: 2-DG which inhibits glycolysis, and oligomycin which inhibits oxidative phosphorylation. Cell death was monitored using DRAQ7 by flow cytometry with neither inhibitor having an effect on non-matched CD4+ or CD8+ T-cell viability (**Figure 5A**). Activation was monitored through CD69

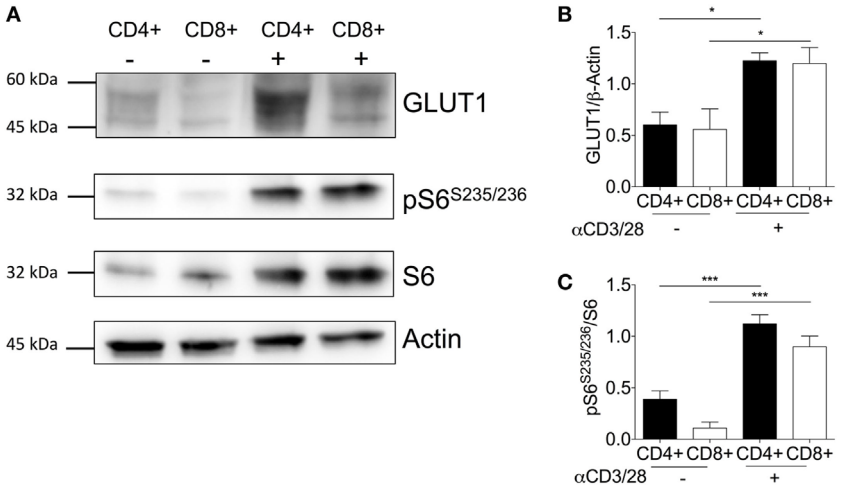

FIGURE 4 | Activation is associated with increased GLUT1 expression in CD4⁺ and CD8⁺ T-cells. **(A)** Protein immunoblot representative of one matched donor, and respective densitometry of all donors showing, **(B)** GLUT1, **(C)** ribosomal proteins; phospho-S6 and total S6 expression levels between unstimulated (–) and activated (+) (anti-CD3; 2 μg/ml and anti-CD28; 20 μg/ml) CD4⁺ and CD8⁺ T-cells for 24 h. Full scan blots are shown in Figure S10 in Supplementary Material. Data are from five independent experiments with donor-matched CD4⁺ and CD8⁺ T-cells **(A–C)**. Data expressed as mean + SEM; $*p \leq 0.05$, $***p \leq 0.001$.

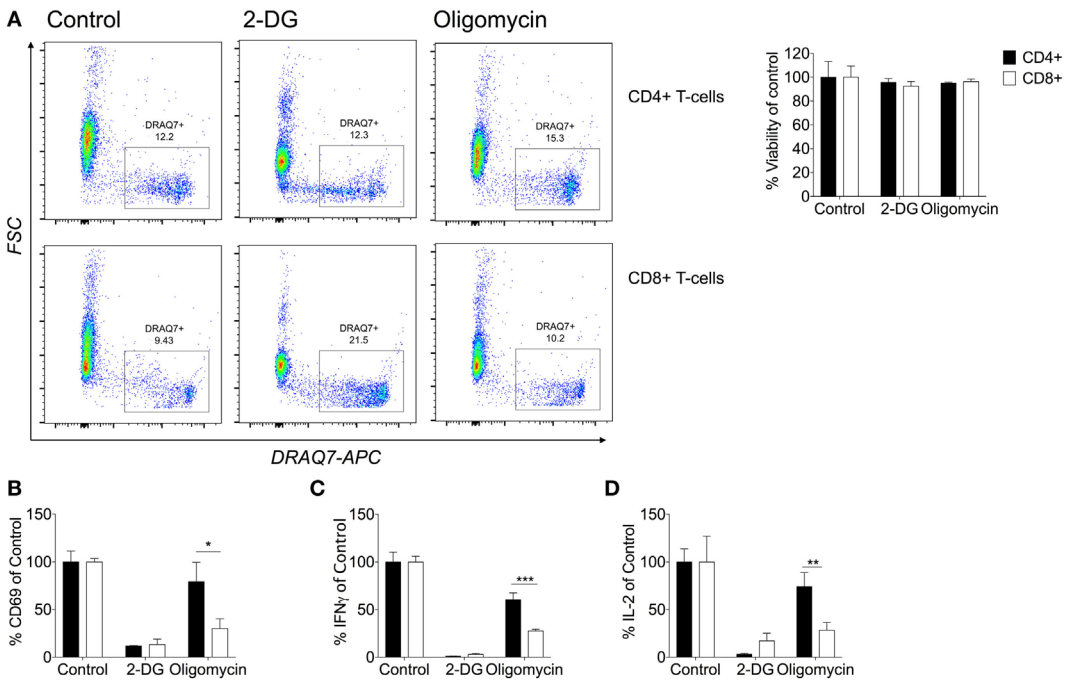

FIGURE 5 | CD8⁺ T-cells are dependent on their mitochondria for cytokine production. **(A)** Viability was determined by DRAQ7 (1 μM) flow cytometry of CD4⁺ and CD8⁺ T-cells stimulated with anti-CD3 (2 μg/ml) and anti-CD28 (20 μg/ml) for 24 h in the presence of 2-deoxy-D-glucose (2-DG; 25 mM), or oligomycin (1 μM). Viability expressed as a percentage of the 24 h activated control **(B)** Percentage CD69 of CD4⁺ and CD8⁺ T-cells (DRAQ7-negative) relative to control. The effects of metabolic inhibitors 2-DG and oligomycin were considered on percentage cytokine production of **(C)** IFNγ and **(D)** IL-2 comparison of CD4⁺ and CD8⁺ T-cells. Data are representative of three to five independent experiments. Data expressed as mean + SEM; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

expression for both subsets, whereby inhibition of glycolysis but not oxidative phosphorylation was associated with reduced expression of CD69 indicating decreased activation of both CD4⁺ and CD8⁺ T-cells if glycolysis is abrogated (**Figure 5B**). Inhibition of oxidative phosphorylation, however, only reduced CD69 expression in CD8⁺ T-cells, indicating a greater contribution of mitochondrial-dependent mechanisms to activation of these cells (**Figure 5B**).

In agreement with other studies, we have shown that limiting glycolysis with 2-DG inhibited production of IFNγ and IL-2 in $CD4^+$ and $CD8^+$ T-cells (**Figures 5C,D**) (7). IFNγ and IL-2 were not detectable in the unstimulated samples (data not shown). The contribution of oxidative phosphorylation to the expression of CD69 and the production of both IFNγ and IL-2 production was significantly greater in $CD8^+$ than $CD4^+$ T-cells. To our knowledge, this is the first time that different metabolic requirements of human $CD4^+$ and $CD8^+$ T-cells have been demonstrated.

We also found that this difference in metabolic requirement was evident for wider cytokine production. The production of IL-13, IL-17, and IL-10 by $CD4^+$ T-cells (Figures S4A–D in Supplementary Material) was reduced significantly after incubation with 2-DG; the decrease in IL-4 was not significant. Only the Th2 cytokines, IL-4 and IL-13, and to a lesser extent IL-10, were decreased by inhibition of oxidative phosphorylation. For $CD8^+$ T-cells, granzyme B and MIP-1β, like IL-2 and IFNγ, were reduced upon treatment with either 2-DG or oligomycin, confirming a greater contribution of mitochondrial metabolism to $CD8^+$ T-cell cytokine and granzyme B production (Figures S5A,B in Supplementary Material). Similar to our findings, murine $CD8^+$ T-cells are more resistant than $CD4^+$ T-cells to knockout of GLUT1 (11). These data further support differential metabolic kinetics of both T-cell subsets that could be important in nutrient competitive and restricted environments.

TCR Peptide HLA Induced T-Cell Activation Stimulates Greater Glycolytic Flux Compared to Non-Natural CD3/CD28 Stimulation

Most studies of T-cell metabolism use anti-CD3/CD28 stimulation to activate T-cells rather than natural ligands although there are a few notable exceptions (7, 29). To address these shortcomings here, for the first time, antigen-specific human T-cell clones were used to investigate T-cell metabolism after stimulation directly through the antigen-specific TCR-pHLA interaction. Two T-cell clones were used: DCD10, a HLA-DRB*0101 restricted, influenza hemagglutinin ($HA_{306–318}$: PKYVKQNTLKLAT) specific $CD4^+$ T-cell clone; and ILA1, a HLA-A*0201-restricted, tumor-associated antigen human telomerase reverse transcriptase ($hTERT_{540–548}$: ILAKFLHWL) specific $CD8^+$ T-cell clone. Extracellular flux analysis relied on the presentation of peptide within T-cell populations, whereby the peptide initially binds to the HLA complex. Initial experiments were performed to optimize peptide concentrations for both clones (Figures S6A–D in Supplementary Material). We confirmed the expression of HLA-DR and MHC class I of DCD10 by flow cytometry (Figure S7A in Supplementary Material). In addition the ability of DCD10 to cross-present peptide was monitored *via* ELISA, whereby overnight cultures with native peptide PKY induced production of IFNγ and MIP-1β in comparison to negative peptide (5T4; Figure S7B in Supplementary Material). The response of the $CD4^+$ DCD10 clone to the PKYVKQNTLKLAT peptide (PKY) was monitored by extracellular flux analysis in comparison with activating antibodies, anti-CD3, and anti-CD28. Both PKY and anti-CD3/anti-CD28 induced an immediate increase in ECAR (albeit to a lesser extent in anti-CD3/anti-CD28 stimulated cells), with 2-DG injection confirming the role of glycolysis (**Figure 6A**). Stimulation of DCD10 to its natural ligand PKY induced a significantly greater ECAR fold change in comparison to anti-CD3/anti-CD28 (**Figure 6B**). The corresponding OCR revealed a substantial increase in oxygen consumption upon injection of PKY, less so with anti-CD3/anti-CD28 (**Figure 6C**). PKY induced a twofold increase in oxygen consumption upon peptide stimulation, whereas anti-CD3/anti-CD28 induced roughly a 1.5-fold change in oxygen consumption (**Figure 6D**). Dual increases in both glycolysis and oxidative phosphorylation (represented as increased ECAR and OCR) *via* use of PKY proved that the use of antigen specificity is a viable option for extracellular flux analysis. We also altered the DCD10 native peptide (PKY) at residue number 11 from threonine to arginine and determined the metabolic response (Figure S8 in Supplementary Material). Here, we found that the residue alteration at position 11 had no effect on peptide recognition for the DCD10 clone using extracellular flux analysis, with ECAR and OCR remaining unchanged (Figure S8A–E in Supplementary Material).

We also compared the ILAKFLHWL index peptide and its metabolic effect on T-cell clone, ILA1. Following index peptide injection, ILA1 also exhibited significantly enhanced glycolysis (**Figure 6E**), in comparison to anti-CD3/anti-CD28 stimulation. This observation was reflected in the ECAR fold change (**Figure 6F**). The oxygen consumption was also monitored throughout the experiment (**Figure 6G**), which revealed a significant 1.5-fold increase after ILA1 peptide interaction, whereas there was no notable OCR increase in anti-CD3/anti-CD28-treated ILA1 clones (**Figure 6H**). Collectively, these data show that naturally recognized peptides produce a different metabolic signature compared to anti-CD3/anti-CD28 stimulation.

TCR Binding Affinity Governs Differential Glycolytic Thresholds in Antigen-Specific T-Cells

In order to investigate whether TCR affinity could tune T-cell metabolism, we used a panel of previously defined APLs that are recognized by the ILA1 TCR with a range of binding affinities (**Figure 7A**) (20). This is the first time T-cell metabolism has been investigated through natural ligands with altered affinity.

The hierarchy of ECAR fold change corresponded to the affinity of the ILA1 TCR for each APL, with the strongest affinity ligand (3G) generating the highest ECAR, and the lowest affinity ligand (8E) generating no ECAR increase (**Figures 7B,C**). This peptide stimulation initiated an increase in ECAR followed by a gradual decrease presumably as glucose in the original seeding media was utilized.

Analysis of OCR revealed that the rate of oxygen consumption was also dependent on the affinity of TCR–pHLA interaction (**Figure 7D**). This was reflected in the OCR fold change where index peptide and 8T APL had similar OCR fold changes and 3G APL had the largest OCR fold change (**Figure 7E**). Thus, we demonstrate, for the first time that TCR binding affinity governs

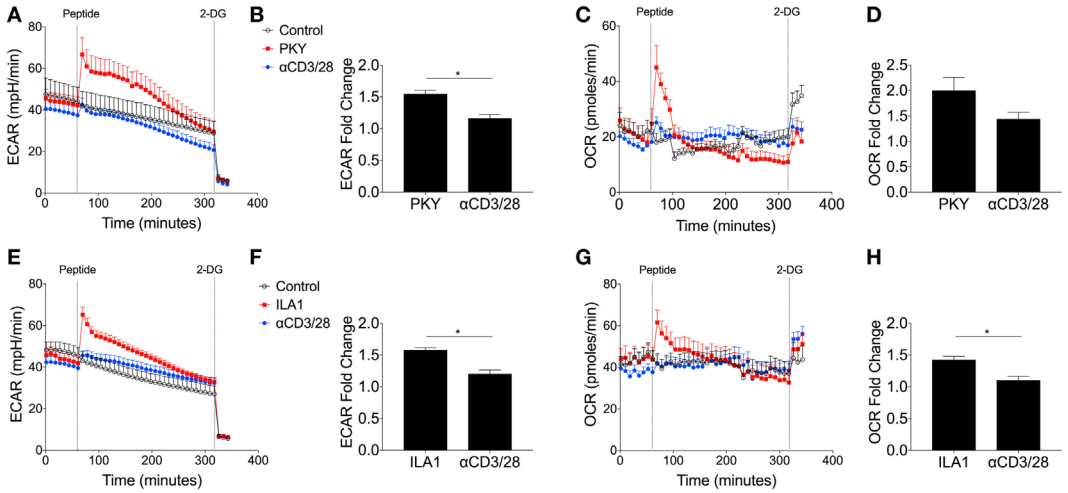

FIGURE 6 | Metabolic response of CD4+ DCD10 and CD8+ ILA T-cell clones to native peptides. Metabolic analysis of CD4+ T-cell clone, DCD10 in response to native Flu1 $HA_{306-318}$, peptide, with sequence; PKYVKQNTLKLAT (PKY). **(A)** Comparative extracellular acidification rate (ECAR) stimulation of DCD10 CD4+ T-cell clone with PKY (10 μM) and anti-CD3 (0.2 μg/ml) and anti-CD28 (20 μg/ml). Final injection of 2-deoxy-D-glucose (100 mM). **(B)** Fold ECAR change calculated with use of single measurement prior to peptide injection and single measurement after peptide injection. **(C)** Corresponding oxygen consumption rate (OCR) and **(D)** OCR fold change after stimulation with PKY or anti-CD3 anti-CD28. **(E)** Metabolic analysis *via* ECAR of CD8+ T-cell clone, ILA1 with ubiquitous tumor-associated antigen human telomerase reverse transcriptase ($hTERT_{540-548}$) peptide ILA1 (ILAKFLHWL; 10 μM) with final injection of 2-DG (100 mM). **(F)** ECAR fold change as calculated previously. **(G)** Corresponding OCR and **(H)** OCR fold change after stimulation with ILA1 or anti-CD3 anti-CD28. Data are representative of four to twelve technical repeats comprising of two to three independent experiments. Data expressed as mean + SEM; $^*p \leq 0.05$.

the metabolic response to antigen, likely a critical step in determining T-cell effector functions.

DISCUSSION

Elucidating metabolic differences of leukocyte subsets and their changes over the life course of a cell is critical to our understanding of both basic immunology and perturbations with disease. Initially, the bioenergetics profile of quiescent, glucose starved CD4+ and CD8+ T-cells were compared to show that upon exposure to glucose T-cells exhibit no glycolytic reserve, although this was in a finite glucose scenario. While both populations were quickly at maximum glycolysis the CD4+ subset had significantly higher basal levels of glycolysis, which could be attributed to elevated glycolytic enzymes, specifically hexokinase isozyme II. These enzymes are important early in glycolysis for phosphorylating glucose to trap it inside the cell (34). Quiescent CD4+ T-cells also had higher oxygen consumption. Significantly higher levels of mitochondria, measured using MitoTracker, in CD4+ than CD8+ T-cells in all likelihood support this which is comparable to what has been reported previously in mice (11). Within the CD8+ T-cell population there was a $MitoTracker^{Hi}$ and a $MitoTracker^{Lo}$ subpopulation that could reflect mitochondrial biogenesis within the CD8+ T-cell population (44).

Like most other hematopoietic cell populations studied to date, human CD4+ and CD8+ T-cells undergo a "Warburg-like" switch to increased glycolytic metabolism upon activation; while accompanied by an increase in oxygen consumption, overall the balance shifts to favor glycolysis immediately upon cell stimulation. This would support ATP and biosynthetic intermediate production to fuel effector functions and we confirm a role for glucose consumption in cytokine production by both CD4+ and CD8+ T-cells. There were, however, some notable differences in the two T cell populations. CD8+ T-cellsexhibited a gradual decrease in glycolysis post-activation and showed greater dependency on mitochondrial metabolism for cytokine production. This gradual decrease in glycolysis by CD8+ T-cells only could reflect metabolites being directed to the mitochondria to support cytokine production and other effector functions. Differential expression of various glycolysis enzymes supports these differences in CD4+ and CD8+ T-cells and could allow CD4+ T-cells to maintain an elevated level of glycolysis. Here, we did not consider whether an alternative fuel switch, such as glutamine utilization as reported in murine CD4+ and CD8+ T-cells, occurs (11, 45). These differential effects of metabolic inhibition on cytokine production could provide targets for the control of inflammatory disease.

Immediate glycolytic switching was also shown using human CD4+ and CD8+ T-cell clones when activated with either the non-specific stimulus anti-CD3/CD28 or in an antigen-specific manner. ECAR and OCR were increased with natural peptide stimulation in comparison to anti-CD3/CD28. Further to this, we studied the ILA1 TCR, which binds with a range of affinities to altered peptides in order to determine whether TCR binding affinity could tune T-cell metabolism. These experiments demonstrated a clear relationship between TCR affinity and metabolic response, with the strongest ILA1 TCR affinity interaction for the 3G peptide inducing the greatest glycolytic change and the weakest, 8E, inducing the smallest glycolytic change. These findings were also consistent when oxygen consumption

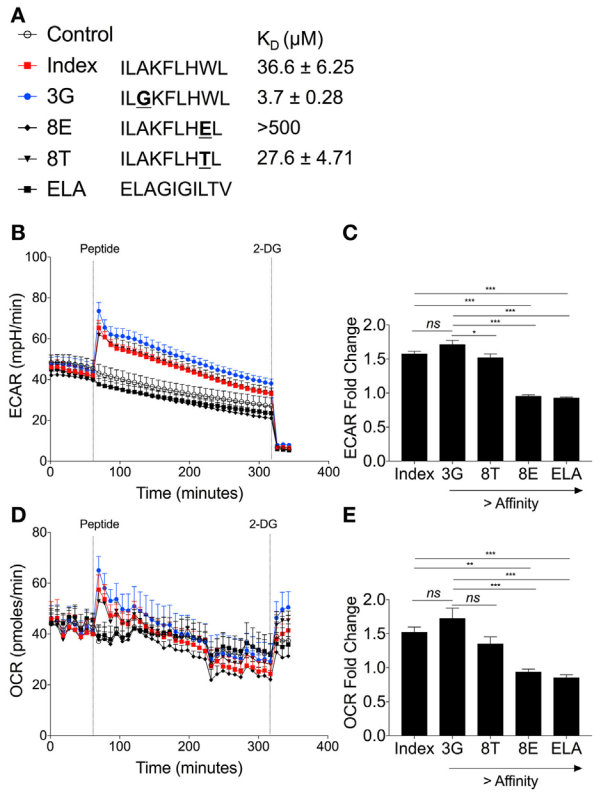

FIGURE 7 | Peptide binding affinity affects underlying metabolic changes in ILA1 CD8⁺ T-cell clones. **(A)** Sequence details and binding affinities of native index peptide ILAKFLHWL of ubiquitous tumor-associated antigen human telomerase reverse transcriptase ($hTERT_{540–548}$) with peptide variants IL**G**KFLHWL (3G), ILAKFLH**T**L (8T) and ILAKFLH**E**L (8E). Negative control peptide ELAGIGILTV (ELA). **(B)** Extracellular acidification rate (ECAR) analysis of ILA1 CD8⁺ T-cell clones stimulated with native index peptide, 3G, 8E, 8T and negative control ELA (all 10 μM). Final injection of 2-DG (100 mM). **(C)** Fold change of index peptide, 3G, 8T, 8E, and ELA. Fold ECAR change calculated with use of single measurement prior to peptide injection and single measurement after peptide injection. **(D)** Oxygen consumption rate (OCR) analysis of ILA1 stimulated with index peptide, 3G, 8E, 8T, and negative control ELA (all 10 μM) including **(E)** fold OCR change as calculated above. Data are representative of 6–10 technical repeats comprised of three independent experiments. Data expressed as mean + SEM; $^{*}p \leq 0.05$, $^{***}p \leq 0.001$.

was investigated. This observation supports previous studies where strength of interaction between TCR and specific pHLA controls murine T-cell responses (46). Fine-tuning the ability of the HLA/peptide to promote post-TCR metabolic changes has implications for therapeutic manipulation of T-cells in cancer and for vaccination (47, 48).

The clinical importance and therapeutic potential of immunometabolism is emerging with modulation of glycolysis and mitochondrial respiration used increasingly to alter cell and disease phenotypes. This includes systemic lupus erythematosus where dual treatment of 2-DG and metformin normalizes CD4⁺ T-cell metabolism, and cancer where microRNA is being used to target glycolysis (49–51). There has also been considerable attention to the use of metformin alongside 2-DG and 6-diazo-5-oxo-L-norleucine (inhibitor of glutaminolysis) in a triple therapy strategy to prolong allograft survival by suppressing autologous T-cell rejection (42). Collectively, our findings illustrate differences in the metabolic activity of CD4⁺ and CD8⁺ T-cells and that TCR-pHLA affinity governs the underlying glycolytic switch and thus T-cell activation status. These findings aid our understanding of metabolically linked T-cell activation thresholds and could potentially improve vaccination strategies *via* the understanding of metabolic profiles during immune cell activation.

ETHICS STATEMENT

All subjects gave written informed consent in accordance with the Declaration of Helsinki. Human blood samples were collected with informed written consent and ethical approval was obtained from Wales Research Ethics Committee 6 (13/WA/0190).

AUTHOR CONTRIBUTIONS

NJ performed the majority of experiments; JC, GD, AJS, SP, and SG performed experiments and provided intellectual discussion. AKS contributed reagents. NF, DC, NJ, and CT designed the experiments. NJ, DC, CT, and NF wrote the manuscript. All authors critically revised and approved the manuscript.

ACKNOWLEDGMENTS

The authors thank staff in the Joint Clinical Research Facility for phlebotomy, S. E. Owens and D. O. F. Skibinski for technical assistance, and all blood donors. AKS is a Wellcome Trust Senior Investigator. DC is a Wellcome Trust Research Career Development Fellow (WT095767). This work was supported with grants awarded by Saint David's Medical Foundation (SDMF) and Life Sciences Research Network Wales (NRN). SP was supported by a Wellcome Trust Vacation Scholarship.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fimmu.2017.01516/full#supplementary-material.

REFERENCES

1. Zloza A, Al-Harthi L. Multiple populations of T lymphocytes are distinguished by the level of CD4 and CD8 coexpression and require individual consideration. *J Leukoc Biol* (2006) 79:4–6. doi:10.1189/jlb.0805455
2. Ma EH, Bantug G, Griss T, Condotta S, Johnson RM, Samborska B, et al. Serine Is an essential metabolite for effector T cell expansion. *Cell Metab* (2017) 25:345–57. doi:10.1016/j.cmet.2016.12.011
3. Ron-Harel N, Santos D, Ghergurovich JM, Sage PT, Reddy A, Lovitch SB, et al. Mitochondrial biogenesis and proteome remodeling promote one-carbon metabolism for T cell activation. *Cell Metab* (2016) 24:104–17. doi:10.1016/j.cmet.2016.06.007
4. Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: beyond ATP. *Nat Rev Immunol* (2017) 17(10):608–20. doi:10.1038/nri.2017.66
5. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. *Nat Rev Immunol* (2016). doi:10.1038/nri.2016.70

6. O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. *Immunity* (2014) 41:75–88. doi:10.1016/j.immuni.2014.06.005
7. Gubser PM, Bantug GR, Razik L, Fischer M, Dimeloe S, Hoenger G, et al. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat Immunol* (2013) 14:1064–72. doi:10.1038/ni.2687
8. Pollizzi KN, Patel CH, Sun IH, Oh MH, Waickman AT, Wen J, et al. mTORC1 and mTORC2 selectively regulate CD8(+) T cell differentiation. *J Clin Invest* (2015) 125:2090–108. doi:10.1172/JCI77746
9. Cui G, Staron MM, Gray SM, Ho PC, Amezquita RA, Wu J, et al. IL-7-induced glycerol transport and TAG synthesis promotes memory CD8+ T cell longevity. *Cell* (2015) 161:750–61. doi:10.1016/j.cell.2015.03.021
10. Henson SM, Lanna A, Riddell NE, Franzese O, Macaulay R, Griffiths SJ, et al. p38 signaling inhibits mTORC1-independent autophagy in senescent human CD8(+) T cells. *J Clin Invest* (2014) 124:4004–16. doi:10.1172/JCI75051
11. Cao Y, Rathmell JC, Macintyre AN. Metabolic reprogramming towards aerobic glycolysis correlates with greater proliferative ability and resistance to metabolic inhibition in CD8 versus CD4 T cells. *PLoS One* (2014) 9:e104104. doi:10.1371/journal.pone.0104104
12. Phan AT, Doedens AL, Palazon A, Tyrakis PA, Cheung KP, Johnson RS, et al. Constitutive glycolytic metabolism supports CD8+ T cell effector memory differentiation during viral infection. *Immunity* (2016) 45:1024–37. doi:10.1016/j.immuni.2016.10.017
13. Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, et al. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab* (2014) 20:61–72. doi:10.1016/j.cmet.2014.05.004
14. Cammann C, Rath A, Reichl U, Lingel H, Brunner-Weinzierl M, Simeoni L, et al. Early changes in the metabolic profile of activated CD8(+) T cells. *BMC Cell Biol* (2016) 17:28. doi:10.1186/s12860-016-0104-x
15. Cretenet G, Clerc I, Matias M, Loisel S, Craveiro M, Oburoglu L, et al. Cell surface Glut1 levels distinguish human CD4 and CD8 T lymphocyte subsets with distinct effector functions. *Sci Rep* (2016) 6:24129. doi:10.1038/srep24129
16. Verbist KC, Guy CS, Milasta S, Liedmann S, Kamiński MM, Wang R, et al. Metabolic maintenance of cell asymmetry following division in activated T lymphocytes. *Nature* (2016) 532:389–93. doi:10.1038/nature17442
17. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* (2012) 36:68–78. doi:10.1016/j.immuni.2011.12.007
18. Wahl DR, Byersdorfer CA, Ferrara JL, Opipari AW Jr, Glick GD. Distinct metabolic programs in activated T cells: opportunities for selective immunomodulation. *Immunol Rev* (2012) 249:104–15. doi:10.1111/j.1600-065X.2012.01148.x
19. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1 alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208:1367–76. doi:10.1084/jem.20110278
20. Laugel B, van den Berg HA, Gostick E, Cole DK, Wooldridge L, Boulter J, et al. Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. *J Biol Chem* (2007) 282:23799–810. doi:10.1074/jbc.M700976200
21. Huang J, Zeng X, Sigal N, Lund PJ, Su LF, Huang H, et al. Detection, phenotyping, and quantification of antigen-specific T cells using a peptide-MHC dodecamer. *Proc Natl Acad Sci U S A* (2016) 113:E1890–7. doi:10.1073/pnas.1602488113
22. Tan MP, Gerry AB, Brewer JE, Melchiori L, Bridgeman JS, Bennett AD, et al. T cell receptor binding affinity governs the functional profile of cancer-specific CD8+ T cells. *Clin Exp Immunol* (2015) 180:255–70. doi:10.1111/cei.12570
23. Tan MP, Dolton GM, Gerry AB, Brewer JE, Bennett AD, Pumphrey NJ, et al. HLA class I-redirected anti-tumour CD4+ T-cells require a higher TCR binding affinity for optimal activity than CD8+ T-cells. *Clin Exp Immunol* (2017) 187(1):124–37. doi:10.1111/cei.12828
24. Acuto O, Michel F. CD28-mediated co-stimulation: a quantitative support for TCR signalling. *Nat Rev Immunol* (2003) 3:939–51. doi:10.1038/nri1248
25. Fracchia KM, Pai CY, Walsh CM. Modulation of T cell metabolism and function through calcium signaling. *Front Immunol* (2013) 4:324. doi:10.3389/fimmu.2013.00324
26. Puleston DJ, Villa M, Pearce EL. Ancillary activity: beyond core metabolism in immune cells. *Cell Metab* (2017) 26:131–41. doi:10.1016/j.cmet.2017.06.019
27. Almeida L, Lochner M, Berod L, Sparwasser T. Metabolic pathways in T cell activation and lineage differentiation. *Semin Immunol* (2016) 28:514–24. doi:10.1016/j.smim.2016.10.009
28. Delmastro-Greenwood MM, Piganelli JD. Changing the energy of an immune response. *Am J Clin Exp Immunol* (2013) 2:30–54.
29. Man K, Miasari M, Shi W, Xin A, Henstridge DC, Preston S, et al. The transcription factor IRF4 is essential for TCR affinity-mediated metabolic programming and clonal expansion of T cells. *Nat Immunol* (2013) 14:1155–65. doi:10.1038/ni.2710
30. Holland CJ, Dolton G, Scurr M, Ladell K, Schauenburg AJ, Miners K, et al. Enhanced detection of antigen-specific CD4+ T cells using altered peptide flanking residue peptide-MHC class II MULTIMERS. *J Immunol* (2015) 195:5827–36. doi:10.4049/jimmunol.1402787
31. Purbhoo MA, Li Y, Sutton DH, Brewer JE, Gostick E, Bossi G, et al. The HLA A*0201-restricted hTERT(540-548) peptide is not detected on tumor cells by a CTL clone or a high-affinity T-cell receptor. *Mol Cancer Ther* (2007) 6:2081–91. doi:10.1158/1535-7163.MCT-07-0092
32. Theaker SM, Rius C, Greenshields-Watson A, Lloyd A, Trimby A, Fuller A, et al. T-cell libraries allow simple parallel generation of multiple peptide-specific human T-cell clones. *J Immunol Methods* (2016) 430:43–50. doi:10.1016/j.jim.2016.01.014
33. Jones N, Piasecka J, Bryant AH, Jones RH, Skibinski DO, Francis NJ, et al. Bioenergetic analysis of human peripheral blood mononuclear cells. *Clin Exp Immunol* (2015) 182:69–80. doi:10.1111/cei.12662
34. Marko AJ, Miller RA, Kelman A, Frauwirth KA. Induction of glucose metabolism in stimulated T lymphocytes is regulated by mitogen-activated protein kinase signaling. *PLoS One* (2010) 5:e15425. doi:10.1371/journal.pone.0015425
35. Ganapathy-Kanniappan S, Geschwind JF. Tumor glycolysis as a target for cancer therapy: progress and prospects. *Mol Cancer* (2013) 12:152. doi:10.1186/1476-4598-12-152
36. Shikhman AR, Brinson DC, Valbracht J, Lotz MK. Cytokine regulation of facilitated glucose transport in human articular chondrocytes. *J Immunol* (2001) 167:7001–8. doi:10.4049/jimmunol.167.12.7001
37. Zhao FQ, Keating AF. Functional properties and genomics of glucose transporters. *Curr Genomics* (2007) 8:113–28. doi:10.2174/138920207780368187
38. Hresko RC, Hruz PW. HIV protease inhibitors act as competitive inhibitors of the cytoplasmic glucose binding site of GLUTs with differing affinities for GLUT1 and GLUT4. *PLoS One* (2011) 6:e25237. doi:10.1371/journal.pone.0025237
39. Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vázquez G, Yurchenko E, et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* (2015) 42:41–54. doi:10.1016/j.immuni.2014.12.030
40. Loreni F, Thomas G, Amaldi F. Transcription inhibitors stimulate translation of 5' TOP mRNAs through activation of S6 kinase and the mTOR/FRAP signalling pathway. *Eur J Biochem* (2000) 267:6594–601. doi:10.1046/j.1432-1327.2000.01753.x
41. Nguyen HD, Chatterjee S, Haarberg KM, Wu Y, Bastian D, Heinrichs J, et al. Metabolic reprogramming of alloantigen-activated T cells after hematopoietic cell transplantation. *J Clin Invest* (2016) 126:1337–52. doi:10.1172/JCI82587
42. Lee CF, Lo YC, Cheng CH, Furtmüller GJ, Oh B, Andrade-Oliveira V, et al. Preventing allograft rejection by targeting immune metabolism. *Cell Rep* (2015) 13:760–70. doi:10.1016/j.celrep.2015.09.036
43. Keppel MP, Saucier N, Mah AY, Vogel TP, Cooper MA. Activation-specific metabolic requirements for NK cell IFN-gamma production. *J Immunol* (2015) 194:1954–62. doi:10.4049/jimmunol.1402099
44. Dimeloe S, Mehling M, Frick C, Loeliger J, Bantug GR, Sauder U, et al. The immune-metabolic basis of effector memory CD4+ T cell function under hypoxic conditions. *J Immunol* (2016) 196:106–14. doi:10.4049/jimmunol.1501766
45. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lym-

phocyte activation. *Immunity* (2011) 35:871–82. doi:10.1016/j.immuni.2011.09.021
46. Zehn D, Lee SY, Bevan MJ. Complete but curtailed T-cell response to very low-affinity antigen. *Nature* (2009) 458:211–4. doi:10.1038/nature07657
47. Buhrman JD, Slansky JE. Improving T cell responses to modified peptides in tumor vaccines. *Immunol Res* (2013) 55:34–47. doi:10.1007/s12026-012-8348-9
48. Comber JD, Philip R. MHC class I antigen presentation and implications for developing a new generation of therapeutic vaccines. *Ther Adv Vaccines* (2014) 2:77–89. doi:10.1177/2051013614525375
49. Yin Y, Choi SC, Xu Z, Perry DJ, Seay H, Croker BP, et al. Normalization of CD4+ T cell metabolism reverses lupus. *Sci Transl Med* (2015) 7:274ra218. doi:10.1126/scitranslmed.aaa0835
50. Weinberg SE, Chandel NS. Targeting mitochondria metabolism for cancer therapy. *Nat Chem Biol* (2015) 11:9–15. doi:10.1038/nchembio.1712
51. Zhao E, Maj T, Kryczek I, Li W, Wu K, Zhao L, et al. Cancer mediates effector T cell dysfunction by targeting microRNAs and EZH2 via glycolysis restriction. *Nat Immunol* (2016) 17:95–103. doi:10.1038/ni.3313

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Immunology

REVIEW
published: 21 July 2017
doi: 10.3389/fimmu.2017.00837

Similarities in the Metabolic Reprogramming of Immune System and Endothelium

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Cellular metabolism has been known for its role in bioenergetics. In recent years, much light has been shed on the reprogrammable cellular metabolism underlying many vital cellular processes, such as cell activation, proliferation, and differentiation. Metabolic reprogramming in immune and endothelial cells (ECs) is being studied extensively. These cell compartments are implicated in inflammation and pathogenesis of many diseases but their similarities in metabolic reprogramming have not been analyzed in detail. One of the most notable metabolic reprogramming is the Warburg-like effect, famously described as one of the hallmarks of cancer cells. Immune cells and ECs can display this phenotype that is characterized by a metabolic switch favoring glycolysis over oxidative phosphorylation (OXPHOS) in aerobic conditions. Though energy-inefficient, aerobic glycolysis confers many benefits to the respiring cells ranging from higher rate of adenosine triphosphate production to maintaining redox homeostasis. Chemical and biological regulators either promote or perturb this effect. In this review, nitric oxide, hypoxia-inducible factor, and adenosine monophosphate-activated protein kinase have been discussed for their common involvement in metabolic reprogramming of both systems. From *in vitro* and animal studies, various discrepancies exist regarding the effects of those regulators on metabolic switch. However, it is generally accepted that glycolysis favors inflammatory reactions while OXPHOS favors anti-inflammatory processes. The reasons for such observation are currently subject of intense studies and not completely understood. Finally, metabolic reprogramming in immune cells and ECs does not limit to the physiological state in health but can also be observed in pathological states, such as atherosclerosis and cancer. These new insights provide us with a better understanding of the similarities in metabolic reprogramming across a number of cell types, which could pave the way for future research and possible metabolic-based therapeutics.

Keywords: metabolism, metabolic reprogramming, glycolysis, oxidative phosphorylation, macrophage, T lymphocyte, endothelial cell

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Immunological Tolerance and Regulation, a section of the journal Frontiers in Immunology

Received: *07 March 2017*
Accepted: *03 July 2017*
Published: *21 July 2017*

Citation:
Tang C-Y and Mauro C (2017) Similarities in the Metabolic Reprogramming of Immune System and Endothelium. Front. Immunol. 8:837. doi: 10.3389/fimmu.2017.00837

INTRODUCTION

Cellular metabolism has been sidelined for many years and it was only seen as the series of sequential pathways involved in converting fuel sources, such as glucose, fatty acids, ketones, and amino acids to generate packets of cellular energy in the form of adenosine triphosphate (ATP). At its infancy, scientists found it difficult to convince the scientific community that cellular metabolism and its

by-products might have wider implications on inflammation and disease pathogenesis (1). In 1997, Shim et al. conducted a study using representational difference analysis and found that lactate dehydrogenase (LDH)-A induction by the oncogene c-myc leads to overproduction of lactate (2). This finding provides a molecular explanation to the metabolism of tumor cells characterized by glucose overutilization and lactate overproduction in normal oxygen conditions, also known as the Warburg effect. This reprogramming of cellular energetics in cancer cells is described as one of the ten hallmarks of cancer by Douglas Hanahan and Robert A. Weinberg in 2011 (3). With regard to lactate, it was recently found that the high levels of lactate present in the inflammatory microenvironment reduce the motility of T cells, hence serving an active retention mechanism and lead to the induction of T helper (T_H)17 responses, which suggests that lactate is not merely a by-product of cellular metabolism, but it has a role in determining specific immune cell responses (4). There is an increasing awareness that many fundamental cellular processes, such as cell differentiation and proliferation have their distinct but reprogrammable metabolic requirements, which many experts would coin as the "renaissance of cellular metabolism."

At the heart of these exciting findings, there is a growing interest in explaining well-established disease pathogenesis in the light of metabolic reprogramming. Inflammation is a tissue response to insult by host defense mechanisms. Although the inflammation is the bodily response to rid invading pathogens and promote healing of injured tissues, often unresolved chronic inflammation or dysregulated autoimmunity can be the core of many disease processes. In inflammation, endothelial cells (ECs) are responsible for controlling immune cell trafficking. Cells of the immune system are recruited to the site of injury *via* dilated tissue vasculature. With the lower shear stress in dilated vessels, immune cells accumulate at the margins of the lumen in proximity to the endothelium. The immune cells roll along and adhere firmly on the endothelium before migrating across the vessel in a process known as diapedesis. There is a major interplay between immune cells and ECs to orchestrate these complex series of events. Extensive studies on the metabolic reprogramming in both cells types are underway but little or no comparisons between these closely related systems exist in the current literature. Hence, this review will analyze and compare the similarities in metabolic reprogramming of endothelial and immune cells in both health and disease state and further discuss about possible therapeutic targets.

Abbreviations: ACC, acetyl-carboxylase A carboxylase; ADMA, asymmetric dimethylarginine; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; ATP, adenosine triphosphate; CARKL, carbohydrate kinase-like protein; cGMP, cyclic guanosine monophosphate; ECs, endothelial cells; eNOS, endothelial nitric oxide synthase; ETC, electron transport chain; FAO, fatty acid oxidation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GLUT, glucose transporter; HIF, hypoxia-inducible factor; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; IDH, isocitrate dehydrogenase; IFN, interferon; iNOS, inducible nitric oxide synthase; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; OXPHOS, oxidative phosphorylation; PFK, phosphofructokinase-1; PPP, pentose phosphate pathway; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SDMA, symmetrical dimethylarginine; TAMs, tumor-associated macrophages; TCA, tricarboxylic acid; T_H, T helper; T_{reg}, regulatory T; VEGF, vascular endothelial growth factor.

METABOLIC CHARACTERISTICS OF MACROPHAGES, T CELLS, AND ECs

Macrophage Metabolism

Macrophages have been traditionally described by two differentiation pathways, which lead to the classical (M1) and the alternative (M2) phenotype (5). M1 and M2 phenotypes, however, are now known to belong to a spectrum of possible differentiation pathways alongside with numerous activation states with characteristic phenotypes. Recently, plaque-specific macrophages, such as M4, Mhem, and Mox, have been identified in atherosclerosis lesions (6–9). M2 macrophages can further be classified into M2a, M2b, M2c, and M2d subtypes (6, 10, 11). Xue et al. stimulated human macrophages with 28 different activation stimuli and analysis of the data has shown a spectrum of macrophage activation states which does not conform to the classical bipolar M1/M2 axis (12). Despite the existence of a myriad of macrophage subsets, the M1/M2 paradigm is still extensively used in the literature and is a useful framework for the purpose of our discussion pertaining to macrophage metabolism and function.

In general, M1 macrophages are pro-inflammatory and they are functionally important for clearance of pathogens. M1 macrophage metabolism is characterized by high glycolysis and relatively low oxidative phosphorylation (OXPHOS), high inducible nitric oxide synthase (iNOS) activity, and nitric oxide (NO) production (13). The tricarboxylic acid (TCA) cycle of M1 macrophages is discontinuous and it has two break-points at isocitrate dehydrogenase and succinate dehydrogenase (SDH) (14). This observation provides explanation for the upstream accumulation of citrate and succinate, both of which influence the polarity of macrophages. Succinate acts as a pro-inflammatory signaling molecule by stabilizing hypoxia-inducible factor (HIF), a transcription factor which upregulates the biosynthetic capacity of cells (15). Citrate is also a known pro-inflammatory signal. Citrate is involved in fatty acid and phospholipid biosynthesis that promotes the production of inflammatory prostaglandins (16). In the cytosol, the metabolism of citrate by ATP-citrate lyase generates nicotinamide adenine dinucleotide phosphate (NADPH) which is a required substrate for the synthesis of NO (17). Unlike naïve cell types, macrophages are terminally differentiated and they do not require energy for proliferation (18). Instead ATPs are used to sustain energy-demanding cellular activities, such as phagocytosis and secretory functions (18).

M2 macrophages, on the other hand, are involved in regenerative roles, such as tissue remodeling, repair, and healing. M2 macrophage metabolism is characterized by OXPHOS, fatty acid oxidation (FAO), and upregulated arginase 1 activity (13). Unlike M1 macrophages, the mitochondrial complexes in M2 macrophages are not occupied by NO and reactive oxygen species (ROS) and, hence, OXPHOS is sufficient to sustain the metabolic demand. M2 macrophages also express PFKFB1, an isoform of 6-phosphofructo-2-kinase capable of metabolizing the glycolytic activator fructose-2,6-bisphosphate (19).

As opposed to the upregulated pentose phosphate pathway (PPP) in M1 macrophage, PPP is suppressed in M2 macrophage by the expression of carbohydrate kinase-like protein (20). L-arginine metabolism *via* arginase produces ornithine, which is important for the synthesis of proline, a component of tissue collagen (21). Consequently, arginase activity might be driving the reparative function of M2 macrophages in tissue remodeling. Lastly, the predilection for OXPHOS and FAO in M2 macrophage is also driven by high adenosine monophosphate-activated protein kinase (AMPK) activity (22).

T Cell Metabolism

The role of T cells in the adaptive immune system is vast and they function by secreting lymphokines to induce immunomodulatory actions ($CD4^+$) or by promoting cytotoxicity ($CD8^+$). $CD4^+$ T cells bind to major histocompatibility complex (MHC) class II ligands on antigen-presenting cells, while the counterpart, $CD8^+$ T cells, bind to MHC class I ligands. There are several subtypes of $CD4^+$ T cell differentiation, including T_H1, T_H2, T_H17, T_H9, regulatory T (T_{reg}) cell, follicular helper T cell, type 1 T regulatory cell, and memory T cells (23–25).

Naïve T cells rely on FAO and OXPHOS for their energy production (26, 27). Upon encountering antigen-presenting cells, naïve T cells undergo activation and subsequently clonal expansion and differentiation. During this period, profound metabolic changes occur. Activated T cells upregulate aerobic glycolysis and the glycolytic branch reaction, PPP (28, 29). This is attained *via* glucose transporter (GLUT) 1 translocation to cell periphery and upregulation of glycolytic enzymes (28, 30). Glutamine metabolism is also enhanced which supplies products for the TCA cycle as well as promoting polyamine synthesis (29, 31).

Distinct metabolic pathways are required for differentiation of activated T cells. The T effector subsets, T_H1, T_H2, and T_H17, are known to prefer glycolysis even in aerobic conditions (32, 33). T_{reg} cells, on the other hand, mainly prefer FAO for energy generation and replicate at moderate levels as opposed to the profound amplification in cell proliferation seen in T effector cells (18, 33, 34). Metabolism of memory T cells is largely similar to that in naïve T cells, aside from the increased mitochondrial mass that is a preemptive measure to prepare for mitochondrial energy generation upon secondary antigen exposure (35).

EC Metabolism

Endothelial cells can be classified into three different subtypes based on their morphology and role in angiogenesis: the highly branched tip cells are primarily migratory and navigate the direction of the vessel sprout; the stalk cells have less branches but are highly proliferative and elongate the sprout during extension; and lastly, phalanx cells, commonly recognized by their cobblestone appearance, are quiescent and line the mature blood vessels (36). In maturity, majority of the blood vessels are relatively quiescent but they retain the capacity to respond to angiopoietin in physiological and pathological states to generate new blood vessels from existing vasculature, in a process known as angiogenesis (37). This sequential and highly coordinated process can be accomplished by either sprouting or non-sprouting angiogenesis (37, 38).

Owing to the low mitochondrial content, ECs rely almost exclusively on glycolysis for energy generation (39). On vascular endothelial growth factor (VEGF) stimulation, the rate of glycolysis is doubled and GLUT 1 expression is upregulated to meet the metabolic demand of cell migration during angiogenesis (40, 41). PFKFB3, a different isoform to PFKFB1 expressed in M2 macrophages, is also involved in the metabolic regulation of ECs. PFKFB3 stimulates glycolysis by synthesizing large quantity of fructose-2,6-bisphosphate, which is a potent glycolytic stimulator (39). Glycolysis is shown to modulate the phenotypic expression of ECs. Increased activity of PFKFB3 could override genetically predestined stalk cells into metabolically active tip cells to further enhance ECs sprouting (39). Conversely, extracellular environment such as laminar shear stress exerted by blood flow lowers PFKFB3 activities and the associated metabolic changes that resultantly sustain cellular quiescence (42). Apart from angiogenesis, ECs are involved in a wide range of vascular homeostatic functions such as vasodilation and the anti-proliferating effect on vascular smooth muscle cells through the action of NO generated by endothelial nitric oxide synthase (eNOS) (43). As in the case with M2 macrophages, AMPK in ECs senses glucose deprivation and promotes the inhibition of acetyl-coenzyme A carboxylase (ACC), resulting in increased FAO (44). Schoors et al. investigated FAO in ECs and found it essential for ECs proliferation during vessel sprouting, unlike glycolysis that regulates both proliferation and migration (45). The targeted control of FAO on ECs proliferation could potentially be a therapeutic option for pathological angiogenesis (**Figure 1**).

METABOLIC REPROGRAMMING IN THE IMMUNE CELLS AND ECs

Cells derive energy from catabolism of the three major biomass sources: glucose, fatty acids, and amino acids mainly through glycolysis and OXPHOS. In theory, a molecule of glucose can yield up to approximately 38 molecules of ATP of which, 2 from glycolysis, 2 from the TCA cycle, and 34 from OXPHOS. Based on the number of moles of ATP produced per mole of glucose, OXPHOS would be the ideal and most efficient bioenergetic pathway. However, glycolysis is the preferred one in cells with high metabolic demands as it confers many benefits. The demand for an expensive amount of energy from metabolically active processes can be met by aerobic glycolysis, which is a faster bioenergetic pathway as compared to OXPHOS, granted that there is a steady stream of glucose supply (46). The metabolic intermediates of glycolysis can also be shunted to branch reactions and synthetic pathways to generate macromolecules and maintain redox homeostasis needed for cell proliferation, migration, and maintaining physiological functions (17, 41, 47). M1-activated macrophages rely on glycolysis for ATP production, while the mitochondrial machinery has been shunted to ROS production necessary for bactericidal activity (48). The enhanced glycolytic branch reaction PPP also augments the production of NADPH required for the production of ROS (49). In $CD4^+$ T cells, it was shown that OXPHOS and aerobic glycolysis can interchangeably be used to sustain cell proliferation

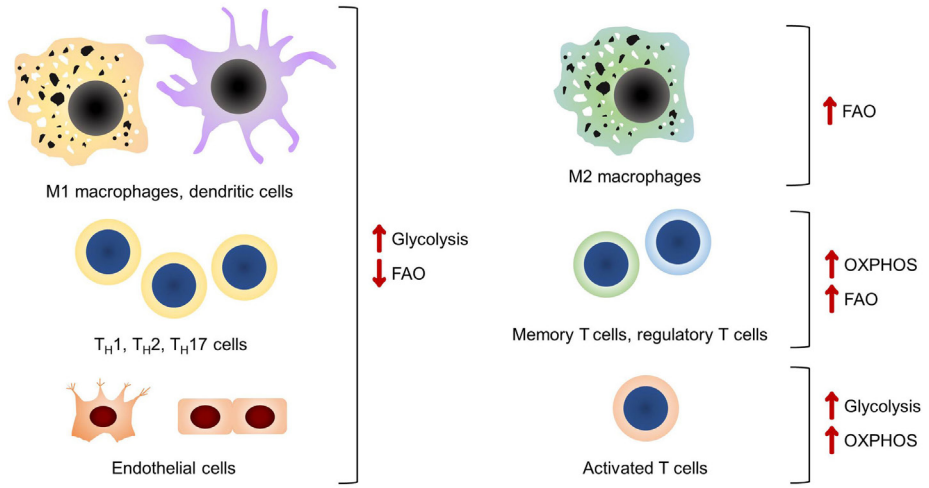

FIGURE 1 | A summary of the main bioenergetic pathways in each of the cell types discussed. It should be noted that glycolysis is the preferred bioenergetic pathway in classical pro-inflammatory cells and endothelial cells.

and survival, but only aerobic glycolysis can adequately lead to the attainment of full effector function (26, 31). ECs invariably utilize glycolysis as the main bioenergetic process and several advantages of aerobic glycolysis have been suggested. Despite the proximity to readily available oxygen sources, glycolysis reduces the need for oxygen and this allows maximum oxygen concentration carried in the blood to tissues perfused by the blood vessels (18). In neovascularization, ECs proliferate in order to perfuse hypoxic tissues. Low oxygen level in hypoxic environment limits the effectiveness of OXPHOS in generating ATP and, hence, glycolysis is preferred (18). Reaction–diffusion models show that the decrease in partial pressure of oxygen is less than the decrease in glucose concentration over distance (18, 50). This suggests that ECs can still undergo anaerobic glycolysis as tip cells get further away from blood vessels. In this review, NO, HIF, and AMPK will be discussed in more detail owing to their common involvement in the regulation of the Warburg-like effect and other forms of bioenergetics seen in all three cell types. Other potent metabolic regulators, such as mTOR, are not within the scope of this review as there is limited literature of its effect on ECs (18, 41). The main similarities and differences in the metabolic pathways of macrophages, T cells, and ECs are summarized in **Table 1**.

Nitric Oxide

Synergistic stimulation of macrophages by lipopolysaccharide (LPS) and interferon (IFN) has been shown to increase the expression of iNOS and NO production and resulting in a preferential switch to glycolysis (17, 51, 52). One of the possible mechanisms is *via* the nitrosylating effect of NO on iron-sulfur proteins of the electron transport chain complex leading to inhibition of cell respiration (75, 76). iNOS-deficient dendritic cells are unresponsive to LPS stimulation but pharmacological reintroduction of exogenous NO results in a metabolic switch from OXPHOS to aerobic glycolysis (75). Several studies have shown that NO can permeate cell membranes to exert its effect on cellular metabolism. Recent studies have shown that low plasma membrane cholesterol and functional connexin-based channels facilitate the transport of NO across cellular membranes (77, 78). These observations suggest that NO generated by ECs could exert its effect on immune cells in a paracrine fashion. In terms of cellular production of NO, the substrate L-arginine can be shunted into two metabolic processes, either the iNOS or the arginase pathway. iNOS metabolizes L-arginine to produce NO and citrulline while arginase, on the other hand, controls the ornithine cycle by catalyzing the formation of ornithine and urea. Both enzymes compete for the same common substrate and, hence, NO production and its effect on metabolism are controlled by the expression of these enzymes. Colegio et al. found that lactate generated from aerobic glycolysis could induce the expression of arginase 1 and M2-like phenotype in tumor-associated macrophages (TAMs) *via* the HIF pathway (79). This finding has implications for cancer cell survival and is further discussed in a later section.

A study on murine thymus shows that the nitrosylating effect of NO is also seen in other enzymes regulating the activity of glycolysis, the TCA cycle, and fatty acid metabolism (80). NO has a toxic effect on T cells, consistent with a role as self-limiting pathway in T_H1 cell-mediated responses *in vivo via* IFN-γ (56, 81). In an experimental study on autoimmune disorder, IFN-γ exerted a protective effect *via* activation of iNOS and production of NO (82). NO in turn triggered T cell apoptosis, thus providing some degree of explanation to the self-limiting effector response of T_H1 cells (55). Addition of L-arginine to $CD4^+$ T cells was shown to induce several metabolic changes, including increased gluconeogenesis, decreased GLUTs and glycolytic enzymes, and a metabolic switch to OXPHOS (53). A possible explanation is the upregulation of the serine biosynthetic pathway which fuels the TCA cycle and subsequently OXPHOS and results in a "reverse" Warburg-like effect (53, 54). T cells have also been shown to display a twofold decrease in glycolytic rate when iNOS is knocked-out (83). Alongside the apparent anti-inflammatory effect of NO on T cells, it was shown

TABLE 1 | The similarities and differences in metabolic reprogramming mediated by nitric oxide (NO), hypoxia-inducible factor (HIF), and adenosine monophosphate-activated protein kinase (AMPK) in macrophages, T lymphocytes and endothelial cells (ECs).

	Macrophages	T lymphocytes	ECs
NO	↑ Glycolysis, ↓ oxidative phosphorylation (OXPHOS) (17, 51, 52)	↓ Glycolysis (53) ↑ OXPHOS (53, 54) ↑ Effector T cell apoptosis (55) ↑ Regulatory T (T_{reg}) population (56)	↑ Glycolysis, ↓ OXPHOS (57, 58) ↓ Glyceraldehyde-3-phosphate dehydrogenase activity (59)
HIF	↑ Glycolysis (60–63)	↑ Glycolysis (29, 64) ↑ T_h17 differentiation, ↓ T_{reg} population (65)	↑ Glycolysis (66, 67)
AMPK	↑ Fatty acid oxidation (FAO), ↓ inflammatory response (68, 69) ↓ M1 macrophage polarization (70) ↑ Glycolysis in monocytes (71)	↓ Glycolysis (72) ↑ OXPHOS (73)	↓ Glycolysis (74) ↑ FAO (74)

On a whole, the metabolic changes induced by these metabolic signals are similar between the immune and endothelial systems. However, NO suppresses glycolysis in T cells, while AMPK upregulates glycolysis in monocytes, both of which do not conform to the general framework.

that NO induces proliferation of functional T_{reg} cell population (56). The metabolic mechanism behind the increased proliferation of T_{reg} cells by NO is not well understood but it may represent a key regulation point of host immune function.

Nitric oxide is arguably the most important synthetic product of ECs as it has a role in modulating vasodilation and inflammation. eNOS is functionally similar to iNOS and it metabolizes L-arginine to citrulline and NO. Aside from its local effect on vascular smooth muscle cells, NO is also an autocrine signaling molecule and exhibits effects on ECs metabolism. In the angiogenic state, the metabolic demand of ECs is greatly increased and this is met by increasing the rate of glycolysis (39–41). Using an NO-donor, it was shown that glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a glycolytic enzyme in bovine aortic ECs is inhibited in a dose-dependent manner but the effect is reversed to near base-line upon removal of the NO-donor, which implies that GAPDH is a target for NO-mediated oxidative stress (59). The same group of researchers conducted another study and found that NO stimulates glycolysis and reversibly impairs mitochondrial reserve capacity (57). This set of data opposes the earlier finding on NO-mediated inhibition of GAPDH, leading to the conclusion that inhibition of this enzyme is not a sensitive biological effect of NO (57). Other studies have also been in support of the action of NO in promoting glycolysis in ECs. Paik et al. treated human umbilical vein ECs with sodium nitroprusside and diethylenetriamine, both of which are NO donors and found that ^{18}F-fluorodeoxyglucose uptake was increased alongside with GLUT 1 expression and hexokinase activity (58). These changes are surrogate markers of increased glycolytic flux in ECs. Despite not many studies support a role for NO-mediated effects *via* rewiring of EC metabolism, NO produced from eNOS is theoretically capable of interacting with the immune system and, hence, it represents a topic of interest for further investigation.

Hypoxia-Inducible Factor

In vitro and *in vivo* studies show that glycolytic flux is upregulated by both M1 activation and hypoxic condition (60). Cellular adaptation to hypoxia is mediated by the transcription factor HIF (84). It is made of two subunits, HIF-1β and HIF-1α of which the latter is degraded by oxygen-dependent mechanisms (84). HIF-1α expression is induced by T_H1 cytokine stimulation and is known to promote the metabolic switch from OXPHOS to glycolysis by regulating the activity of GLUT 1 and several enzymes in the glycolytic pathway (61). HIF-1α also increases LDH and reduces pyruvate dehydrogenase activity, collectively shunting the production of acetyl-CoA for the TCA cycle to the production of lactate inducing the Warburg-like effect in macrophages (62, 63). Interestingly, Takeda et al. found that in T_H1 cytokine-induced M1 macrophages, HIF-1α induces the expression of iNOS but not arginase, while the converse is true for HIF-2α in M2 macrophages (85).

Wang et al. found that glycolysis, glutamine metabolism, and FAO were not affected by short-term (24 h) deletion of HIF-1α but mild glycolysis impairment was shown after 72 h with moderate downregulation of LDH-A and hexokinase-2 gene expression (29). Despite the data suggesting a limited role of HIF-1α in promoting the reprogramming of T cell metabolism, HIF-1α-deficient CD4$^+$ T cells cultured in T_H17-stimulating conditions show lower expression of genes encoding for GLUTs, LDH-A, and other glycolytic enzymes (64). Interestingly, HIF has also been shown to regulate the T_H17/T_{reg} balance by favoring T_H17 differentiation and suppressing T_{reg} population (65). This is also apparent in T_H17-mediated autoimmune disease as *in vivo* studies showed that mice with HIF-1α-knock out T cells were resistant to experimental autoimmune encephalitis (65). It is known that T_H17 cells favor glycolysis, while T_{reg} cells rely on OXPHOS and FAO for energy generation. This experiment provides indirect evidence for a possible link between HIF and the Warburg-like effect seen in T cells.

Similar metabolic adaptation to hypoxia is also seen in ECs. An experiment on bovine aorta and human umbilical ECs shows that, in hypoxic conditions, ECs increase glucose uptake and lactate generation, both of which are surrogates for glycolysis activity (66). The team then inhibited OXPHOS under aerobic conditions and an upregulation of GLUTs in ECs was observed over several hours. The authors suggest that the lag period is observed as a result of a series of events involving mRNA transcription and protein translation of the GLUTs (66). Oswald et al. studied the effect of hypoxia on the metabolism of ECs from three different sources, namely umbilical, dermal, and aortic. The expression of HIF and VEGF mRNA as markers of experimental hypoxia was measured and they found that hypoxia is

associated with increased uptake of ^{18}F-fluorodeoxyglucose by ECs from all tissue sources (67). The authors concluded that the low oxygen tension stabilizes HIF-1α that is involved in the upregulation of GLUT-1 expression (67).

Adenosine Monophosphate-Activated Protein Kinase

Adenosine monophosphate-activated protein kinase is known as the metabolite-sensing kinase and is activated in conditions of low energy and oxygen reserve (17, 86). The breakdown product of ATP, adenosine monophosphate (AMP), and adenosine diphosphate (ADP) bind to AMPK making it more susceptible to activation by upstream kinases (17, 87). AMPK has been shown to play a role in regulating mitochondrial bioenergetic reactions. One proposed pathway is by directly upregulating mitochondrial biogenesis through the induction of its transcriptional coactivator, proliferator-activated receptor-γ-coactivator-1 that induces mitochondrial biogenesis and respiration (88, 89). AMPK also induces the activity of SDH in the TCA cycle that fuels OXPHOS (90). Another notable function of AMPK is the modulation of mTOR as one of its downstream targets. mTOR acts as an intracellular sensor to metabolic cues and directs the rate of cell growth and proliferation (91). Depletion of ATP activates AMPK that in turns inhibits mTOR and subsequent protein synthesis in order to conserve energy (92). Aside from that, AMPK also has a role in upregulating FAO by inhibiting ACC, a rate-limiting enzyme for the carboxylation reaction of acetyl-CoA to malonyl-CoA (93). However, the study outcomes on the importance of AMPK in FAO have not been always congruent. Recent evidence shows that skeletal muscles with AMPK kinase-dead mutant display ACC phosphorylation, reduction in malonyl-CoA, and FAO rate similar to those in controls (94). Nonetheless, these data are not retrieved from studies on immune cells or ECs but rather conducted in skeletal muscles for their abundance in mitochondria content.

Stimulation by M2 stimuli, such as IL-10 and TGF-β rapidly activates AMPK (70). Inhibition of AMPK by genetic deletion of its subunits leads to a heightened inflammatory response and reduction in FAO, a key bioenergetic pathway in M2 macrophages (68, 69). These observations are in concordance with the role of AMPK in opposing the polarization of M1-activated macrophages (70). In one occasion, AMPK has been shown to increase glycolysis in monocytes during hypoxia by activating the glycolytic stimulator, PFKFB3 (71). O'Neill and Hardie proposed that this observation could be explained as an ATP-generating function of AMPK by activated macrophages during hypoxia (22). Another interesting theory from Luo et al. based on their work on tumor cells describes that AMPK induces glycolysis and FAO in acute stress, whereas chronic AMPK stimulation dampens glycolysis *via* inhibition of mTOR and its action on p53 tumor suppressor protein, albeit the evidence for the latter is limited (95). The relevance of this dual effect of AMPK on the metabolism of macrophages requires further investigation.

Although T_H1, T_H2, and T_H17 subtypes of activated $CD4^+$ T cells adopt glycolysis as their main form of energy generation, T_{reg} cells, on the other hand, rely mainly on fatty acid metabolism (18, 33, 34). Metformin, a known inducer of AMPK, was shown to increase the number of T_{reg} cells in murine studies (33). To find out the direct role of AMPK on T_{reg} cells metabolism, genetic methods were employed and it was shown that knock out of the AMPKα1 subunit increases GLUT-1 expression and hexokinase activity (72). This resulted is a threefold increase in basal glycolytic rate observed in T-cells supporting the role of AMPK in hindering the switch to aerobic glycolysis (72). However, the cells did not display any polarization toward pro-inflammatory subtypes despite an increase in glycolysis (72). Mayer et al. found that T cells in AMPK-deficient mice displayed increased rate of cell death and reduced aerobic glycolysis as compared to wild-type T cells when mitochondrial respiration is artificially inhibited, which suggests the role of AMPK as a response mechanism to metabolic stress (96). T cell activation and cytokine production, on the other hand, remain intact in AMPK-deficient mice (96). Blagih et al. conducted another study by subjecting T cells to glucose-scarce environment, which inhibited glycolysis. They found that AMPK is essential for glutamine-dependent OXPHOS when glucose supply is limited, providing further evidence for the role of AMPK in T cell response during metabolic stress (73). Functionally, although AMPK-deficient T cells display full proliferative capacity when glucose supply is not a limiting factor, the same scenario is not true when glucose supply is low (73). *In vivo* experiments show that the T cell population was relatively smaller in AMPK-deficient mice as compared to wild-type controls (73). There was no difference observed in viral load but there was a reduction in bacterial clearance in the AMPK-deficient group (73). Taken together, these observations show that despite the AMPK-induced metabolic reprogramming seen in T cells, AMPK might not display an overt *in vitro* effect on T cell function, such as activation and differentiation. However, AMPK could be essential for T cell function in metabolic stress and *in vivo* environment where the cellular micromilieu is more variable.

Although the name suggests otherwise, AMP has not been shown to be responsible for the activation of AMPK in ECs. Alternative stimuli, such as the increase in ADP/ATP ratio, tumor suppressor gene product LKB1, shear stress exposure, and Ca^{2+}-elevating agonists, such as bradykinin and thrombin, have been proposed (87, 97–99). Similar to the observations seen in M1 macrophages and T_{reg} cells, AMPK activation was shown to reduce glucose uptake and glycolysis in ECs (74). ECs are known to generate up to 85% of their total ATP through glycolysis (39). Therefore, endothelial mitochondria have been proposed to serve other functions other than solely being the energy powerhouse of the ECs. The expression of mitochondrial antioxidant enzymes induced by AMPK has shown to confer protective benefit to ECs against oxidative stress (100). Apart from its effect on glucose metabolism, AMPK activation has been shown to increase FAO in human umbilical vein ECs (74). Specifically, palmitate oxidation is heightened from the activation of AMPK by bradykinin, suggesting that AMPK activation may mitigate lipotoxicity secondary to fatty acid accumulation in the initial stages of atherosclerosis (98) (**Figure 2**).

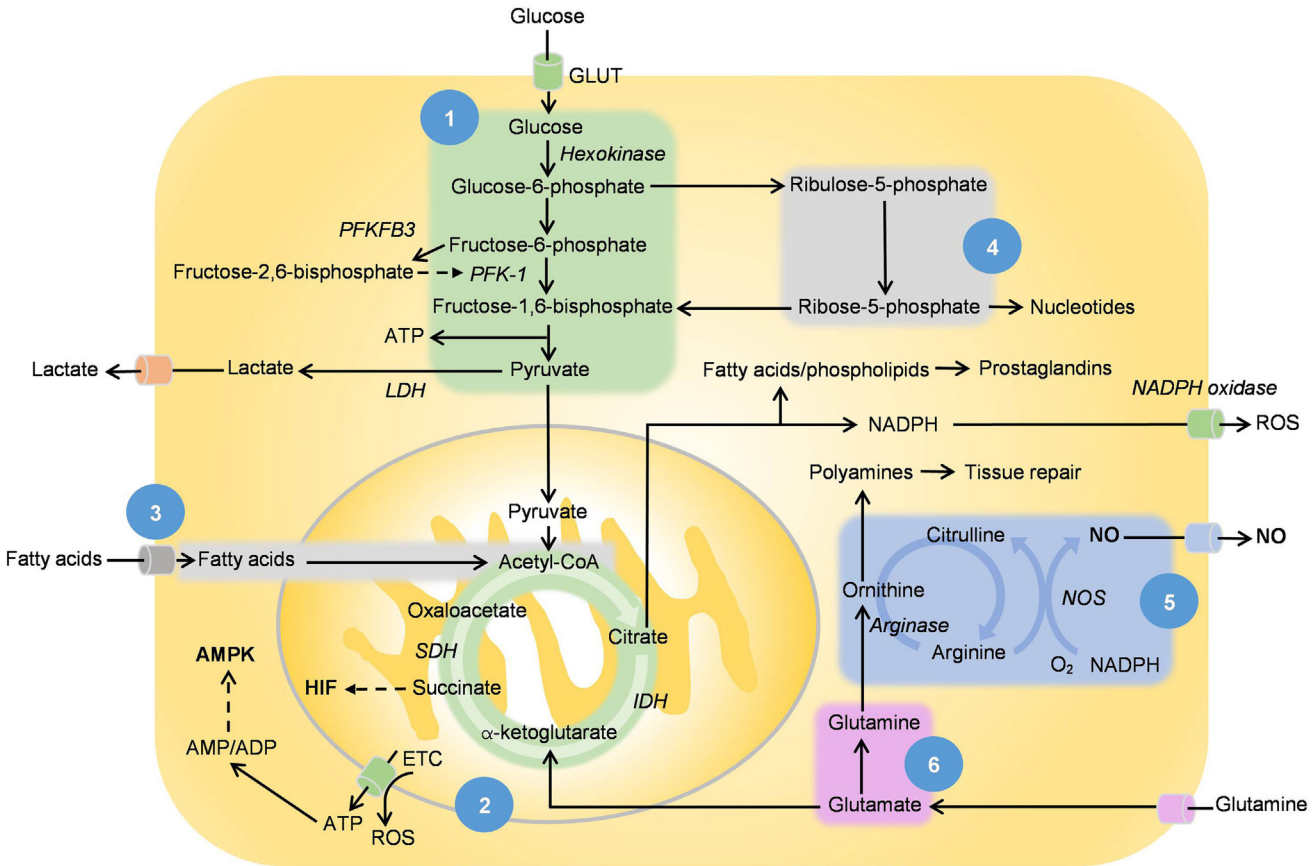

FIGURE 2 | [1] Glycolysis, [2] tricarboxylic acid (TCA) cycle and oxidative phosphorylation, [3] fatty acid oxidation, [4] pentose phosphate pathway, [5] ornithine cycle, and [6] glutamine metabolism. This figure shows the main metabolic pathways in relation with nitric oxide (NO), hypoxia-inducible factor (HIF), and adenosine monophosphate-activated protein kinase (AMPK). NO is produced by NOS with L-arginine as the substrate while succinate, the intermediate of the TCA cycle stabilizes and activates HIF. Adenosine triphosphates (ATPs) are hydrolyzed to adenosine diphosphate (ADP), while some are converted to adenosine monophosphate (AMP) *via* adenylyl cyclase. The increase in AMP/ADP:ATP ratio as well as other extracellular metabolic stressors activate AMPK. The effect of these mediators on metabolic reprogramming is listed in **Table 1**. PFKFB3 converts fructose-6-phosphate to fructose-2,6-bisphosphate, which in turn activates phosphofructokinase-1 and promotes the rate of glycolysis. Citrate metabolism produces acetyl-CoA, which is converted to malonyl-CoAs for fatty acid synthesis. Arachidonic acid and its derived inflammatory prostaglandins are produced from the same pathway. Arginase regulates the ornithine cycle, which is involved in the production of polyamines, a prominent feature of metabolism in M2 macrophages.

CELLULAR METABOLISM IN INFLAMMATION

Atherosclerosis from a Perspective of Cellular Metabolism

Despite being largely seen as a lifestyle disease, a large part of atherosclerosis stems from inflammation. The earliest evidence of atherosclerosis in a human's lifetime is fatty streaks, which are inflammatory lesions found in blood vessels, even in those of infants and young children. As described earlier, NO has several homeostatic functions in the circulatory system, including modulating vasodilation, inhibiting platelet aggregation, and smooth muscle proliferation, all of which if dysfunctional will result in atherosclerosis. In ECs, an imbalance of antiatherogenic NO versus its counterpart, the proatherogenic ROS such as superoxides, is often described in early atherosclerosis (101–103). eNOS uncoupling also occurs when L-arginine supply is limited, generating ROS instead of NO (104). On the other hand, administration of L-arginine over 13 weeks has also been shown to regress atherosclerotic lesions in rabbits with hypercholesterolemia, suggesting reversibility in atherosclerosis progression through the restoration of NO action (105). In a study, plasma level of L-arginine, asymmetric dimethylarginine (ADMA), and symmetrical dimethylarginine in 49 patients with hypercholesterolemia were compared with the controls and it was found that increased ADMA associates with impaired vasodilation and reduction in urinary nitrate (106). These effects were shown to be reversible upon L-arginine administration in a double-blinded, randomized, placebo-controlled cross over study and the researchers concluded that ADMA can serve as a novel risk factor for cardiovascular disease (106). It is postulated that ADMA is a competitive inhibitor of NOS, which at high levels results in reduction of NO synthesis (107). This competitive inhibition could then be overcome by increasing the plasma level of L-arginine.

The inflammatory process of atherosclerosis is dependent on the balance of pro-inflammatory and anti-inflammatory cells (108, 109). In early atherogenesis, T_H1 cytokines, such as IFN-γ, and lipoprotein, activate M1 macrophages that liberate more pro-inflammatory cytokines (110). M2 macrophages, on the other hand, were shown to counteract inflammation and promote healing (21). Regulation of this balance could be a potential therapeutic option for atherosclerosis. Some regulators such as peroxisome proliferator-activated receptor gamma coactivators 1β were shown to induce OXPHOS and FAO in macrophage population and to enhance the maturation of anti-inflammatory M2 macrophages (110). Induction of M2 macrophages can also be achieved *via* AMPK activation (111). Accumulation of M2 macrophages *in vivo* was found to decreases the size of atherosclerotic plaques in mice with hypercholesterolemia (111).

Although most T effector responses aggravate atherosclerosis, T_{reg} cells activity was shown to reduce the size of lesions and extent of inflammation (112, 113). In a murine study, injection of anti-CD3 antibody was shown to induce the T_{reg} cell population and promote regression of atherosclerotic lesions. This beneficial effect was abolished soon after injection of anti-CD25 antibody that depleted T_{reg} cells (114). Macrophages with increased expression of enzymes involved in the synthesis of retinoic acid, such as aldehyde dehydrogenase 1A2 and retinal dehydrogenase were shown to increase T_{reg} cell population (111). This is in tandem with the role of retinoic acid in supporting differentiation of T_{reg} cells from naïve T cells (115).

Patients with cardiovascular risks are classically treated with statins, a drug that inhibits HMG-CoA reductase, a rate-controlling enzyme of the mevalonate pathway that leads to the production of cholesterol. HMG-CoA reductase inhibition *via* statins, however, causes reduced immunosuppressive activity by T_{reg} cells, an effect that could limit the therapeutic effect of statins (116, 117). Restoration of T_{reg} cell function can be achieved through administration of mevalonate, the product of 3-hydroxy-3-methylglutaryl-CoA reductase enzymatic reaction (116, 117). The role of mevalonate as an adjunctive treatment for lipid-lowering statin regimen is also an interesting topic for clinical research. These discoveries are exciting as they open new ways of treating this extensively studied condition, which has plagued the humanity since the beginning of modernization.

The Survival Mechanisms of Cancer Cells

In neoplasm, growing tumors proliferate at an exceedingly fast rate, rendering the micromilieu deprived of glucose and amino acids essential for T cell function (118–120). This is in concordance with the importance of metabolic pathways in controlling cellular functions. T cells assume an effector status by upregulating glycolytic and anabolic activities through increased glucose uptake, glutamine oxidation, and oxygen consumption (27). The glucose-scarce micromilieu perturbs these energy-expensive processes and sends $CD4^+$ T cells into dormancy known as the anergy state and, subsequently, T cell dysfunction (119–121). $CD4^+$ T cells that have undergone metabolic inhibition and anergy fail to proliferate even upon re-challenging with costimulation, which is the signal required for T cells to achieve full effector status and maximal expression of cytokines (121). Furthermore, lactate accumulation secondary to tumor glycolysis from the Warburg effect leads to acidosis in the microenvironment (122). High levels of lactate have been shown to inhibit T cell motility and glycolysis *via* distinct transporters exhibited by $CD4^+$ and $CD8^+$ T cells (4). This inhibition coupled with HIF-induced upregulation of programmed cell death-ligand 1 grants tumor cells immunity against cytotoxic effect of T cells (119, 120, 123). In terms of NO, T cell cytotoxicity can be hindered as a result of peroxynitrite formation from the rapid interaction of ROS and NO generated by tumor iNOS from L-arginine metabolism (118).

To contribute to the existing complexity, M2 TAMs are also implicated in tumor cell survival. As mentioned before, M2 TAMs have heightened arginase activity that converts L-arginine to ornithine and urea, further depleting the limited local source of L-arginine. Ornithine induces the polarization of more M2 TAMs and, hence, this leads to the development of a vicious circle (124). The competition for L-arginine hampers the ability of M1 TAMs to generate NO that has antitumor properties (124). The scarcity of L-arginine also has a knock-on effect on T cell function as they also depend on L-arginine for NO and protein synthesis, both of which are required for T cell activation. In short, tumor cells have developed a distinctive reprogrammed metabolism that provides survival advantage by fast-tracking energy production and anabolic processes while in the process also creates the perfect microenvironment to hinder the metabolism and antitumor function of immune cells.

Tumor ECs, on the other hand, have overexpression of GLUTs, which is indicative of rapid glucose uptake and glycolytic activity (40, 41). Perivascular NO gradient has been shown *in vitro* to facilitate blood vessel normalization and maturation (125). As seen in the competition with immune cells for metabolic substrate, increased uptake of L-arginine by cancer cells reduces NO generated by eNOS. This results in the formation of abnormal vessels in terms of organization, structure, and function, proving a challenge for delivering antitumor drugs to the perfused cancer cells (125). Recently, upregulation of PFKFB3, a key regulator of ECs glycolysis, was shown to lead to the development of immature and dysfunctional vasculature in tumor angiogenesis (39, 41, 126). Further to that, inhibition of the enzyme results in normalization of blood vessels and, hence, it could be a potential therapeutic target as an adjunctive treatment for effective delivery of modern cytotoxic agents to tumor cells (39, 41, 126). At the same time, heightened glycolysis in tumor cells is PFKFB3-dependent and inhibition of the same enzymes was shown to reduce glucose uptake and proliferation of human hematopoietic and adenomatous cancer cell lines (127).

The clinical implication of cell metabolism in oncology is vast. Since the year 2000, lactate accumulations have been reported by several teams as prognostic predictors of poor outcome in patients with solid malignant tumors (128). Several glycolytic inhibitors have also been shown to be effective therapeutic adjuncts against cancers in hypoxic environment and those with mitochondrial defects that are resistant to conventional chemoradiotherapies (129). Although the theoretical advantage of glycolysis inhibitors is to devoid tumor cells of ATP by inhibiting their main mode of bioenergetics, it is postulated that cancer cells with intact mitochondria could still generate ATP through OXPHOS. The

other advantage of inhibiting glycolysis is to normalize the acidic tumor micromilieu and the subsequent reestablishment of tumor-suppressing immune function. Arginine metabolism has also attained great interest in cancer therapeutics and, interestingly, both upregulation and inhibition of arginase action can lead to tumor-suppressing activities. Tumor growth displayed dose-dependent suppression on administration of arginase inhibitor and the effect is not seen in mice with dysfunctional adaptive immune system, suggesting that the antitumor activity of arginase inhibitor is immune mediated (130). On the other hand, supplementation of recombinant human arginase was shown to induce cell apoptosis in non-small cell lung cancers through mitochondrial-derived ROS production (131). However, arginase supplementation reduces L-arginine required for the T-cell proliferation and cell–cycle progression from the accumulation of myeloid-derived suppressor cells (MDSC), suggesting a need for co-targeting MDSC accumulation in arginase inhibitor cancer treatments (132). At the moment, the knowledge of cellular metabolism is pushing the boundaries of modern oncology but the *in vivo* effect of metabolic reprogramming remains ambiguous in certain conditions.

PERSPECTIVE AND CONCLUSION

The impact of metabolism on cell function is an area with great future prospect for research. For example, NO has been shown to selectively induce differentiation of naïve CD^+ 4 T cells into T_H1 phenotype (56). Although both T_H1 and T_H2 cells utilize aerobic glycolysis as their main source of bioenergetics, the reasons for NO to selectively induce T_H1 differentiation are not completely understood (33). It is exciting and equally challenging to find out methods to translate our knowledge on metabolic reprogramming into therapeutics of human diseases. Seeing the fact that bioenergetic mechanisms are ubiquitous in all cell lines, often the big question in therapeutics may be the systemic side effects of metabolic-based treatments as a result of unintended involvement of bystander cells. To illustrate, a partial and reversible reduction of glycolysis can be achieved through PFKFB3 blockade with an intention to reduce proliferation, migration, and sprouting of ECs (133). Potentially, the enzyme blockade could sufficiently reduce pathological progression of atherosclerosis while sparing healthy vasculature from permanent glycolytic dysfunction. Furthermore, pathogenesis of some well-described diseases could be seen from a different light with new understanding in metabolic reprogramming. Recently, novel research on endothelial dysfunction in rheumatoid arthritis is underway (134). ROS is found to be directly implicated in synovitis associated with rheumatoid arthritis (135). Patients with rheumatoid arthritis also have raised plasma ADMA, a known cause of NO-mediated endothelial dysfunction (136). These new findings could lead to the development of treatment adjuncts to couple with the current regimen largely based on immunosuppressants such as methotrexate. These examples are milestones we have achieved in a relatively short span of time, a testimony of the tremendous potential in the field of cellular metabolism. Cellular functions are highly dependent on the metabolic requirement; understanding the metabolic pathways will inform us with novel approaches to exploit these functions in the light of therapeutic and translational opportunities.

AUTHOR CONTRIBUTIONS

C-YT and CM conceived and wrote the manuscript.

FUNDING

This work was supported by the British Heart Foundation (Fellowship FS/12/38/29640 and Project Grant PG/15/105/31906) and the Fondazione Cariplo (PG 2015-0552), and forms part of the research themes contributing to the translational research portfolio of Barts and the London Cardiovascular Biomedical Research Unit, which is supported and funded by the National Institutes of Health Research. C-YT would like to thank CM for his academic supervision and Jed Yu Jie Lee for the editorial advice.

REFERENCES

1. Frezza C, Mauro C. Editorial: the metabolic challenges of immune cells in health and disease. *Front Immunol* (2015) 6:293. doi:10.3389/fimmu.2015.00293
2. Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA, et al. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. *Proc Natl Acad Sci U S A* (1997) 94(13):6658–63. doi:10.1073/pnas.94.13.6658
3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* (2011) 144(5):646–74. doi:10.1016/j.cell.2011.02.013
4. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, et al. Lactate regulates metabolic and pro-inflammatory circuits in control of T cell migration and effector functions. *PLoS Biol* (2015) 13(7):e1002202. doi:10.1371/journal.pbio.1002202
5. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep* (2014) 6:13. doi:10.12703/P6-13
6. Colin S, Chinetti-Gbaguidi G, Staels B. Macrophage phenotypes in atherosclerosis. *Immunol Rev* (2014) 262(1):153–66. doi:10.1111/imr.12218
7. Gleissner CA, Shaked I, Little KM, Ley K. CXC chemokine ligand 4 induces a unique transcriptome in monocyte-derived macrophages. *J Immunol* (2010) 184(9):4810–8. doi:10.4049/jimmunol.0901368
8. Boyle JJ, Johns M, Kampfer T, Nguyen AT, Game L, Schaer DJ, et al. Activating transcription factor 1 directs Mhem atheroprotective macrophages through coordinated iron handling and foam cell protection. *Circ Res* (2012) 110(1):20–33. doi:10.1161/CIRCRESAHA.111.247577
9. Kadl A, Meher AK, Sharma PR, Lee MY, Doran AC, Johnstone SR, et al. Identification of a novel macrophage phenotype that develops in response to atherogenic phospholipids via Nrf2. *Circ Res* (2010) 107(6):737–46. doi:10.1161/CIRCRESAHA.109.215715
10. Sironi M, Martinez FO, D'Ambrosio D, Gattorno M, Polentarutti N, Locati M, et al. Differential regulation of chemokine production by Fcgamma receptor engagement in human monocytes: association of CCL1 with a distinct form of M2 monocyte activation (M2b, type 2). *J Leukoc Biol* (2006) 80(2):342–9. doi:10.1189/jlb.1005586
11. Jetten N, Verbruggen S, Gijbels MJ, Post MJ, De Winther MP, Donners MM. Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo. *Angiogenesis* (2014) 17(1):109–18. doi:10.1007/s10456-013-9381-6
12. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* (2014) 40(2):274–88. doi:10.1016/j.immuni.2014.01.006
13. Galván-Peña S, O'Neill LAJ. Metabolic reprograming in macrophage polarization. *Front Immunol* (2014) 5:420. doi:10.3389/fimmu.2014.00420

14. Jha AK, Huang SC, Sergushichev A, Lampropoulou V, Ivanova Y, Loginicheva E, et al. Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity* (2015) 42(3):419–30. doi:10.1016/j.immuni.2015.02.005
15. Tannahill G, Curtis A, Adamik J, Palsson-McDermott E, McGettrick A, Goel G, et al. Succinate is a danger signal that induces IL-1β via HIF-1α. *Nature* (2013) 496(7444):238–42. doi:10.1038/nature11986
16. O'Neill LA, Pearce EJ. Immunometabolism governs dendritic cell and macrophage function. *J Exp Med* (2016) 213(1):15–23. doi:10.1084/jem.20151570
17. Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* (2015) 25(7):771–84. doi:10.1038/cr.2015.68
18. Ghesquiere B, Wong BW, Kuchnio A, Carmeliet P. Metabolism of stromal and immune cells in health and disease. *Nature* (2014) 511(7508):167–76. doi:10.1038/nature13312
19. Rodriguez-Prados JC, Traves PG, Cuenca J, Rico D, Aragones J, Martin-Sanz P, et al. Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. *J Immunol* (2010) 185(1):605–14. doi:10.4049/jimmunol.0901698
20. Haschemi A, Kosma P, Gille L, Evans C, Burant C, Starkl P, et al. The sedoheptulose kinase CARKL directs macrophage polarization through control of glucose metabolism. *Cell Metab* (2012) 15(6):813–26. doi:10.1016/j.cmet.2012.04.023
21. Gordon S. Alternative activation of macrophages. *Nat Rev Immunol* (2003) 3(1):23–35. doi:10.1038/nri978
22. O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* (2013) 493(7432):346–55. doi:10.1038/nature11862
23. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood* (2008) 112(5):1557–69. doi:10.1182/blood-2008-05-078154
24. Fazilleau N, Mark L, McHeyzer-Williams LJ, McHeyzer-Williams MG. Follicular helper T cells: lineage and location. *Immunity* (2009) 30(3):324–35. doi:10.1016/j.immuni.2009.03.003
25. Zeng H, Zhang R, Jin B, Chen L. Type 1 regulatory T cells: a new mechanism of peripheral immune tolerance. *Cell Mol Immunol* (2015) 12(5):566–71. doi:10.1038/cmi.2015.44
26. Wahl DR, Byersdorfer CA, Ferrara JLM, Opipari AW, Glick GD. Distinct metabolic programs in activated T cells: opportunities for selective immunomodulation. *Immunol Rev* (2012) 249(1):104–15. doi:10.1111/j.1600-065X.2012.01148.x
27. MacIver NJ, Michalek RD, Rathmell JC. Metabolic regulation of T lymphocytes. *Annu Rev Immunol* (2013) 31:259–83. doi:10.1146/annurev-immunol-032712-095956
28. Jacobs SR, Herman CE, MacIver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol* (2008) 180(7):4476–86. doi:10.4049/jimmunol.180.7.4476
29. Wang R, Dillon CP, Shi LZ, Milasta S, Carter R, Finkelstein D, et al. The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. *Immunity* (2011) 35(6):871–82. doi:10.1016/j.immuni.2011.09.021
30. Kaminski MM, Sauer SW, Kaminski M, Opp S, Ruppert T, Grigaravicius P, et al. T cell activation is driven by an ADP-dependent glucokinase linking enhanced glycolysis with mitochondrial reactive oxygen species generation. *Cell Rep* (2012) 2(5):1300–15. doi:10.1016/j.celrep.2012.10.009
31. Chang CH, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional control of T cell effector function by aerobic glycolysis. *Cell* (2013) 153(6):1239–51. doi:10.1016/j.cell.2013.05.016
32. Maciver NJ, Jacobs SR, Wieman HL, Wofford JA, Coloff JL, Rathmell JC. Glucose metabolism in lymphocytes is a regulated process with significant effects on immune cell function and survival. *J Leukoc Biol* (2008) 84(4):949–57. doi:10.1189/jlb.0108024
33. Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, MacIver NJ, Mason EF, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J Immunol* (2011) 186(6):3299–303. doi:10.4049/jimmunol.1003613
34. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol* (2012) 13(10):907–15. doi:10.1038/ni.2386
35. van der Windt GJ, Everts B, Chang CH, Curtis JD, Freitas TC, Amiel E, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* (2012) 36(1):68–78. doi:10.1016/j.immuni.2011.12.007
36. Mazzone M, Dettori D, Leite de Oliveira R, Loges S, Schmidt T, Jonckx B, et al. Heterozygous deficiency of PHD2 restores tumor oxygenation and inhibits metastasis via endothelial normalization. *Cell* (2009) 136(5):839–51. doi:10.1016/j.cell.2009.01.020
37. Risau W. Mechanisms of angiogenesis. *Nature* (1997) 386(6626):671–4. doi:10.1038/386671a0
38. Ribatti D, Crivellato E. "Sprouting angiogenesis", a reappraisal. *Dev Biol* (2012) 372(2):157–65. doi:10.1016/j.ydbio.2012.09.018
39. De Bock K, Georgiadou M, Schoors S, Kuchnio A, Wong BW, Cantelmo AR, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. *Cell* (2013) 154(3):651–63. doi:10.1016/j.cell.2013.06.037
40. Yeh WL, Lin CJ, Fu WM. Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia. *Mol Pharmacol* (2008) 73(1):170–7. doi:10.1124/mol.107.038851
41. Eelen G, de Zeeuw P, Simons M, Carmeliet P. Endothelial cell metabolism in normal and diseased vasculature. *Circ Res* (2015) 116(7):1231–44. doi:10.1161/CIRCRESAHA.116.302855
42. Doddaballapur A, Michalik KM, Manavski Y, Lucas T, Houtkooper RH, You X, et al. Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arterioscler Thromb Vasc Biol* (2015) 35(1):137–45. doi:10.1161/ATVBAHA.114.304277
43. Forstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* (2012) 33(7):829–37, 837a–d. doi:10.1093/eurheartj/ehr304
44. Dagher Z, Ruderman N, Tornheim K, Ido Y. Acute regulation of fatty acid oxidation and amp-activated protein kinase in human umbilical vein endothelial cells. *Circ Res* (2001) 88(12):1276–82. doi:10.1161/hh1201.092998
45. Schoors S, Bruning U, Missiaen R, Queiroz KC, Borgers G, Elia I, et al. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. *Nature* (2015) 520(7546):192–7. doi:10.1038/nature14362
46. Locasale JW, Grassian AR, Melman T, Lyssiotis CA, Mattaini KR, Bass AJ, et al. Phosphoglycerate dehydrogenase diverts glycolytic flux and contributes to oncogenesis. *Nat Genet* (2011) 43(9):869–74. doi:10.1038/ng.890
47. Palmer CS, Ostrowski M, Balderson B, Christian N, Crowe SM. Glucose metabolism regulates T cell activation, differentiation, and functions. *Front Immunol* (2015) 6:1. doi:10.3389/fimmu.2015.00001
48. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signaling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* (2011) 472(7344):476–80. doi:10.1038/nature09973
49. Freemerman AJ, Johnson AR, Sacks GN, Milner JJ, Kirk EL, Troester MA, et al. Metabolic reprogramming of macrophages: glucose transporter 1 (GLUT1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem* (2014) 289(11):7884–96. doi:10.1074/jbc.M113.522037
50. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nat Rev Cancer* (2004) 4(11):891–9. doi:10.1038/nrc1478
51. Pantel A, Teixeira A, Haddad E, Wood EG, Steinman RM, Longhi MP. Direct type I IFN but not MDA5/TLR3 activation of dendritic cells is required for maturation and metabolic shift to glycolysis after poly IC stimulation. *PLoS Biol* (2014) 12(1):e1001759. doi:10.1371/journal.pbio.1001759
52. Lowenstein CJ, Alley EW, Raval P, Snowman AM, Snyder SH, Russell SW, et al. Macrophage nitric oxide synthase gene: two upstream regions mediate induction by interferon gamma and lipopolysaccharide. *Proc Natl Acad Sci U S A* (1993) 90(20):9730–4. doi:10.1073/pnas.90.20.9730
53. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine modulates T cell metabolism and enhances survival and anti-tumor activity. *Cell* (2016) 167(3):829–42.e13. doi:10.1016/j.cell.2016.09.031
54. Possemato R, Marks KM, Shaul YD, Pacold ME, Kim D, Birsoy K, et al. Functional genomics reveals serine synthesis is essential in PHGDH-amplified breast cancer. *Nature* (2011) 476(7360):346–50. doi:10.1038/nature10350
55. Dalton DK, Haynes L, Chu CQ, Swain SL, Wittmer S. Interferon gamma eliminates responding CD4 T cells during mycobacterial infection by inducing apoptosis of activated CD4 T cells. *J Exp Med* (2000) 192(1):117–22. doi:10.1084/jem.192.1.117

56. Niedbala W, Cai B, Liew FY. Role of nitric oxide in the regulation of T cell functions. *Ann Rheum Dis* (2006) 65(Suppl 3):iii37–40. doi:10.1136/ard.2006.058446
57. Diers AR, Broniowska KA, Darley-Usmar VM, Hogg N. Differential regulation of metabolism by nitric oxide and S-nitrosothiols in endothelial cells. *Am J Physiol Heart Circ Physiol* (2011) 301(3):H803–12. doi:10.1152/ajpheart.00210.2011
58. Paik JY, Lee KH, Ko BH, Choe YS, Choi Y, Kim BT. Nitric oxide stimulates 18F-FDG uptake in human endothelial cells through increased hexokinase activity and GLUT1 expression. *J Nucl Med* (2005) 46(2):365–70.
59. Broniowska KA, Hogg N. Differential mechanisms of inhibition of glyceraldehyde-3-phosphate dehydrogenase by S-nitrosothiols and NO in cellular and cell-free conditions. *Am J Physiol Heart Circ Physiol* (2010) 299(4):H1212–9. doi:10.1152/ajpheart.00472.2010
60. Tawakol A, Singh P, Mojena M, Pimentel-Santillana M, Emami H, MacNabb M, et al. HIF-1α and PFKFB3 mediate a tight relationship between pro-inflammatory activation and anaerobic metabolism in atherosclerotic macrophages. *Arterioscler Thromb Vasc Biol* (2015) 35(6):1463–71. doi:10.1161/ATVBAHA.115.305551
61. Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A. Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. *J Biol Chem* (2001) 276(12):9519–25. doi:10.1074/jbc.M010144200
62. Semenza GL, Jiang BH, Leung SW, Passantino R, Concordet JP, Maire P, et al. Hypoxia response elements in the aldolase A, enolase 1, and lactate dehydrogenase A gene promoters contain essential binding sites for hypoxia-inducible factor 1. *J Biol Chem* (1996) 271(51):32529–37. doi:10.1074/jbc.271.51.32529
63. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* (2006) 3(3):177–85. doi:10.1016/j.cmet.2006.02.002
64. Shi LZ, Wang R, Huang G, Vogel P, Neale G, Green DR, et al. HIF1α-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. *J Exp Med* (2011) 208:1367–76. doi:10.1084/jem.20110278
65. Dang EV, Barbi J, Yang HY, Jinasena D, Yu H, Zheng Y, et al. Control of TH17/Treg balance by hypoxia-inducible factor 1. *Cell* (2011) 146(5):772–84. doi:10.1016/j.cell.2011.07.033
66. Loike JD, Cao L, Brett J, Ogawa S, Silverstein SC, Stern D. Hypoxia induces glucose transporter expression in endothelial cells. *Am J Physiol* (1992) 263 (2 Pt 1):C326–33.
67. Oswald J, Treite F, Haase C, Kampfrath T, Mading P, Schwenzer B, et al. Experimental hypoxia is a potent stimulus for radiotracer uptake in vitro: comparison of different tumor cells and primary endothelial cells. *Cancer Lett* (2007) 254(1):102–10. doi:10.1016/j.canlet.2007.02.016
68. Galic S, Fullerton MD, Schertzer JD, Sikkema S, Marcinko K, Walkley CR, et al. Hematopoietic AMPK beta1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity. *J Clin Invest* (2011) 121(12):4903–15. doi:10.1172/JCI58577
69. Mounier R, Theret M, Arnold L, Cuvellier S, Bultot L, Goransson O, et al. AMPKalpha1 regulates macrophage skewing at the time of resolution of inflammation during skeletal muscle regeneration. *Cell Metab* (2013) 18(2):251–64. doi:10.1016/j.cmet.2013.06.017
70. Sag D, Carling D, Stout RD, Suttles J. AMP-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J Immunol* (2008) 181(12):8633–41. doi:10.4049/jimmunol.181.12.8633
71. Marsin AS, Bouzin C, Bertrand L, Hue L. The stimulation of glycolysis by hypoxia in activated monocytes is mediated by AMP-activated protein kinase and inducible 6-phosphofructo-2-kinase. *J Biol Chem* (2002) 277(34):30778–83. doi:10.1074/jbc.M205213200
72. MacIver NJ, Blagih J, Saucillo DC, Tonelli L, Griss T, Rathmell JC, et al. The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J Immunol* (2011) 187(8):4187–98. doi:10.4049/jimmunol.1100367
73. Blagih J, Coulombe F, Vincent EE, Dupuy F, Galicia-Vazquez G, Yurchenko E, et al. The energy sensor AMPK regulates T cell metabolic adaptation and effector responses in vivo. *Immunity* (2015) 42(1):41–54. doi:10.1016/j.immuni.2014.12.030
74. Dagher Z, Ruderman N, Tornheim K, Ido Y. The effect of AMP-activated protein kinase and its activator AICAR on the metabolism of human umbilical vein endothelial cells. *Biochem Biophys Res Commun* (1999) 265(1):112–5. doi:10.1006/bbrc.1999.1635
75. Everts B, Amiel E, van der Windt GJW, Freitas TC, Chott R, Yarasheski KE, et al. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* (2012) 120(7):1422–31. doi:10.1182/blood-2012-03-419747
76. Baseler WA, Davies LC, Quigley L, Ridnour LA, Weiss JM, Hussain SP, et al. Autocrine IL-10 functions as a rheostat for M1 macrophage glycolytic commitment by tuning nitric oxide production. *Redox Biol* (2016) 10:12–23. doi:10.1016/j.redox.2016.09.005
77. Figueroa XF, Lillo MA, Gaete PS, Riquelme MA, Saez JC. Diffusion of nitric oxide across cell membranes of the vascular wall requires specific connexin-based channels. *Neuropharmacology* (2013) 75:471–8. doi:10.1016/j.neuropharm.2013.02.022
78. Miersch S, Espey MG, Chaube R, Akarca A, Tweten R, Ananvoranich S, et al. Plasma membrane cholesterol content affects nitric oxide diffusion dynamics and signaling. *J Biol Chem* (2008) 283(27):18513–21. doi:10.1074/jbc.M800440200
79. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* (2014) 513(7519):559–63. doi:10.1038/nature13490
80. Doulias PT, Tenopoulou M, Greene JL, Raju K, Ischiropoulos H. Nitric oxide regulates mitochondrial fatty acid metabolism through reversible protein S-nitrosylation. *Sci Signal* (2013) 6(256):rs1. doi:10.1126/scisignal.2003252
81. Feuerer M, Eulenburg K, Loddenkemper C, Hamann A, Huehn J. Self-limitation of Th1-mediated inflammation by IFN-gamma. *J Immunol* (2006) 176(5):2857–63. doi:10.4049/jimmunol.176.5.2857
82. Tarrant TK, Silver PB, Wahlsten JL, Rizzo LV, Chan CC, Wiggert B, et al. Interleukin 12 protects from a T helper type 1-mediated autoimmune disease, experimental autoimmune uveitis, through a mechanism involving interferon gamma, nitric oxide, and apoptosis. *J Exp Med* (1999) 189(2):219–30. doi:10.1084/jem.189.2.219
83. Douguet L, Cherfils-Vicini J, Bod L, Lengagne R, Gilson E, Prevost-Blondel A. Nitric oxide synthase 2 improves proliferation and glycolysis of peripheral gammadelta T cells. *PLoS One* (2016) 11(11):e0165639. doi:10.1371/journal.pone.0165639
84. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* (2003) 3(10):721–32. doi:10.1038/nrc1187
85. Takeda N, O'Dea EL, Doedens A, Kim J, Weidemann A, Stockmann C, et al. Differential activation and antagonistic function of HIF-α isoforms in macrophages are essential for NO homeostasis. *Genes Dev* (2010) 24(5):491–501. doi:10.1101/gad.1881410
86. Marsin AS, Bertrand L, Rider MH, Deprez J, Beauloye C, Vincent MF, et al. Phosphorylation and activation of heart PFK-2 by AMPK has a role in the stimulation of glycolysis during ischaemia. *Curr Biol* (2000) 10(20):1247–55. doi:10.1016/S0960-9822(00)00742-9
87. Fisslthaler B, Fleming I. Activation and signaling by the AMP-activated protein kinase in endothelial cells. *Circ Res* (2009) 105(2):114–27. doi:10.1161/CIRCRESAHA.109.201590
88. Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, et al. AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci U S A* (2002) 99(25):15983–7. doi:10.1073/pnas.252625599
89. St-Pierre J, Lin J, Krauss S, Tarr PT, Yang R, Newgard CB, et al. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. *J Biol Chem* (2003) 278(29):26597–603. doi:10.1074/jbc.M301850200
90. Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol (1985)* (2000) 88(6):2219–26.
91. Cobbold SP, Adams E, Farquhar CA, Nolan KF, Howie D, Lui KO, et al. Infectious tolerance via the consumption of essential amino acids and mTOR signaling. *Proc Natl Acad Sci U S A* (2009) 106(29):12055–60. doi:10.1073/pnas.0903919106
92. Deldicque L, Theisen D, Francaux M. Regulation of mTOR by amino acids and resistance exercise in skeletal muscle. *Eur J Appl Physiol* (2005) 94(1–2):1–10. doi:10.1007/s00421-004-1255-6
93. Munday MR, Campbell DG, Carling D, Hardie DG. Identification by amino acid sequencing of three major regulatory phosphorylation sites on rat

acetyl-CoA carboxylase. *Eur J Biochem* (1988) 175(2):331–8. doi:10.1111/j.1432-1033.1988.tb14201.x
94. Dzamko N, Schertzer JD, Ryall JG, Steel R, Macaulay SL, Wee S, et al. AMPK-independent pathways regulate skeletal muscle fatty acid oxidation. *J Physiol* (2008) 586(Pt 23):5819–31. doi:10.1113/jphysiol.2008.159814
95. Luo Z, Zang M, Guo W. AMPK as a metabolic tumor suppressor: control of metabolism and cell growth. *Future Oncol* (2010) 6(3):457–70. doi:10.2217/fon.09.174
96. Mayer A, Denanglaire S, Viollet B, Leo O, Andris F. AMP-activated protein kinase regulates lymphocyte responses to metabolic stress but is largely dispensable for immune cell development and function. *Eur J Immunol* (2008) 38(4):948–56. doi:10.1002/eji.200738045
97. Boyle JG, Logan PJ, Ewart MA, Reihill JA, Ritchie SA, Connell JM, et al. Rosiglitazone stimulates nitric oxide synthesis in human aortic endothelial cells via AMP-activated protein kinase. *J Biol Chem* (2008) 283(17):11210–7. doi:10.1074/jbc.M710048200
98. Mount PF, Lane N, Venkatesan S, Steinberg GR, Fraser SA, Kemp BE, et al. Bradykinin stimulates endothelial cell fatty acid oxidation by CaMKK-dependent activation of AMPK. *Atherosclerosis* (2008) 200(1):28–36. doi:10.1016/j.atherosclerosis.2007.12.003
99. Stahmann N, Woods A, Carling D, Heller R. Thrombin activates AMP-activated protein kinase in endothelial cells via a pathway involving Ca2+/calmodulin-dependent protein kinase kinase beta. *Mol Cell Biol* (2006) 26(16):5933–45. doi:10.1128/MCB.00383-06
100. Valle I, Alvarez-Barrientos A, Arza E, Lamas S, Monsalve M. PGC-1alpha regulates the mitochondrial antioxidant defense system in vascular endothelial cells. *Cardiovasc Res* (2005) 66(3):562–73. doi:10.1016/j.cardiores.2005.01.026
101. Katusic ZS. Vascular endothelial dysfunction: does tetrahydrobiopterin play a role? *Am J Physiol Heart Circ Physiol* (2001) 281(3):H981–6.
102. Chang GJ, Woo P, Honda HM, Ignarro LJ, Young L, Berliner JA, et al. Oxidation of LDL to a biologically active form by derivatives of nitric oxide and nitrite in the absence of superoxide. Dependence on pH and oxygen. *Arterioscler Thromb* (1994) 14(11):1808–14. doi:10.1161/01.ATV.14.11.1808
103. Lloyd-Jones DM, Bloch KD. The vascular biology of nitric oxide and its role in atherogenesis. *Annu Rev Med* (1996) 47:365–75. doi:10.1146/annurev.med.47.1.365
104. Kawashima S. Malfunction of vascular control in lifestyle-related diseases: endothelial nitric oxide (NO) synthase/NO system in atherosclerosis. *J Pharmacol Sci* (2004) 96(4):411–9. doi:10.1254/jphs.FMJ04006X6
105. Candipan RC, Wang BY, Buitrago R, Tsao PS, Cooke JP. Regression or progression. Dependency on vascular nitric oxide. *Arterioscler Thromb Vasc Biol* (1996) 16(1):44–50. doi:10.1161/01.ATV.16.1.44
106. Boger RH, Bode-Boger SM, Szuba A, Tsao PS, Chan JR, Tangphao O, et al. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation* (1998) 98(18):1842–7. doi:10.1161/01.CIR.98.18.1842
107. Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. *J Cardiovasc Pharmacol* (1992) 20(Suppl 12):S60–2. doi:10.1097/00005344-199204002-00018
108. Binder CJ, Chang MK, Shaw PX, Miller YI, Hartvigsen K, Dewan A, et al. Innate and acquired immunity in atherogenesis. *Nat Med* (2002) 8(11):1218–26. doi:10.1038/nm1102-1218
109. Buono C, Binder CJ, Stavrakis G, Witztum JL, Glimcher LH, Lichtman AH. T-bet deficiency reduces atherosclerosis and alters plaque antigen-specific immune responses. *Proc Natl Acad Sci U S A* (2005) 102(5):1596–601. doi:10.1073/pnas.0409015102
110. Vats D, Mukundan L, Odegaard JI, Zhang L, Smith KL, Morel CR, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab* (2006) 4(1):13–24. doi:10.1016/j.cmet.2006.08.006
111. Ouimet M, Ediriweera HN, Gundra UM, Sheedy FJ, Ramkhelawon B, Hutchison SB, et al. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. *J Clin Invest* (2015) 125(12):4334–48. doi:10.1172/JCI81676
112. Ketelhuth DF, Gistera A, Johansson DK, Hansson GK. T cell-based therapies for atherosclerosis. *Curr Pharm Des* (2013) 19(33):5850–8. doi:10.2174/1381612811319330003
113. Ait-Oufella H, Salomon BL, Potteaux S, Robertson AK, Gourdy P, Zoll J, et al. Natural regulatory T cells control the development of atherosclerosis in mice. *Nat Med* (2006) 12(2):178–80. doi:10.1038/nm1343
114. Kita T, Yamashita T, Sasaki N, Kasahara K, Sasaki Y, Yodoi K, et al. Regression of atherosclerosis with anti-CD3 antibody via augmenting a regulatory T-cell response in mice. *Cardiovasc Res* (2014) 102(1):107–17. doi:10.1093/cvr/cvu002
115. Broadhurst MJ, Leung JM, Lim KC, Girgis NM, Gundra UM, Fallon PG, et al. Upregulation of retinal dehydrogenase 2 in alternatively activated macrophages during retinoid-dependent type-2 immunity to helminth infection in mice. *PLoS Pathog* (2012) 8(8):e1002883. doi:10.1371/journal.ppat.1002883
116. Zeng H, Chi H. Metabolic control of regulatory T cell development and function. *Trends Immunol* (2015) 36(1):3–12. doi:10.1016/j.it.2014.08.003
117. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish Treg cell function. *Nature* (2013) 499(7459):485–90. doi:10.1038/nature12297
118. Molon B, Calì B, Viola A. T cells and cancer: how metabolism shapes immunity. *Front Immunol* (2016) 7:20. doi:10.3389/fimmu.2016.00020
119. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic competition in the tumor microenvironment is a driver of cancer progression. *Cell* (2015) 162(6):1229–41. doi:10.1016/j.cell.2015.08.016
120. Ho PC, Bihuniak JD, Macintyre AN, Staron M, Liu X, Amezquita R, et al. Phosphoenolpyruvate is a metabolic checkpoint of anti-tumor T cell responses. *Cell* (2015) 162(6):1217–28. doi:10.1016/j.cell.2015.08.012
121. Zheng Y, Delgoffe GM, Meyer CF, Chan W, Powell JD. Anergic T cells are metabolically anergic. *J Immunol* (2009) 183(10):6095–101. doi:10.4049/jimmunol.0803510
122. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-associated lactic acid production blunts tumor immunosurveillance by T and NK cells. *Cell Metab* (2016) 24(5):657–71. doi:10.1016/j.cmet.2016.08.011
123. Parry RV, Chemnitz JM, Frauwirth KA, Lanfranco AR, Braunstein I, Kobayashi SV, et al. CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. *Mol Cell Biol* (2005) 25(21):9543–53. doi:10.1128/MCB.25.21.9543-9553.2005
124. Mills CD. M1 and M2 macrophages: oracles of health and disease. *Crit Rev Immunol* (2012) 32(6):463–88. doi:10.1615/CritRevImmunol.v32.i6.10
125. Kashiwagi S, Tsukada K, Xu L, Miyazaki J, Kozin SV, Tyrrell JA, et al. Perivascular nitric oxide gradients normalize tumor vasculature. *Nat Med* (2008) 14(3):255–7. doi:10.1038/nm1730
126. Teuwen LA, Draoui N, Dubois C, Carmeliet P. Endothelial cell metabolism: an update anno 2017. *Curr Opin Hematol* (2017) 24(3):240–7. doi:10.1097/MOH.0000000000000335
127. Clem B, Telang S, Clem A, Yalcin A, Meier J, Simmons A, et al. Small-molecule inhibition of 6-phosphofructo-2-kinase activity suppresses glycolytic flux and tumor growth. *Mol Cancer Ther* (2008) 7(1):110–20. doi:10.1158/1535-7163.MCT-07-0482
128. Hirschhaeuser F, Sattler UG, Mueller-Klieser W. Lactate: a metabolic key player in cancer. *Cancer Res* (2011) 71(22):6921–5. doi:10.1158/0008-5472.CAN-11-1457
129. Pelicano H, Martin DS, Xu RH, Huang P. Glycolysis inhibition for anticancer treatment. *Oncogene* (2006) 25(34):4633–46. doi:10.1038/sj.onc.1209597
130. Rodriguez PC, Quiceno DG, Zabaleta J, Ortiz B, Zea AH, Piazuelo MB, et al. Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. *Cancer Res* (2004) 64(16):5839–49. doi:10.1158/0008-5472.CAN-04-0465
131. Shen W, Zhang X, Fu X, Fan J, Luan J, Cao Z, et al. A novel and promising therapeutic approach for NSCLC: recombinant human arginase alone or combined with autophagy inhibitor. *Cell Death Dis* (2017) 8(3):e2720. doi:10.1038/cddis.2017.137
132. Fletcher M, Ramirez ME, Sierra RA, Raber P, Thevenot P, Al-Khami AA, et al. L-Arginine depletion blunts anti-tumor T cell responses by inducing myeloid-derived suppressor cells. *Cancer Res* (2015) 75(2):275–83. doi:10.1158/0008-5472.CAN-14-1491
133. Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquiere B, Cauwenberghs S, et al. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. *Cell Metab* (2014) 19(1):37–48. doi:10.1016/j.cmet.2013.11.008

134. Chimenti MS, Triggianese P, Conigliaro P, Candi E, Melino G, Perricone R. The interplay between inflammation and metabolism in rheumatoid arthritis. *Cell Death Dis* (2015) 6:e1887. doi:10.1038/cddis.2015.246
135. Datta S, Kundu S, Ghosh P, De S, Ghosh A, Chatterjee M. Correlation of oxidant status with oxidative tissue damage in patients with rheumatoid arthritis. *Clin Rheumatol* (2014) 33(11):1557–64. doi:10.1007/s10067-014-2597-z
136. Dimitroulas T, Sandoo A, Veldhuijzen van Zanten JJ, Smith JP, Hodson J, Metsios GS, et al. Predictors of asymmetric dimethylarginine levels in patients with rheumatoid arthritis: the role of insulin resistance. *Scand J Rheumatol* (2013) 42(3):176–81. doi:10.3109/03009742.2012.747627

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