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Review

Amino Assets: How Amino Acids Support Immunity

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Amino acids are fundamental building blocks supporting life. Their role in protein synthesis is well defined, but they contribute to a host of other intracellular metabolic pathways, including ATP generation, nucleotide synthesis, and redox balance, to support cellular and organismal function. Immune cells critically depend on such pathways to acquire energy and biomass and to reprogram their metabolism upon activation to support growth, proliferation, and effector functions. Amino acid metabolism plays a key role in this metabolic rewiring, and it supports various immune cell functions beyond increased protein synthesis. Here, we review the mechanisms by which amino acid metabolism promotes immune cell function, and how these processes could be targeted to improve immunity in pathological conditions.

Introduction

Amino acids are fundamental units that supported the earliest life on earth. Individual amino acids can be created abiotically and have been detected in extraterrestrial sources such as meteorites (Burton et al., 2012). A major evolutionary step was the ability to combine amino acids into peptides and then proteins, which mediate cellular functions. In addition to protein synthesis, amino acids are used for many other processes driving growth and proliferation. Amino acid metabolism has profound consequences for cell function, not the least of which include that of immune cells, which are critically dependent on metabolic status due to their dynamic activation states as they respond to infections and changes in their tissue environments (Buck et al., 2017).

Amino acids are a key nutrient for immune cells, and amino acid supply instructs immune cell function. Immune cells have specific amino acid requirements, while growth factor stimulation and activation of T cells, which induce their rapid proliferation, increase amino acid transporter expression (Geiger et al., 2016; Ron-Harel et al., 2019; Sinclair et al., 2019), illustrating the need for enhanced amino acid uptake during this process. Upon activation, T cells rapidly proliferate and undergo increased gene transcription-translation for critical immune-related responses, such as cytokines and adhesion molecules, and thus must upregulate protein synthesis. Such responses are metabolically demanding, as new DNA, proteins, nucleotides, and other biomolecules must be produced before cells can divide and mount an immune response. However, it is now appreciated that amino acids have multiple signaling roles and supply material for more than just energy and protein synthesis.

In this review, we explore how amino acids go beyond merely supporting protein synthesis to control immune cell function. We examine how different immune cells selectively acquire amino acids, rather than their indiscriminate uptake, downstream of antigen and cytokine signaling and the control of amino acid transporter expression. We then discuss how amino acids support the metabolic reprogramming critical for immune cell activation. In addition, we expand on the idea that amino acids are not just used for the end result of this reprogramming, i.e., increased biosynthesis to drive proliferation and effector molecule production. Instead, they control these metabolic switches by feeding different ATP-producing pathways, such as glycolysis and mitochondrial metabolism, via the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OXPHOS). And as reactive oxygen species (ROS) production is a key step in immune cell activation, we describe how amino acids are central regulators of redox balance within immune cells, and how sulfur supply from cysteine and methionine may determine continued metabolic and translational activity in cells with altered redox status. Further, we review how amino acids tune immune effector protein activity by supplying intermediates, such as methyl and acetyl groups, for post-translational modification (PTM), and how such intermediates also epigenetically regulate acute immune responses and immune cell memory. Finally, we investigate amino acid competition between immune and tumor cells, and explore how targeting amino acid metabolism could modulate immune responses in cancer, infection, and autoimmunity.

Acquisition and Sensing of Amino Acids

An amino acid is an organic molecule containing an amino group and a carboxyl group attached to a carbon atom. Each amino acid also has a unique side chain, which bestows different properties and functions (Wu, 2009). A limited set of 10 amino acids, produced by abiotic chemical or physical reactions, may have been used for very early proteinogenesis before cellular biosynthesis evolved (Frenkel-Pinter et al., 2019; Longo and Blaber, 2012). A consensus set of these 10 early amino acids comprises alanine, aspartate, glutamate, glycine, isoleucine, leucine, proline, serine, threonine, and valine (Doi et al., 2005b), while the more complex aromatic amino acids tryptophan and phenylalanine, and the sulfurous amino acid methionine, arose later (Longo et al., 2015). Mammals use a set of 20 amino acids for protein synthesis, though many more exist in nature and influence cell function (Schuller-Levis and Park, 2003). Nine of these 20 proteinogenic amino acids are known as essential amino acids, as they cannot be sufficiently synthesized by the body and must be acquired from dietary sources (Wu, 2013). In contrast, non-essential amino acids can be synthesized to sufficient levels in the body and do not need to be acquired, but can become conditionally essential under conditions in which demand exceeds the body's capacity for synthesizing them

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(Combs and DeNicola, 2019; Wu, 2013). This dependence occurs in rapidly proliferating cells (Curthoys and Watford, 1995), which need amino acids for protein synthesis and increased biomass (Hosios et al., 2016). Given the marked changes in cell growth and proliferation that are characteristic of immune cells responding to alterations in their extracellular environment, it is likely that for these cells non-essential amino acids may become conditionally essential during an immune response.

Acquisition of Amino Acids

Amino acid acquisition is a point of control for cell function. Uptake from the external environment relies on transporters, while intracellularly, amino acids are recycled and donate functional groups for other amino acids. For example, glutamine supplies amino groups for other amino acids via transamination and transamidation, and methionine is metabolized to cysteine in the methionine cycle (Sinclair et al., 2019). Lysosomes also contain amino acid transport machinery (Sagné et al., 2001; Wyant et al., 2017), and can act as intracellular amino acid depots (Abu-Remaileh et al., 2017), important for cell function. For example, the lysosomal histidine transporter Slc15a4 is needed for Toll-like receptor (TLR)-induced type I interferon (IFN-I) production in plasmacytoid dendritic cells (pDCs), and IFN-I and immunoglobulin (Ig)G production in B cells (Kobayashi et al., 2014). During starvation, amino acids are sequestered in the lysosome, possibly to prevent their inappropriate use during this nutrient-restricted period. One possible consequence of such lysosomal amino acid storage is induction of autophagy, which promotes longevity, in part by antagonizing the age-related decline in $CD8^+$ T cell function and immune responses to infection and cancer (Pietrocola et al., 2016; Puleston et al., 2014). Lysosomal storage could also provide the cell with a bank of amino acids that can be mobilized when protein synthesis resumes, and autophagy would presumably increase amino acid availability in this context. Amino acids could be selectively released to drive particular gene expression programs to adapt to prolonged starvation or nutrient restoration; for example, by influencing epigenetic modifications.

Amino Acid Transporters Control Immune Cell Function

Amino acid uptake via transporters is a tightly regulated process that is critical for immune cell activation and function. A comprehensive proteomic analysis of human immune cells illustrated the vast diversity of transporters and receptors expressed by different immune cell types, and how these transporters change expression upon antigen stimulation and cytokine signaling (Rieckmann et al., 2017). This has been more specifically explored in a proteomic analysis of $CD4^+$ and $CD8^+$ T cells, which illustrated how antigen and cytokine stimulation changes expression of amino acid transporters to alter nutrient uptake and specifically enable effector function in activated T cells (Howden et al., 2019). Activation of $CD8^+$ T cells by T cell receptor (TCR) stimulation in the presence of growth factor cytokines, e.g., interleukin (IL)-2, increases the density of Slc1a5 and Slc7a5 on the cell surface compared to naive $CD8^+$ T cells. Activated T cells require a supply of amino acids to support proliferation, and this activation-dependent enhancement of transporter expression ensures that cells can acquire these nutrients according to their demand. It is not only the type, but the level of transporter expression, that influences T cell function. While activated $CD4^+$ and $CD8^+$ T cells express a similar nutrient transporter repertoire, $CD4^+$ T cells have a lower copy number of these transporters and less nutrient transport (Howden et al., 2019; Sinclair et al., 2013; Swamy et al., 2016). This difference, in combination with the lower levels of ribosomes and translational machinery in activated $CD4^+$ versus $CD8^+$ T cells, could underlie the lower cell mass and proliferative capacity of activated $CD4^+$ T cells compared to $CD8^+$ T cells. Such a detailed study of changes in amino acid transporter expression in other immune cell types would be highly informative, as their activation also depends on amino acid transporter activity. Further, both classical and alternative macrophage activation depend on arginine uptake via cationic amino acid transporter (CAT)2 (Slc7a2) (Yeramian et al., 2006b), while resting macrophages use the y^+L (Slc7a6, Slc7a7) system for arginine uptake, likely via CAT1 (Slc7a1) (Yeramian et al., 2006a). Lipopolysaccharide (LPS) increases Slc7a5 expression and leucine uptake to support human pro-inflammatory macrophage cytokine production (Yoon et al., 2018). These examples illustrate how innate immune cell activation depends on remodeling of amino acid transporter activity.

Multiple signaling pathways in turn control transporter expression. Myc, a central transcription factor driving T cell activation, increases Slc1a5, Slc7a1, Slc7a5, Slc38a1, and Slc38a2 on activated $CD4^+$ and $CD8^+$ T cells (Marchingo et al., 2020). Myc deficiency blocks activated T cell growth by reducing expression of these transporters to that of naive cells. TCR stimulation also activates the mitogen-activated protein kinase (MAPK) family member extracellular signal-regulated kinase (ERK) to increase glutamine uptake (Carr et al., 2010), possibly by increasing surface sodium-coupled neutral amino acid transporter (SNAT)2 (Slc38a2) expression (Franchi-Gazzola et al., 1999). Multiple pathways control expression of individual transporters. Slc7a5 expression also depends on calcium-calcineurin signaling (Sinclair et al., 2013), and both ERK and c-Jun N-terminal kinase (JNK) control SNAT2 (Hyde et al., 2007). Differential regulation of transporters and use of signaling pathways underlie the varying effects of cytokines on T cell growth and proliferation. IL-2 and IL-15 both signal through CD122 and activate Janus kinase (JAK)1, JAK3, and signal transducer and activator of transcription (STAT)5, but with different consequences for antigen-activated T cells. IL-2 promotes $CD8^+$ effector T (T_{eff}) cell formation and cell growth, while IL-15 is needed for the formation of $CD8^+$ memory T (T_{mem}) cells, which are smaller in size. T cells maintained in IL-2 have higher CD98 (Slc7a5/Slc3a2) expression than those in IL-15, and increase amino acid uptake and protein synthesis, underlying the different effects of these cytokines on T cell growth (Cornish et al., 2006). These effects depend on sustained phosphoinositide-3-kinase (PI3K) signaling in T cells cultured in IL-2 versus more transient PI3K signaling in IL-15-cultured cells. More work is needed to understand how divergent effects of cytokines on signaling pathway activities and kinetics control immune cell growth via modulation of amino acid transporters.

Asymmetric Distribution of Amino Acid Transporters Influences T Cell Fate

During an infection, a T cell population must generate T_{eff} cells to fight the infection, as well as T_{mem} cells to provide long-lasting immunity. Asymmetric cell division, in which the two daughter cells generated by a cell division event adopt different fates, is one model proposed for T cells to achieve this goal (Arsenio et al., 2014, 2015; Reiner and Adams, 2014). In asymmetric cell

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division, protein, RNA, and organelles are unequally inherited by the daughter cells, laying the foundation for their different fates. Antigen presentation by antigen-presenting cells (APCs) stimulates activation and proliferation of naive T cells. The T cell proximal to the APC adopts an effector-like fate, while the distal T cell assumes a memory-like fate, based on the asymmetric inheritance of factors, including CD3, CD4, CD8, CD62L, transcription factors, and mechanistic target of rapamycin (mTOR), between the daughter cells (Chang et al., 2007). Slc1a5 is one such asymmetrically inherited factor. Slc1a5 preferentially accumulates in the proximal cell, which also exhibits increased amino acid abundance, glutamine uptake, glycolysis, c-Myc, and mTOR complex (mTORC)1 activity (Verbist et al., 2016). The direct effect of Slc1a5 on c-Myc levels was not established in this system, but amino acid deprivation or blockade of glutaminolysis reduces the differences in c-Myc between the daughter cells, and reducing c-Myc levels pushes the daughter cells toward a memory-like phenotype (Verbist et al., 2016). This study established that metabolic asymmetry, specifically in terms of amino acid acquisition, may underlie different T cell fates. Similarly, daughter $CD8^+$ T cells with increased Slc7a5 expression have increased CD8 expression, mTORC1 activity, Myc levels, and glycolysis, while cells with lower CD8 and Slc7a5 expression have increased mitochondrial mass. Slc7a5 inhibition, but not mTORC1 inhibition, decreases mTORC1 lysosomal translocation in $CD8^{hi}$ daughter cells, indicating amino acid supply through Slc7a5 as an upstream regulator of the asymmetric division of mTORC1 activity (Pollizzi et al., 2016). Together, these results implicate amino acid metabolism and cellular metabolic profiles as determinants of cell fate.

Sensing Amino Acid Supply

Multiple mechanisms sense amino acids to control immune cell metabolism. mTORC1 is a central cellular signaling hub that drives protein synthesis, cell growth, and proliferation, while the general controlled non-repressed kinase 2 (GCN2) senses amino acid starvation by detecting uncharged tRNA. The precise mechanisms of amino acid sensing by these molecules have been extensively reviewed elsewhere (Bar-Peled et al., 2013; Kedersha et al., 2002; Saxton and Sabatini, 2017; Taniuchi et al., 2016). mTORC1 licenses immune cell activation only when enough resources are present. Active mTORC1 promotes the differentiation of $CD8^+$ cytotoxic T cells (CTLs) (Pollizzi et al., 2015; Rao et al., 2010), controls T_{mem} cell formation (Araki et al., 2009a; Pearce et al., 2009), and stimulates helper T (T_h)1 and T_h17 differentiation while restraining regulatory T (T_{reg}) generation (Delgoffe et al., 2011). In macrophages, pharmacologically activating GCN2 and the amino acid starvation response dampens IL-1β production (Battu et al., 2018), and inhibits inflammasome-mediated gut inflammation (Ravindran et al., 2016). Upon activation, both innate and adaptive immune cells must sense their amino acid supplies in order to engage the new biosynthetic programs that accompany this activation, and mTORC1 and GCN2 are central regulators of amino acid usage in this context.

Amino Acids Support Immune Cell Metabolic Reprogramming

Immune cell activation by receptor ligation and cytokine signaling induces drastic changes in transcription and translation, for cytokine and effector molecule production, as well as extensive proliferation, which requires acquisition of biomass for cell division. Immune cells reprogram their metabolism to support these exceptionally high metabolic demands. Distinct metabolic pathways fuel different immune cells, and metabolic reprogramming dictates immune cell survival, differentiation, and function (Geltink et al., 2018; O'Neill et al., 2016).

A major requirement of activated immune cells is energy in the form of ATP. Glycolysis, the TCA cycle, and OXPHOS cooperate to produce ATP, and amino acids regulate these interconnected processes. Glycolysis processes glucose to pyruvate, generating ATP and NADH, a cofactor for a multitude of enzymes, including those of mitochondrial metabolism. Pyruvate-derived acetyl-CoA enters the TCA cycle in the mitochondria to generate the reducing equivalents NADH and $FADH_2$ that provide electrons for OXPHOS. Mitochondrial fatty acid oxidation (FAO) yields acetyl-CoA, NADH, and $FADH_2$, further driving mitochondrial metabolism and ATP production. Amino acid metabolism contributes to these pathways (Figure 1), the differential use of which dictates immune cell function (Box 1).

Glutamine Supports Metabolic Rewiring

Glutamine provides intermediates for many metabolic pathways. Glutaminolysis is a major energy-producing process for proliferating cells, including activated T cells (Newsholme et al., 1999), by supplying α-ketoglutarate (αKG) to the TCA cycle, via glutamate. Glutamine is required for T cell activation (Yaqoob and Calder, 1997), as T cells cultured without glutamine cannot proliferate or produce IL-2 or IFN-γ (Carr et al., 2010). Supplementation with asparagine, proline, or glutamate, which can be metabolized to glutamine, does not recue proliferation or cytokine defects due to glutamine withdrawal, indicating that acquisition of extracellular glutamine, and not its intracellular generation, is the key regulatory event. This explains why naive T cell activation induces glutamine uptake, dependent on SNAT1, SNAT2 (Slc38a2) (Carr et al., 2010) and the alanine, serine, cysteine-preferring transporter 2 (ASCT2; Slc1a5) (Nakaya et al., 2014). ASCT2 disruption inhibits T_h1 and T_h17 differentiation, though proliferation and IL-2 production are normal (Nakaya et al., 2014). The increased glycolysis and mitochondrial metabolism necessary to support T cell differentiation is also impaired in *Asct2*$^{-/-}$ $CD4^+$ T cells, as they have decreased glucose uptake, lactate production, and oxygen consumption. Glutamine addition rescues these effects, though other amino acids transported by ASCT2 could theoretically also contribute to the phenotype.

While glutamine supports both T_h1 and T_h17 cell differentiation, glutamine degradation by glutaminolysis may be more critical specifically for T_h17 cells, while other downstream effects of glutamine may influence T_h1 cell differentiation (Johnson et al., 2018). This is illustrated in mice lacking glutaminase 1 (Gls1) (Kono et al., 2018), which metabolizes glutamine to glutamate. In cells from wild-type mice cultured in T_h17-polarizing conditions, the inducible cAMP early repressor (ICER) isoform of the cAMP response element modulator transcription factor binds to the *Il17a* promoter and drives T_h17 cell generation. ICER reinforces this differentiation by also binding to the *Gls1* promoter, increasing Gls1 levels and consequent glutamine utilization (Kono et al., 2018). Gls1 deficiency impairs T_h17 cell differentiation by limiting αKG supply. αKG is a cofactor for DNA and histone demethylases, which modify chromatin methylation to impact gene expression (Xiao et al., 2012). Gls1 disruption

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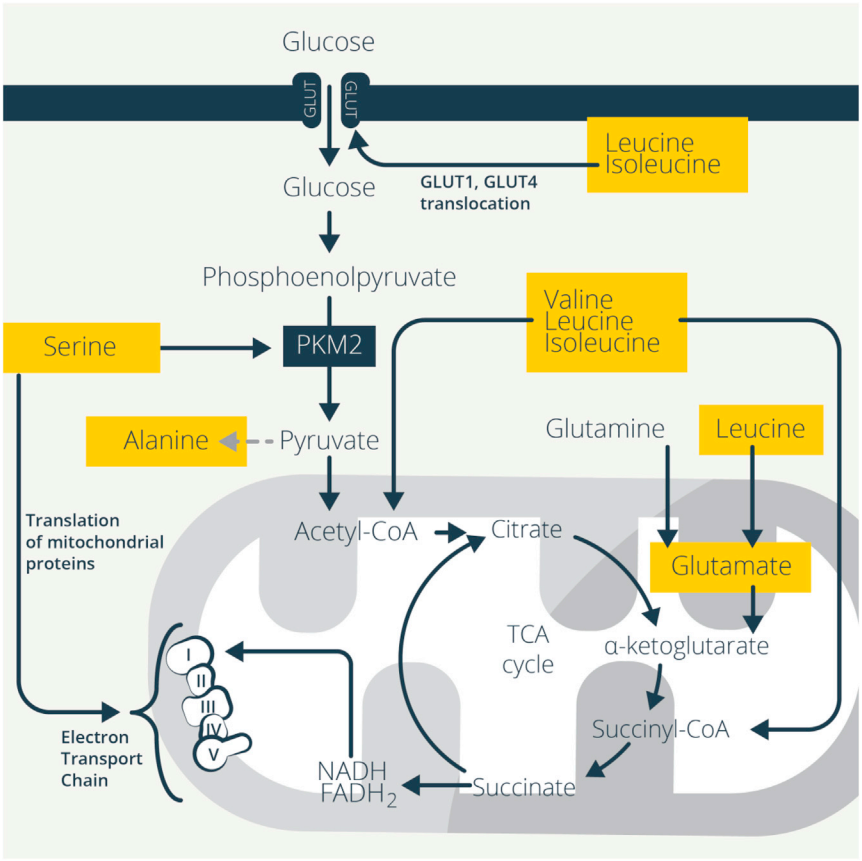

Figure 1. Amino Acids Support Glycolysis and Mitochondrial Metabolism

Amino acids influence cellular metabolism by contributing to glycolysis, and the tricarboxylic acid (TCA) cycle and OXPHOS in the mitochondria. Leucine and isoleucine increase translocation of the glucose transporters GLUT1 and GLUT4 to the cell surface in muscle cells and provide CoA intermediates to the TCA cycle, as does valine. Serine activates the key glycolytic enzyme PKM2 in macrophages and can increase translation of mitochondrial proteins in T cells, including those of the electron transport chain. PKM2 activity is also needed for T cell differentiation and cytokine production. While pyruvate can be used to make alanine, activated T cells decrease alanine synthesis from pyruvate, in order to preserve pyruvate metabolism to acetyl-CoA for TCA cycle activity. In macrophages and plasma cells, glutamine supplies the TCA cycle by anaplerosis to α-ketoglutarate, via glutamate.

restrains mTORC1 and IL-2 signaling, and consequent T cell differentiation, by altering the effects of αKG on chromatin (Johnson et al., 2018). At the same time, Gls1 deficiency increases the transcription factor T-bet to promote differentiation and effector function of T_h1 and $CD8^+$ CTLs. Restricting glutaminolysis may increase glutamine availability for other processes, or may limit αKG to generate an epigenetic landscape supporting T-bet-mediated T_h1 cell differentiation and function. Therapeutically, Gls1 inhibition ameliorates hyperinflammation in experimental autoimmune encephalomyelitis (EAE) (Kono et al., 2018) and in a model of rheumatoid arthritis (Takahashi et al., 2017). Downstream of glutamine uptake, glutamine degradation by glutaminolysis is a major catabolic pathway supporting the function of specific T cell subsets.

Glutamine metabolism differs between different immune cell types. IL-2/IL-12-induced Myc-dependent natural killer (NK) cell activation requires Slc7a5-mediated glutamine uptake (Loftus et al., 2018). Glutamine acquisition, not degradation, is important in this context, as glutamine withdrawal, but not glutaminolysis blockade, inhibits NK cell activation. NK cells could instead use glutamine for glutathione or hexosamine synthesis. In contrast, glutaminolysis supports antibody production by activated B cells. Overexpression of the let-7adf microRNA cluster represses ASCT2 and Gls1 expression in activated B cells, decreasing IgM and IgG production (Jiang et al., 2018). Gls1 inhibition may be the important event here, as B cells lacking ASCT2 develop and proliferate normally, and antibody production in ASCT2-deficient mice in response to *Bordetella pertussis* is normal (Masle-Farquhar et al., 2017).

Anaplerosis is a mechanism of replenishing TCA cycle intermediates that have been removed for biosynthetic reactions. Glutamine anaplerosis to αKG is increased in pro-inflammatory M1-like macrophages, and, due to a break in the TCA cycle, causes a build-up of succinate, which drives IL-1β production. Nitric oxide (NO)-mediated inhibition of pyruvate dehydrogenase and aconitase-2 limits entry of metabolites into the TCA cycle, promoting this glutamine anaplerosis to αKG in inflammatory macrophages (Palmieri et al., 2020). Glutamine is needed to support LPS induction of IL-1 production by macrophages (Wallace and Keast, 1992), though it does not appear to be required for M1-like macrophage development. Glutamine flux into the TCA cycle underlies M2-like macrophage polarization by IL-4 (Jha et al., 2015; Liu et al., 2017). Plasma cells also use glutamine for anaplerotic glutamate and aspartate replacement (Lam et al., 2018). In contrast, activated NK cells use some glutamine to replenish TCA cycle intermediates and increase OXPHOS, but the glucose-supplied citrate-malate shuttle has a bigger role in driving TCA cycle activity in these cells (Loftus et al., 2018). NKT cells, a cell type that shares features of both innate and adaptive immune cells and express a highly restricted TCR repertoire, need glutamine for survival and proliferation, though how this glutamine is used is unknown (Kumar et al., 2019). Clearly, many immune cells increase glutamine uptake. Glutaminolysis supports the function of some of these immune cells, while others funnel glutamine into other metabolic pathways. Improved understanding of glutamine utilization in different cell types will enable more specific targeting of glutamine metabolism in a therapeutic setting.

Branched-Chain Amino Acids Provide TCA Cycle Intermediates

Glutamine is not the only amino acid that supports metabolic reprogramming by supplying TCA cycle metabolites. The

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Box 1. How Metabolism Links to Immune Cell Activation and Function

Immune cell activation induces metabolic reprogramming compared to unstimulated or naive immune cells. Pro-inflammatory M1-like macrophages dampen OXPHOS and rely on glycolysis, the pentose phosphate pathway, and fatty acid synthesis (Krawczyk et al., 2010; Newsholme et al., 1986; Tannahill et al., 2013). M2-like macrophages, which promote anti-helminth responses and resolve inflammation, rely more on the TCA cycle to support OXPHOS, as well as FAO (Huang et al., 2014; Vats et al., 2006). DCs also upregulate glycolysis upon activation (Wculek et al., 2019). Upon TCR stimulation, naive T cells proliferate and differentiate into different subtypes. Glycolysis and OXPHOS drive proliferation and cytokine production by T_{eff} cells (Chang et al., 2013; Sena and Chandel, 2012), while $CD8^+$ T_{mem} cell generation and survival depend on OXPHOS and FAO (Pearce et al., 2009; van der Windt et al., 2012). Naive and regulatory $CD4^+$ T cells engage FAO (Beier et al., 2015), while glycolysis predominates in T_h1, T_h2, and T_h17 effector $CD4^+$ T cells (Gerriets et al., 2015). B cell receptor (BCR) stimulation of naive B cells increases glycolysis and OXPHOS (Akkaya et al., 2018), while antibody-secreting cells rely on sustained glutamine consumption (Garcia-Manteiga et al., 2011). These pathways are interlinked and co-regulated, and increased use of a particular pathway does not mean that other pathways are completely abandoned. Acquisition of energy and use of metabolites is fundamental to cell function, and immune cells can quickly switch between metabolic pathways to support various activities. Nutrient supply and transporter expression dictate metabolic pathway utilization, underlying how varying microenvironments and signals can alter immune cell function.

branched-chain amino acids (BCAAs) leucine, isoleucine, and valine provide the coenzyme A (CoA) derivatives acetyl-CoA and succinyl-CoA, which enter the TCA cycle (Neinast et al., 2019). Leucine transamination also produces glutamate, which feeds the TCA cycle via αKG. Acetyl-CoA condenses with oxaloacetate to form the TCA intermediate citrate. Similar to glutamine transporters, large neutral amino acid transporters are increased upon immune cell activation. L-type amino acid transporter 1 (LAT1; Slc7a5) is an excellent example of how an amino acid transporter can link pathogen infection to the immune response. *In vivo* listeria infection increases Slc7a5 expression on T cells, and IL-2 sustains this expression, maintaining continuous BCAA supply to the activated T cells (Sinclair et al., 2013). TCR stimulation of human T cells upregulates Slc7a5 (Hayashi et al., 2013), as does IL-18 stimulation of NK cells (Almutairi et al., 2019). Slc7a5 inhibition dampens IFN-γ and IL-17 production (Hayashi et al., 2013), and T_h1 and T_h17 cell development, but T_{reg} cells develop normally (Sinclair et al., 2013). Slc7a5-deficient T cells cannot undergo the mTORC1- and Myc-dependent increase in glycolysis necessary for activation (Sinclair et al., 2013). Strikingly, knockout of Slc7a5 alone largely phenocopies the effect of Myc deficiency on the activated $CD4^+$ T cell proteome, and impairs T cell growth (Marchingo et al., 2020). The fact that loss of a single transporter has effects as drastic as knocking out such a central regulator as Myc illustrates the importance of amino acid transport and acquisition for T cell function.

Slc7a5 also underlies macrophage metabolic rewiring. LPS increases BCAA transporter expression in macrophages, and macrophages lacking leucine transport via Slc7a5 have reduced glycolysis and IL-1β production (Yoon et al., 2018). CD98 is needed for Foxp3+ T_{reg} (Ikeda et al., 2017) formation, proliferation of T and B cells (Cantor et al., 2009, 2011), and NK cell cytokine production (Jensen et al., 2017). CD98 expression is high in antibody-secreting plasma cells, and its absence impairs antibody production. Cell surface expression of CD98 correlates with plasma cell longevity (Shi et al., 2015; Tellier et al., 2016), and plasma cells with high surface levels of CD98 secrete more antibodies than short-lived plasma cells with low surface CD98 expression. Interestingly, these populations with very different longevities have similar transcriptional profiles, but metabolic parameters such as CD98 expression, glucose uptake, and pyruvate-dependent mitochondrial respiration are better able to distinguish between long- and short-lived plasma cells (Lam et al., 2016, 2018). Understanding plasma cell longevity and persistence is beneficial in terms of generating long-lived immune responses after vaccination (White et al., 2015). Given these clear differences in metabolic signatures, manipulation of plasma cell metabolism is an intriguing potential method to increase the duration of humoral immunity to pathogens.

Branched-chain amino acid aminotransferase (BCAT1), the first enzyme of BCAA catabolism, transaminates BCAAs to branched-chain α-keto acids, which are decarboxylated to form CoA derivatives (Brosnan and Brosnan, 2006). BCAT1 inhibition restrains both glycolysis and oxygen consumption, and limits production of itaconate (Papathanassiu et al., 2017), an anti-inflammatory metabolite (Mills et al., 2018). Older work showed that mice fed diets lacking BCAAs generate defective antibody and cytotoxic T cell responses in response to *Salmonella typhimurium* (Petro and Bhattacharjee, 1981) or mammary adenocarcinoma (Jose and Good, 1973), while BCAA supplementation enhanced liver $CD8^+$ T cell activity in a murine model of liver cirrhosis (Tsukishiro et al., 2000), and increased lymphocyte numbers and responses to skin antigens in patients with post-operative trauma or sepsis (Cerra et al., 1984). Valine boosts DC IL-12 production in cirrhotic patients (Kakazu et al., 2007). BCAAs could also support metabolic reprogramming of immune cells by stimulating glucose uptake to promote glycolysis. In rat muscle cells, leucine and isoleucine increase translocation of the glucose transporters (GLUTs) GLUT1 and GLUT4 to the cell surface to increase glucose uptake (Nishitani et al., 2005), in a process that may depend on PI3K and protein kinase C (Doi et al., 2005a). Leucine-supplied acetyl-CoA could also acetylate and activate mTORC1 (Son et al., 2019), further reinforcing enhanced glycolysis.

Serine Promotes Glycolysis and Mitochondrial Metabolism

Serine also increases glycolytic flux. It ligates and allosterically activates pyruvate kinase M2 (PKM2) (Chaneton et al., 2012), which converts phosphoenolpyruvate to pyruvate in the last step of glycolysis. LPS induces PKM2 in macrophages to drive the metabolic switch toward glycolysis and IL-1β induction (Palsson-McDermott et al., 2015). TCR stimulation of $CD4^+$

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T cells increases nuclear translocation of PKM2 (Wang et al., 2011), which increases glycolysis to support T_h1 and T_h17 cell generation, and tumor necrosis factor (TNF) and IL-17 production. Inhibition of PKM2 nuclear translocation by inducing its tetramerization limits T_h1 and T_h17 generation and cytokine production, and inhibits the development of EAE, a murine model of neural inflammation including multiple sclerosis (Angiari et al., 2020). Serine limitation also inhibits PKM2 to decrease excessive macrophage activation in atherosclerosis (Shirai et al., 2016), and inhibition of serine synthesis decreases IL-1β and TNF production in LPS-induced endotoxemia (Rodriguez et al., 2019).

Serine also supports mitochondrial metabolism. The catabolic enzyme serine hydroxymethyltransferase (Shmt2) is needed for mitochondrial translation and respiratory activity (Minton et al., 2018), and Shmt2-deficient mice have respiratory defects (Tani et al., 2018). This effect depends on generation of one-carbon (1-C) units from serine (Stover and Schirch, 1990). 1-C units are used for processes including nucleotide synthesis and methionine recycling, and enzymes of mitochondrial 1-C metabolism are upregulated in proliferative tissues (Mejia and MacKenzie, 1985; Nilsson et al., 2014). Mitochondrial translation initiation depends on the modified tRNA, N-formylmethionine-tRNAMet (fMet-tRNAMet), formed from serine via Shmt2. 1-C unit supplementation rescues defects in respiration and mitochondrial translation in Shmt2-deficient Jurkat cells. This pathway seems to be especially important in low-glucose conditions that induce cells to increase mitochondrial metabolism in a compensatory manner, so it may also be important for immune cells that switch from glycolytic to mitochondrial metabolism. However, even T cells with sufficient glucose to support increased glycolysis, OXPHOS, and effector function rely on 1-C metabolism downstream of serine (Ma et al., 2017). Methylene tetrahydrofolate dehydrogenase 2 catalyzes intermediate steps in mitochondrial fMet-tRNAMet formation, generating NADH. In pancreatic tissue with impaired mitochondrial respiration, this NADH can accumulate to toxic levels, inhibiting cell growth (Yang et al., 2020). It would be intriguing to investigate whether cells that switch away from mitochondrial metabolism concomitantly decrease this serine catabolism to avoid such toxicity and permit continued growth.

While other amino acids can fuel glycolysis and the TCA cycle, sometimes T cells do not use them for these pathways. Extracellular alanine is necessary for early activation of T cells (Ron-Harel et al., 2019). While alanine can be metabolized to pyruvate, the end product of glycolysis, it is not used for this purpose in activated T cells, and is instead used for protein synthesis. Further, although alanine can be synthesized from glucose via alanine aminotransferase, expression of this enzyme is low in T cells early after activation, which instead take up extracellular alanine via SNAT1 (Matheson et al., 2015). This preserves pyruvate for acetyl-CoA production, TCA cycle activity, and OXPHOS, while the extracellular alanine is used for protein synthesis.

Amino Acids Control Sulfur and Redox Metabolism

Immune cell activation frequently depends on increased ROS production (Kamiński et al., 2012; Sena et al., 2013), which can be generated by the mitochondria and cytoplasmic NADPH oxidases. LPS induces ROS production in macrophages to drive cytokine production (Bulua et al., 2011) and bacterial killing (West et al., 2011). ROS can activate the nuclear factor of activated T cells (NFAT), a T cell-specific transcription factor that induces IL-2 production and cell-cycle engagement, increasing T cell proliferation (Sena et al., 2013). Elevated ROS drive calcium signaling, promoting nuclear translocation of NFAT to increase transcription of target genes, including Myc, needed for T cell activation and metabolic reprogramming toward increased glycolysis and glutaminolysis. The balance of ROS is critical, as an excess of these free radicals can cause cell damage and subsequent pathology (Chouchani et al., 2014; Sena and Chandel, 2012). BCR stimulation of naive B cells also induces massive ROS production and calcium-induced mitochondrial dysfunction. This causes activation-induced cell death unless the B cell also receives costimulatory signals such as TLR or CD40 engagement, which prevent the mitochondrial dysfunction through an uncharacterized mechanism (Akkaya et al., 2018). Thus, cells have multiple antioxidant mechanisms to control ROS levels, and amino acids are crucial for maintaining these defenses and redox balance (Figure 2). Major cellular antioxidants include glutathione, thioredoxins, glutaredoxins, superoxide dismutase, and catalase (Sies et al., 2017; Zhang and Hannink, 2003). For example, glutathione peroxidase 4 prevents lipid oxidation to support T cell survival and activation (Matsushita et al., 2015). Immune cells have harnessed these antioxidant systems to modulate activation and cytokine production.

Glutathione Is Needed for T Cell and Macrophage Function

Glutathione is a small molecule composed of glycine, glutamate, and cysteine. Thus, supply of these amino acids dictates glutathione levels. The reduced form of glutathione (GSH) is oxidized to form a disulfide bridge with another GSH molecule, becoming oxidized glutathione (GSSG). GSSG is recycled to GSH by glutathione reductase (GSR), maintaining an intracellular GSH pool that detoxifies ROS (Lu, 2009). Increased ROS production upon immune cell activation increases demand for GSH, and such increased GSH synthesis requires a supply of its component amino acids. GSH levels are indeed increased upon T cell activation (Mak et al., 2017), and *de novo* GSH synthesis, rather than recycling from GSSG, is important for T cell differentiation and function (Lian et al., 2018). Deficiency of the GSH synthetic enzyme glutamate cysteine ligase (GCLC), but not that of the GSH recycling enzyme GSR, reduces activated T cell viability, proliferation, and expression of the activation marker CD25 due to aberrant ROS balance (Lian et al., 2018). Glutamine supplies glutamate for such *de novo* GSH synthesis, and Gls1 inhibition antagonizes T cell differentiation and function. Glutathione also impacts the increased glycolysis and glutamine utilization underlying T cell activation. Conditional *Gclc* deletion abolishes mTORC1 activation, the nuclear accumulation of NFAT, and the Myc-dependent switch toward glycolysis and glutaminolysis in activated $CD4^+$ and $CD8^+$ T cells (Mak et al., 2017). These cells initially activate normally but cannot meet the metabolic demands for expansion and fail to proliferate (Mak et al., 2017). In contrast, *Gclc*-deficient T_{reg} cells increase mTOR activity, which inhibits Foxp3 expression and T_{reg} suppressive function (Kurniawan et al., 2020). *Gclc*-deficient T_{reg} cells increase serine import and synthesis, possibly in an attempt to generate glycine for GSH, via Shmt enzymes. In this context of impaired GSH

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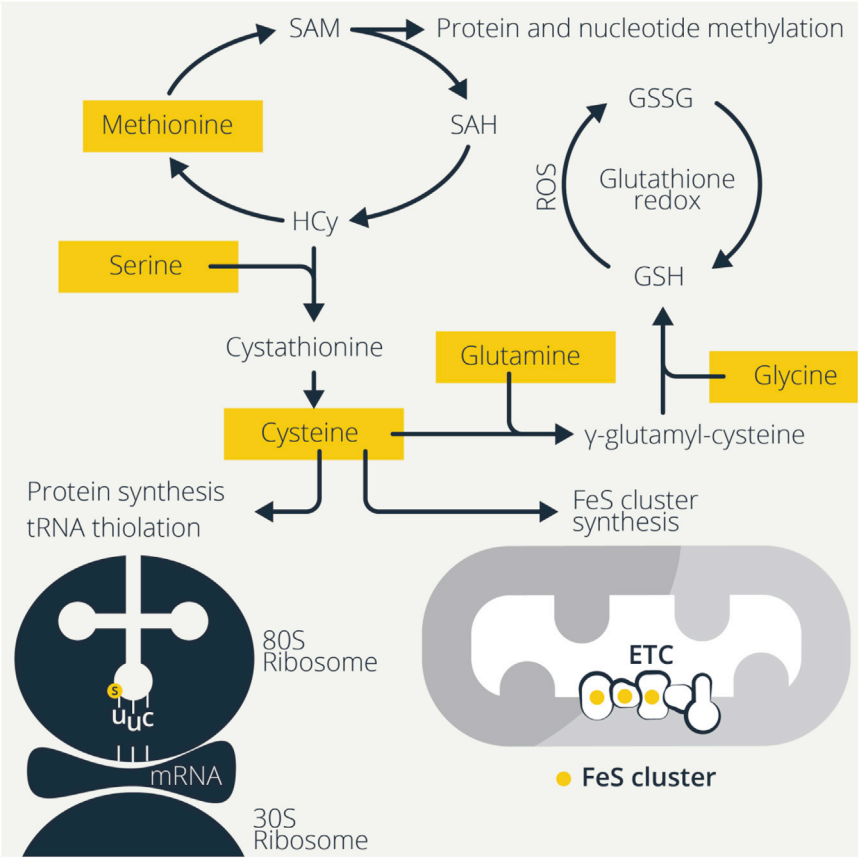

Figure 2. Sulfurous Amino Acids Maintain Redox Balance and Drive Protein Synthesis

Cysteine, glutamine, and glycine make the antioxidant glutathione (GSH), which is oxidized to GSSG to detoxify reactive oxygen and maintain intracellular redox balance. T cells increase GSH synthesis upon activation, and B cells and macrophages also need GSH to regulate redox status after ROS production upon activation. DCs can aid this process by providing cysteine to other immune cells. Methionine and serine can be metabolized to cysteine, with methionine also generating S-adenosylmethionine (SAM), which provides methyl groups to modify immune effector proteins and nucleic acids, facilitating cytokine gene expression in T cells and macrophages, and both innate and adaptive immune memory. Cysteine is a sulfur donor for processes including tRNA thiolation, which aids translation, and iron sulfur (FeS) cluster synthesis. FeS clusters are key for the activity of a variety of enzymes, including components of the electron transport chain.

synthesis, serine instead activates mTOR, which inhibits Foxp3 expression. Serine limitation restores Foxp3 expression and T_{reg} suppressive activity, decreasing lethal auto-inflammation *in vivo*. So one function of GSH in T_{reg} cells is to restrict serine metabolism, thereby maintaining their suppressive function. Macrophages also use serine to generate glycine for GSH, needed for LPS-induced IL-1β mRNA expression (Rodriguez et al., 2019). This serine-glycine-GSH regulatory loop illustrates how amino acids and small peptides regulate the metabolism of other amino acids.

Cysteine/cystine import supports immune cell function, at least in part by facilitating GSH synthesis. Resting B cells do not express xCT (Slc7a11) but, upon activation, increase xCT expression and cystine import, possibly to produce GSH to counter the ROS production that would otherwise cause activation-induced cell death (Vené et al., 2010). LPS activation of DCs increases system x_c^- cystine-glutamate antiporter activity (D'Angelo et al., 2010), which may be needed to increase GSH synthesis to counter the increased ROS production upon activation. DCs can also export GSH to shape the extracellular redox environment, particularly around the immune synapse between DCs and T cells. This GSH is broken down to cysteine, which decreases extracellular redox potential and promotes $CD4^+$ T_{eff} cell proliferation, possibly as $CD4^+$ T_{eff} cells acquire this cysteine for GSH production and consequent proliferation (Yan et al., 2009). It alters the redox status of proteins on the T cell surface, with as yet unknown consequences. Further, co-culture of T_{reg} cells with DCs and $CD4^+$ T_{eff} cells depletes extracellular cysteine, possibly explaining some of the suppressive effect of T_{reg} cells on T_{eff} cell proliferation. Thus, innate immune cells modulate amino acids in order to regulate adaptive immune cell function.

Cysteine Supports Sulfur-Dependent Metabolism

Cysteine is a key amino acid for GSH function, as it supplies the sulfur necessary for formation of the disulfide bridge in GSSG, but its roles extend beyond GSH synthesis. Cysteine predominantly exists in its oxidized form, cystine, in the oxidative extracellular environment. Cystine uptake is needed for T cell activation (Srivastava et al., 2010), proliferation (Levring et al., 2012), and DNA synthesis (Levring et al., 2015). Blockade of cystine uptake by T cells is protective against EAE (Evonuk et al., 2015). Similar to B cells, resting T cells do not express xCT, but activated $CD4^+$ and $CD8^+$ human T cells increase xCT to promote cystine uptake (Siska et al., 2016). Blockade of this antiporter in monocytes inhibits their differentiation into DCs in response to IL-4 and granulocyte-macrophage colony-stimulating factor, and its inhibition in mature DCs impairs antigen presentation to $CD4^+$ T cells, as well as cross-presentation to $CD8^+$ T cells (D'Angelo et al., 2010).

Only two of the proteinogenic amino acids contain sulfur, and they are intimately related. Methionine transulfuration to cysteine maintains cysteine-dependent processes when extracellular cysteine is in limited supply. Via S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), methionine can be used to produce homocysteine, which forms cystathionine when combined with serine by cystathionine-β-synthase (Stipanuk, 1986). This in turn can be metabolized by cystathionine-γ-lyase to form cysteine. Cysteine limitation induces expression of these transulfuration enzymes (Zhu et al., 2019). This process is known to occur in the liver and has now been demonstrated in cancer cells (Zhu et al., 2019), though it has not yet been investigated in an immune cell context.

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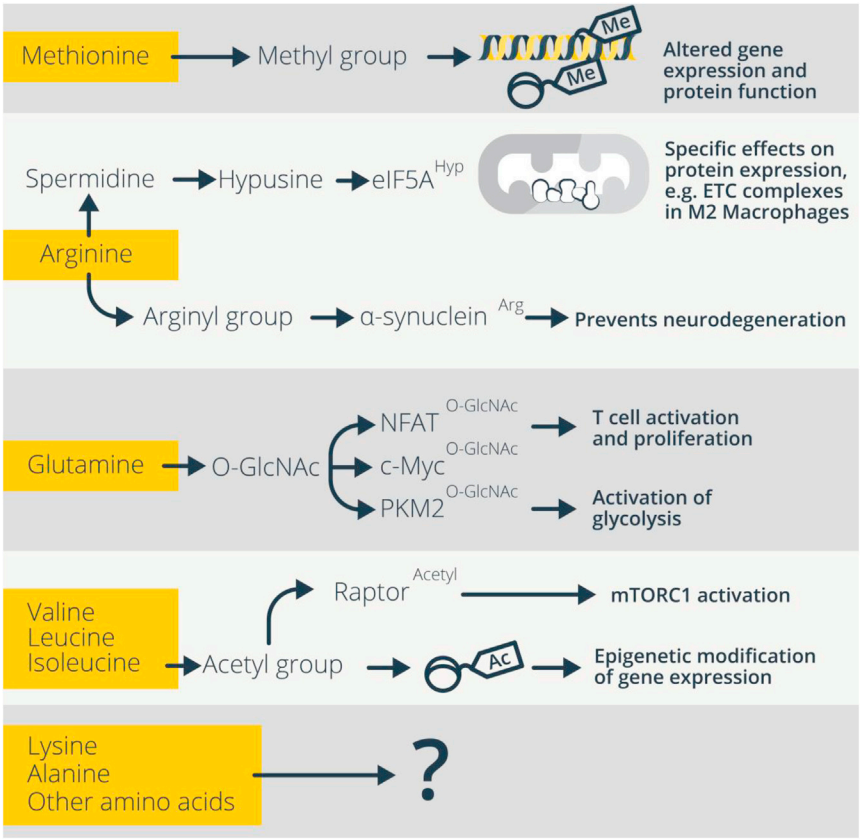

Figure 3. Amino Acids Influence Cell Function by Supplying Intermediates for Protein and Nucleotide Modifications

Amino acids provide a variety of functional groups that can be conjugated to intracellular targets. Methionine, arginine, glutamine, and the branched-chain amino acids provide intermediates for post-translational modifications (PTMs) of proteins. Such PTMs can alter the activity of metabolic pathways and immune cell function by changing the activity of target proteins. Many amino acids have not yet been investigated in this context but are likely to provide intermediates that can modify proteins and thus modulate cellular function.

Sulfur supply from cysteine is also critical for maintaining mitochondrial metabolism via the synthesis of iron-sulfur (FeS) clusters (Lill, 2009), key components of many enzymes, including some electron transport chain (ETC) subunits. The cysteine desulfurase NFS1 removes sulfur from cysteine for FeS clusters, and cysteine limitation decreases FeS cluster synthesis. Loss of FeS clusters can itself impact metabolic pathways, as the FeS cluster-containing enzyme aconitase can no longer metabolize citrate, leading to a buildup of this TCA cycle intermediate and its use in fatty acid synthesis (Crooks et al., 2018). Arginine-derived NO destabilizes FeS clusters (Drapier, 1997), demonstrating a mechanism by which NO may antagonize mitochondrial metabolism in M1-like macrophages. Besides decreasing mitochondrial metabolism, decreasing FeS cluster synthesis leads to an excess of free iron, as it is not being used for these clusters. This can drive ferroptosis, an iron-dependent form of cell death (Dixon et al., 2012). Cystine-GSH crosstalk regulates amino acid signaling and ferroptosis (Yu and Long, 2016). Indeed, maintaining expression of NFS1 can protect against ferroptosis, as occurs in tumor cells (Alvarez et al., 2017).

NFS1 also uses the sulfur atom of cysteine to thiolate tRNA, as shown in *Saccharomyces cerevisiae* (Nakai et al., 2004). Lysine, glutamine, and glutamate tRNAs can be thiolated on the uridine nucleotide in the wobble position (U34), easing translation by aiding tRNA translocation through the ribosome (Phelps et al., 2004; Yarian et al., 2002). Genes enriched in lysine, glutamine, and glutamate codons occur frequently in proteins important for translation and growth. During sulfur limitation, i.e., methionine and cysteine limitation, such tRNA thiolation is decreased, slowing translation when nutrients are limiting (Laxman et al., 2013). FeS cluster synthesis and tRNA thiolation have not yet been investigated in immune cells, but may have a role to play here, as maintenance of mitochondrial metabolism and increased translation underlie immune cell activation. It is easy to imagine a situation in which sulfur could become limiting in T cells and macrophages, which increase ROS upon activation, and consequently induce production of antioxidants, including GSH (Mak et al., 2017). Prioritizing such GSH production could limit the use of sulfur for translation, and eventually curb immune cell proliferation and effector function. Such sulfur competition could be exacerbated in sulfur-deficient microenvironments, and could contribute to differences in proliferation in different environments; for example, the continued T cell expansion in lymph nodes versus decreased proliferation in tissue populations.

Amino Acids Support Nucleic Acid and Protein Modification

Amino Acids Supply Methyl Groups for Methylation

Besides its contribution to sulfur metabolism, methionine donates methyl groups for methylation of proteins and nucleotides to modulate their function (Figures 2 and 3). Histone and DNA methylation can promote or inhibit transcription by altering the accessibility of DNA to transcriptional machinery (Allis and Jenuwein, 2016). Both inhibitory (histone H3 lysine 27, H3K27) and activating (H3K4) histone methylation occur upon T cell activation, underlying transcriptional remodeling (Henning et al., 2018; Sinclair et al., 2019). RNA methylation controls such processes as mRNA binding to translation initiation factors, mRNA stability, and splicing (Aregger and Cowling, 2017; Varshney et al., 2018). Adenosine methylation to control mRNA stability is important for T cell homeostasis, and T cells lacking the N6-methyladenosine methylation (m6A) of RNA do not expand and differentiate (Li et al., 2017). Methionine supplies methyl groups by producing SAM, the methyl donor for DNA, RNA, and protein methyltransferases (Lu, 2000), which transfer the methyl group

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from SAM to a substrate, generating SAH and a methylated substrate (Figure 2). S-adenosylhomocysteine hydrolase then metabolizes SAH to homocysteine (Hcy). Hcy can be used to generate cysteine, a process that is increased in activated $CD4^+$ T cells (further illustrating the demand for cysteine in activated T cells) or can be recycled to methionine. T_h17 cells starved of methionine or subjected to methionine cycle inhibition exhibit reduced H3K4 methylation at the *Il17a* promoter and decrease IL-17 production, while methionine-starved T_h1 cells produce less IFN-γ (Roy et al., 2020). Further, dietary methionine restriction decreases the numbers of IL-17- and IFN-γ-producing cells in mice with EAE, delaying the onset of disease and reducing its severity.

As well as its acute impact on immune cell function, methionine status can have longer-lasting effects by epigenetically modifying immune cell memory. Trained immunity is a program of long-term change that occurs in innate immune cells due to pathogen exposure, facilitating increased responses upon restimulation (Netea et al., 2016). β-glucan training of human peripheral blood mononuclear cells enhances H3K4 trimethylation at the promoters of genes for cytokines and immune signaling factors, including MyD88, TNF, and IL-6 (Quintin et al., 2012). This may underlie the boost in cytokine production by these cells upon re-exposure to *Candida albicans*. Adaptive immune memory also relies on histone methylation. Activating histone marks for cytokines (IL-17, IFN-γ) and T cell-specific transcription factors (T-bet, ROR-γ) are enriched in memory $CD4^+$ T cells compared to naive $CD4^+$ T cells, and this poised chromatin state leads to more rapid induction of these cytokines in memory T cells upon stimulation (Barski et al., 2017; Durek et al., 2016). Similarly, the genome-wide pattern of both stimulatory (H3K4) and inhibitory (H3K27) methylation in memory $CD8^+$ T cells is more similar to T_{eff} cells than to naive T cells (Crompton et al., 2016; Russ et al., 2014). Genes for proteins underlying $CD8^+$ memory T cell formation, such as the transcription factor B-lymphocyte-induced maturation protein (BLIMP)1, and cytotoxic effector molecules granzme A and perforin, are hypermethylated on histone H3 (Araki et al., 2009b), facilitating increased expression of these genes and illustrating how epigenetic marks support memory T cell function. Histone methyltransferases mediate histone methylation, using SAM as a methyl donor and producing SAH. SAH can act as a competitive inhibitor of SAM, and thus the balance between SAM and SAH, and flux through the methionine cycle, could impact the extent of histone methylation.

Methionine Transport Underlies T Cell Activation

Antigen-stimulated T cells increase and sustain methionine transport into the cell, which is a rate-limiting step for the supply of methyl donors and methylation of targets in activated T cells (Sinclair et al., 2019). Expression of methionine transporters is restricted to activated T cells, so even though naive T cells have enzymes of methionine metabolism, they cannot transport sufficient methionine into the cell to increase protein synthesis. Thus, control of cellular methionine levels modulates T cell status. Only T cells that can increase methionine transport will have sufficient methionine to drive methylation, protein synthesis, and proliferation. Slc7a5 is the main methionine transporter in activated T cells and is important for T cell differentiation (Sinclair et al., 2013, 2019). Methionine supply is sensed by SAMTOR, which detects SAM and consequently modulates mTORC1 signaling (Gu et al., 2017). This illustrates how an amino acid metabolite, rather than the amino acid itself, is sensed by mTORC1 as a readout of amino acid levels. TCR stimulation promotes flux through the methionine cycle, demonstrated by increased production of SAH and Hcy, and $CD4^+$ T cells activated in the absence of methionine have proliferation defects, but normal expression of activation markers, and lower frequency of IFN-γ-producing cells (Sinclair et al., 2019). Methionine restriction limits T cell activation by blunting Myc induction, which is critical for T cell activation (Wang et al., 2011).

Arginine Contributes to Polyamine Metabolism

Methylation is not the only PTM controlled by amino acid supply (Figure 3). Arginine metabolism also leads to structural modification of proteins to influence immune cell function (Geiger et al., 2016; Puleston et al., 2019). Arginase I metabolizes arginine to ornithine, which feeds polyamine synthesis, including spermidine production. Highlighting the inherent interconnectedness of metabolic pathways, spermidine production also requires SAM. Thus, its synthesis may report that there is adequate nutrient supply to multiple metabolic pathways, and may signal that various metabolites and building blocks are present in sufficient levels to coordinately fuel biosynthesis, and allow proliferation to progress. Spermidine is used to make the unusual amino acid hypusine, which post-translationally modifies only one known target, eukaryotic translation initiation factor 5a (eIF5a) (Park et al., 1981), which drives translation elongation and termination (Saini et al., 2009; Schuller et al., 2017). This hypusination is critical for eIF5a to maintain expression of ETC enzymes and OXPHOS activity (Puleston et al., 2019), a process that is upregulated in IL-4-stimulated M2 macrophages. Arginase I activity is in fact a marker of alternative activation, as its activity is highly induced in M2-like macrophages, but reduced in M1-like macrophages, and arginine usage differs markedly in M2- versus M1-like macrophages (Rath et al., 2014). Mouse M1-like macrophages metabolize arginine via inducible NO synthase (iNOS) to produce citrulline and NO, which inhibits OXPHOS. As a consequence, glycolysis increases and supports pro-inflammatory macrophage function, including cytokine production. In M2-like macrophages, arginase I metabolizes arginine to ornithine and urea. This use of arginine by macrophages may also limit arginine to inhibit T cell activation (Pesce et al., 2009; Rodriguez et al., 2004). While arginase I activity is reduced in M1-like macrophages, it is not absent, and ornithine metabolism to polyamines by ornithine decarboxylase (ODC) limits M1-like macrophage activation and inflammation during bacterial infection. ODC deletion increases inflammatory gene expression due to altered histone methylation and acetylation, and re-addition of putrescine reverses these chromatin modifications and avoids the hyperinflammation caused by ODC deficiency (Hardbower et al., 2017). Polyamine production supports the function of M2-like macrophages while limiting inflammatory activity of M1-like macrophages.

Macrophages take up arginine from apoptotic cells, which is metabolized via arginase I and, in a putrescine-dependent manner, activates the actin regulatory protein Rac1 to enable further apoptotic cell internalization, ensuring a supply of metabolites for the macrophage (Yurdagul et al., 2020). Myeloid-derived suppressor cells (MDSCs) also increase arginine metabolism via arginase to limit arginine supply to T cells

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(Fletcher et al., 2015), and inhibit NK cell function and IFN-γ production in a similar manner (Goh et al., 2016; Lamas et al., 2012). Arginine-starved NK cells have reduced viability, expression of NK cell activating receptors NKp46 and NKp30, and diminished IFN-γ (Lamas et al., 2012). This phenomenon is exploited in chronic hepatitis C infection by the virus to limit antiviral immune responses. Similarly, T_h1 polarization promotes arginase I activity. Arginine drives a metabolic switch toward oxygen consumption and increases spare respiratory capacity in activated T cells, and supports $CD4^+$ and $CD8^+$ T cell survival (Geiger et al., 2016). Polyamine replacement partially rescues the detrimental effects of glutamine deprivation on T cell activation, indicating that one use of glutamine may be its metabolism to arginine for polyamine synthesis (Wang et al., 2011). Spermidine also has anti-aging effects (Madeo et al., 2018), which may result from its enhancement of immune cell function. Spermidine ameliorates the aging-induced decline in $CD8^+$ T cell responses to infection and vaccination in an autophagy-dependent manner (Puleston et al., 2014), and enhances anti-cancer immune responses (Pietrocola et al., 2016). As spermidine levels decrease with age (Pucciarelli et al., 2012), dietary supplementation of spermidine (Kiechl et al., 2018) or enhancing spermidine production by intestinal microbes (Kibe et al., 2014) may prove beneficial in extending lifespan, possibly by maintaining immune cell activity.

Glutamine Fuels the Hexosamine Biosynthesis Pathway

The hexosamine biosynthesis pathway (HBP) metabolizes glutamine to uridine diphosphate N-acetyl-glucosamine (UDP-GlcNAc). Glycosyltransferases use UDP-GlcNAc to glycosylate targets including proteins and lipids, to form glycoproteins, proteoglycans, and glycolipids (Lairson et al., 2008). Glutamine starvation or increased glutaminolysis limits glutamine supply to the HBP, thus reducing intracellular UDP-GlcNAc levels, with consequences for T cell function (Grigorian et al., 2007; Lau et al., 2007). N-linked glycosylation modulates TCR signaling and interaction with co-stimulatory molecules such as CD4 and CD8 (Demotte et al., 2008; Grigorian et al., 2009). Increased N-glycan branching of the TCR reduces its activity, inhibiting T cell proliferation (Demetriou et al., 2001), while N-glycan branching of the inhibitory molecule cytotoxic T-lymphocyte-associated protein (CTLA)4 promotes its retention on the cell surface, compounding this anti-growth effect (Chen et al., 2009). Flux through the HBP and N-linked glycosylation also regulate T_h17 cell differentiation. High glycolysis and glutaminolysis in T_h17 cells restrict HBP activity, limiting N-glycan branching and promoting T_h17 cell generation (Araujo et al., 2017). Convincingly, addition of glutamine or GlcNAC, or glutaminolysis inhibition, reverses these effects and blocks T_h17 cell production while driving T_{reg} cell differentiation. Antibody-secreting plasma cells also glycosylate antibodies to expand the antibody repertoire, as differential glycosylation of antibodies changes protein folding and interactions of antibodies with Fc receptors (Jennewein and Alter, 2017). It remains unknown whether different plasma cell subsets, or plasma cells in different nutrient environments, vary in terms of glutamine uptake and HBP usage, thereby generating different antibodies in a context-dependent manner.

UDP-GlcNAc is also the donor substrate for O-GlcNAc transferase (OGT), which catalyzes O-GlcNAcylation, a PTM in which O-GlcNAc is added to target proteins (Hart et al., 2007). TCR stimulation and c-Myc activation promote O-GlcNAcylation in $CD4^+$ T cells, which requires an increased supply of UDP-GlcNAc and therefore increased glutamine uptake. NFAT and c-Myc themselves are activated and stabilized by this modification (Golks et al., 2007; Swamy et al., 2016). OGT is needed for thymic T cell self-renewal, and T cell activation and expansion (Swamy et al., 2016), as well as nascent RNA synthesis, IL-2 production, and proliferation in primary human T cells (Lund et al., 2016). O-GlcNAcylation of PKM2 promotes its nuclear translocation and activation of glycolysis in leukemic cells and solid tumors (Wang et al., 2017b). Glutamine, as well as glycine and cysteine, can also be used for the S-glutathionylation modification, adding a glutathione tripeptide to target proteins (Dalle-Donne et al., 2009). It is unknown whether and how this modification functions in immune cells. Overall, it is clear that TCR stimulation increases UDP-GlcNAc production, which itself requires an increased supply of glutamine, again highlighting transport of glutamine into the cell as a point of control of T cell function.

BCAAs Support Acetylation

BCAA metabolism provides acetyl-CoA derivatives that can be used for acetylation. Leucine-derived acetyl-CoA is used to acetylate the mTORC1 regulator Raptor via the EP300 acetyltransferase in HeLa cells (Son et al., 2019). This activates mTOR, but it remains to be seen how specifically this mechanism reports on leucine status, as numerous other inputs can generate acetyl-CoA. Histone acetylation epigenetically modulates immune cell activation. In general, histone acetylation makes chromatin more accessible to transcriptional machinery and is associated with increased transcription, while histone deacetylation is associated with repression of transcription (Kouzarides, 2007). Broad alterations in H3K27 acetylation accompany macrophage differentiation, and differences in histone acetylation contribute to the induction of innate immune tolerance (decreased responsiveness to restimulation) or training (increased responsiveness to restimulation) (Saeed et al., 2014). Genes involved in glycolysis and mTOR signaling exhibit altered acetylation patterns upon β-glucan training of human primary monocytes (Cheng et al., 2014). Modulation of HDAC activity and promoter acetylation can both positively and negatively regulate inflammatory gene expression. For example, broad-spectrum HDAC inhibition limits LPS induction of TNF and IL-6 in macrophages, but increases IFN-β production (Roger et al., 2011). More specific HDAC inhibition reveals that different HDACs can have opposing effects. HDAC1 and HDAC8 inhibit IFN-β expression, while HDAC6 promotes enhancer activity for this cytokine (Nusinzon and Horvath, 2006). H3K9 deacetylation mediated by HDAC3 limits M2-like macrophage activation by repressing IL-4 induction of genes characteristic of M2-like macrophages (Mullican et al., 2011).

Non-histone protein acetylation also impacts immune cell function. Activity of the central pro-inflammatory transcription factor nuclear factor (NF) κB requires its acetylation (Yeung et al., 2004), and NOD-, LRR-, and pyrin domain-containing protein (NLRP)3 acetylation promotes inflammasome activation (He et al., 2020). Acetylation likely underlies the metabolic switches accompanying immune cell activation, as the majority of glycolytic and TCA cycle enzymes can be acetylated, with consequences for enzymatic function. For example, glyceraldehyde 3-phosphate dehydrogenase acetylation increases its enzymatic activity in memory $CD8^+$ T cells, boosting glycolysis to

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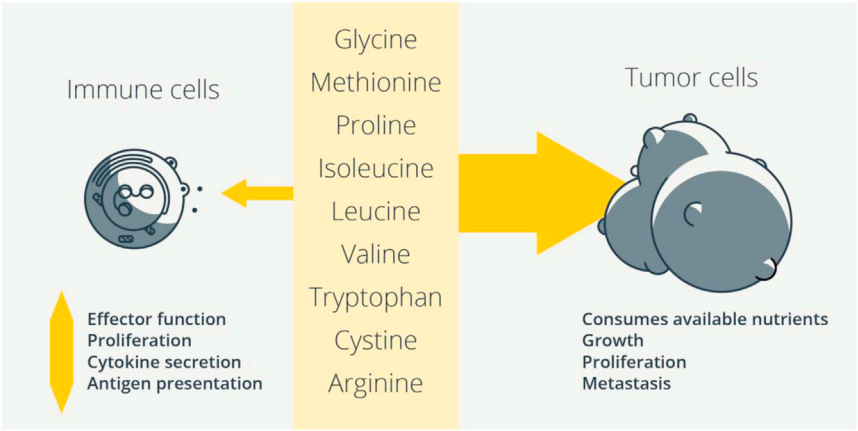

Figure 4. Do Immune Cells and Tumor Cells Compete for Amino Acids in the Tumor Microenvironment?

Within the tumor microenvironment, cancer cells and immune cells may compete for limited amino acid resources. Restricting amino acid supply to some immune cells, such as $CD8^+$ T_{eff} cells, decreases their proliferation and effector function, thereby decreasing anti-tumor immunity. At the same time, limitation of amino acid supply to suppressive immune cells, such as T_{reg} cells or MDSCs, could antagonize their suppression of effector cell function, thereby boosting anti-tumor immunity. Increased consumption of amino acids by tumor cells fuels their proliferation, growth, and metastasis. Understanding and targeting amino acid consumption and metabolism by tumor cells and tumor-associated immune cells is likely to be beneficial in cancer treatment.

support rapid recall responses and cytokine production (Balmer et al., 2016). These memory $CD8^+$ T cells increase acetate uptake to expand their acetyl-CoA pool to support this increased acetylation, but increased BCAA supply could have the same effect. Conversely, deacetylation mediated by HDACs can be anti-inflammatory, and so limiting supply of BCAAs for acetylation could achieve similar results.

Amino Acids Are Used for Immune Cell Nucleotide Synthesis

Nucleotides are needed to make DNA and RNA during cell division and transcription (Sigoillot et al., 2003). Both purine and pyrimidine nucleotides promote activated T cell progression through the cell cycle (Quéméneur et al., 2003), and Myc increases expression of nucleotide synthetic genes (Liu et al., 2008). Aspartate and glutamine provide carbon skeletons for pyrimidine ring formation, while glycine and tetrahydrofolate (THF) from serine-glycine metabolism provide carbon for purine synthesis (Lane and Fan, 2015). Nucleotides can partially rescue the detrimental effects of glutamine deprivation on T cell activation, indicating that one function of glutamine in immune cell activation is provision of nucleotides (Wang et al., 2011).

$CD8^+$ T_{eff} cells increase expression of components of the serine, glycine, one-carbon (SGOC) network upon activation, and serine limitation impairs T cell proliferation, but not expression of the activation markers CD69, CD25, and CD44, or production of IFN-γ (Ma et al., 2017). These T cells need extracellular serine to support purine nucleotide production, and provision of serine-deprived T cells with formate and glycine is sufficient to resume purine biosynthesis and T cell proliferation during serine starvation (Ma et al., 2017). Serine is a major carbon donor to the 1-C pathway. As already mentioned, the mitochondrial form of the Shmt enzyme (Shmt2) is critical for mitochondrial translation. The cytoplasmic form of this enzyme, Shmt1, supports *de novo* nucleotide biosynthesis. Glycine has also been reported to have anti-proliferative effects on T cells, by opening a glycine-gated channel in the plasma membrane to reduce intracellular calcium levels, which are necessary for T cell activation and proliferation (Stachlewitz et al., 2000). These differential effects of glycine may be due to the dose of glycine used, or the timing of glycine supplementation.

Aspartate is also needed for nucleotide synthesis. Jurkat cells with inhibited ETC activity have decreased aspartate synthesis, which slows proliferation in these cells (Birsoy et al., 2015). The cytosolic aspartate aminotransferase GOT1 transaminates aspartate to glutamate to transfer reducing equivalents to the mitochondrial matrix (Toney, 2014) but, upon ETC inhibition, acts in reverse to generate aspartate, to partially compensate for the loss in mitochondrial aspartate synthesis. This reversal supports proliferation of Jurkat cells with ETC inhibition (Sullivan et al., 2015). It is intriguing to speculate that this process may occur in cells that intentionally shut down mitochondrial metabolism, for example via intracellular NO production in murine M1-like macrophages. Mitochondrial metabolism also supports aspartate synthesis by providing electron acceptors, such as O_2. Aspartate synthesis from glutamine requires electron acceptors generated by mitochondrial metabolism. In the absence of such electron acceptors, aspartate synthesis and nucleotide production are decreased (Sullivan et al., 2015).

Do Immune Cells and Cancer Cells Compete for Amino Acids?

While it is clear that all cells need amino acids for protein synthesis and function, the rapid and dramatic changes in immune cell status have very particular amino acid requirements. Restricting amino acid supply can compromise immune cell function, a situation that may arise in a tumorigenic setting to limit anti-tumor immunity. Cancer cells have a high metabolic demand and could compete with immune cells for amino acid resources (Figure 4).

Tumor Cells Have Diverse Amino Acid Requirements

Tumor cells take up amino acids from the external environment and, like T cells, rely on glutamine uptake (DeBerardinis et al., 2007). Glycine fuels serine-glycine exchange via Shmt2, and glycine consumption correlates with cancer cell proliferation (Jain et al., 2012). Increased Shmt2 activity drives serine metabolism (Yang et al., 2018), which may support cancer cell proliferation by supplying nucleotides. Cancer cells indeed increase activity of the SGOC network, in which serine is metabolized to glycine with the concomitant generation of 5, 10-meTHF, a 1-C donor for nucleotide synthesis (Mehrmohamadi et al., 2014). Tumor-initiating cells have increased methionine cycle activity and transmethylation rates (Wang et al., 2019b), making these cells

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dependent on extracellular methionine uptake. Inhibition of methionine metabolism or limitation of extracellular methionine enhances cancer cell death. Proline metabolism supports metastasis formation (Elia et al., 2017), as does pyruvate uptake by breast cancer cells, which use this metabolite to drive collagen hydroxylation to remodel the extracellular matrix, facilitating metastasis (Elia et al., 2019). This collagen modification depends on pyruvate metabolism to αKG, which is accompanied by alanine secretion, which can drive initial activation of naive T cells and re-stimulation of T_{mem} cells (Ron-Harel et al., 2019). On the other hand, pyruvate can also increase expression of the immune checkpoint receptor ligand programmed death ligand (PD-L)1 in macrophages (Watanabe et al., 2017). It would be intriguing to examine if any of the pyruvate taken up by cancer cells is used to increase PD-L1 expression and dampen the anti-tumor response. Increased BCAA catabolism via BCAT1 promotes glioblastoma growth (Tönjes et al., 2013), possibly by fueling energy-producing metabolic pathways, yet hepatocellular carcinoma progression is associated with decreased BCAT1-mediated BCAA catabolism (Ericksen et al., 2019). This preserves BCAAs to activate mTOR. Thus, it will be important to delineate the amino acid demands of different cancer types.

Altered amino acid supply in the tumor microenvironment could have both pro- and anti-tumor effects, as a result of amino acid metabolism in both tumor cells and various tumor-associated immune cells. Deprivation of amino acids in T_{reg} cells, for example, could abolish their suppressive effect on T_{eff} function, thereby promoting anti-cancer activity of T_{eff} cells. TCR stimulation increases expression of amino acid transporters in T_{reg} cells (Do et al., 2020), and arginine and leucine promote mTORC1 activity in T_{reg} cells, via the small G proteins RagA/B and Rheb1/2 (Shi et al., 2019). This signaling drives suppressive activity of T_{reg} cells, including inducible T cell costimulator (ICOS) and CTLA4 expression. RagA deficiency impairs T_{reg} cell proliferation and accumulation in tumors in a B16 melanoma model, allowing expansion of tumor-infiltrating $CD8^+$ T cells with increased granzyme B levels, enhancing antitumor immunity (Do et al., 2020). Similarly, different B cell subtypes can produce immunogenic and immunosuppressive cytokines and thereby exert either pro- or anti-tumor effects, so amino acid supply to different B cells subsets could either drive or inhibit anti-tumor responses (Tsou et al., 2016).

It will be important to investigate exactly which cell types take up amino acids in the tumor microenvironment, and whether transporter repertoire and copy number vary, giving particular cells an advantage in terms of amino acid uptake. Glutamate has differential effects on T cells, dependent on transporter expression. Gls1 is overexpressed in many cancers, which could increase glutamate levels. Breast cancer cells secrete glutamate (Briggs et al., 2016), as do macrophages and DCs (Pacheco et al., 2007), which often infiltrate tumors. Glutamate inhibits the x_c^- cystine-glutamate antiporter, which could restrict cystine supply to T cells, decreasing GSH, ROS detoxification, proliferation, and activation. Naive T cells express the glutamate transporter mGlu5R, which inhibits TCR-mediated activation and proliferation (Pacheco et al., 2006), but activated T cells express an alternative glutamate transporter, mGlu1R, which counters the anti-proliferative activity of mGlu5R via MEK-ERK1/2 and promotes proliferation, IL-2, and IFN-γ production (Pacheco et al., 2004, 2006). The balance between these two transporters may determine whether glutamate has a pro- or anti-tumor immunity effect. B cells and DCs also express glutamate receptors, allowing for their function to be modulated by glutamate. Glutamate signals via the kainate receptor to increase immunoglobulin production by activated B cells (Sturgill et al., 2011). Future research will be needed to provide direct evidence for any competition between tumors and immune cells for amino acids, and how this may ultimately modulate protective immunity in cancer.

Tryptophan and Arginine in Anti-Tumor Immunity

Inhibitors of the tryptophan metabolic enzyme indoleamine-2,3-dioxygenase 1 (IDO-1) have been tested against various types of cancer, both alone and in combination with checkpoint inhibitors (Komiya and Huang, 2018). IDO-1 catabolizes tryptophan to kynurenine, and its expression is increased in both cancer cells and activated immune cells (Yoshida and Hayaishi, 1978), again illustrating an overlapping requirement between these cell types. T cells require tryptophan for proliferation and activation (Lee et al., 2002; Munn et al., 1999), and using IDO-1 inhibitors to target cancer cells may dampen T cell-mediated immunity by limiting tryptophan metabolism, which can induce GCN2 signaling. It is thus crucial to target IDO-1 inhibitors specifically to cancer cells. Overexpression of IDO in tumor cells can also impair T cell responses, possibly by driving tryptophan degradation in cancer cells and limiting tryptophan supply for T cells (Holmgaard et al., 2013). Increased kynurenine can modulate T cell responses, inducing apoptosis (Fallarino et al., 2002), decreasing TCR expression (Fallarino et al., 2006), and promoting T_{reg} cell differentiation (Opitz et al., 2011). Kynurenine may outcompete the transport of leucine and methionine by Slc7a5/System L in T cells (Sinclair et al., 2018), possibly pushing a more regulatory phenotype by limiting leucine and methionine supply. Kynurenine also inhibits NK cell proliferation (Frumento et al., 2002) and cytokine production (Della Chiesa et al., 2006).

Tumor-associated pDCs accumulate in tumor-draining lymph nodes and cause antigen-specific T cell anergy via IDO (Friberg et al., 2002; Munn et al., 2004). Tumor-associated DCs also consume arginine and limit its use by T cells, and arginine metabolism to spermidine by DCs further pushes IDO expression (Mondanelli et al., 2017). This contrasts with the production of spermidine in T cells, which enhances their anti-tumor effect (Pietrocola et al., 2016). Collectively, these actions of DCs limit anti-tumor immunity by T cells. Inhibition of DC arginase metabolism has a greater effect on restoring T cell proliferation in a fibrosarcoma model than restoration of arginine supply alone (Norian et al., 2009), indicating that it is not just restriction of arginine supply to T cells that mediates the suppressive effects of DCs. MDSCs inhibit $CD8^+$ T cell function by targeting intracellular arginine, rather than arginine uptake. In a recent report, it was shown that MDSCs transfer the glycine-derived metabolite methylglyoxal to $CD8^+$ T cells, which forms glycation products with arginine, thereby depleting free intracellular arginine needed for $CD8^+$ T cell activation (Baumann et al., 2020). Methylglyoxal also opposes the increase in glycolysis supporting $CD8^+$ T cell function.

Arginine depletion also dampens IFN-γ production and proliferation of human NK cells (Oberlies et al., 2009). Tumor-associated macrophages (TAMs) expressing arginase are associated with pro-tumor growth, whereas TAMs with low arginase expression drive tumor regression by promoting macrophage and NK cell-mediated anti-tumor immunity

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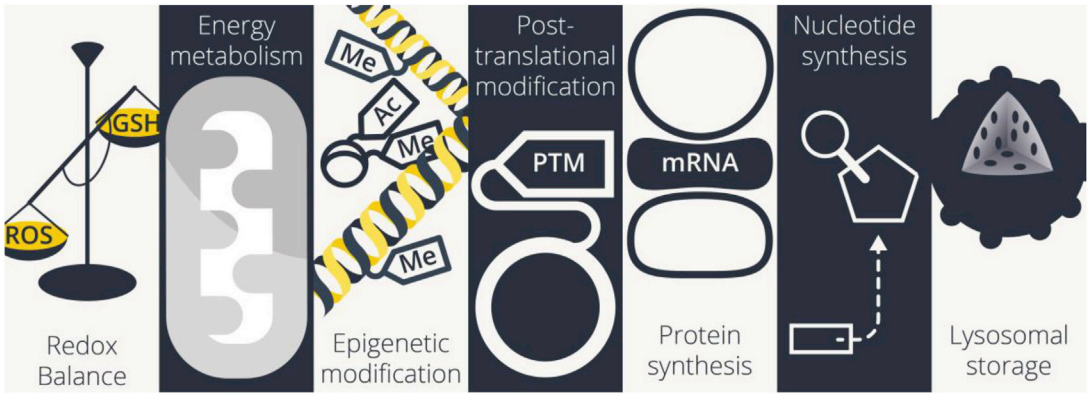

Figure 5. Amino Acids Support Immune Cell Function by Multiple Mechanisms
While amino acids are known to be used for protein synthesis, they are critical for many other cellular processes. They supply intermediates that drive metabolic rewiring upon immune cell activation, and are used to make antioxidants such as glutathione, maintaining redox balance. They provide methyl and acetyl groups to epigenetically modify DNA and histones to facilitate specific gene expression programs in immune cells. These intermediates, and others, can also be used to post-translationally modify proteins and affect their function. Amino acids drive proliferation and growth by fueling nucleotide synthesis and driving translation, and can also be stored in lysosomes, driving autophagy as a protective mechanism in times of stress.

(Hagemann et al., 2008). Both direct targeting of intracellular arginine metabolism by suppressive immune cells and limitation of extracellular arginine supply limit anti-tumor T cell immunity, illustrating how immune cells regulate each other via amino acid metabolism in a tumor setting.

Modulation of Cysteine in Cancer Immunotherapy

Cysteine modulation also controls anti-tumor immunity. Tumor cells are particularly sensitive to ferroptosis, and $CD8^+$ T cells activate ferroptosis in tumors in cancer immunotherapy to drive lipid peroxidation and tumor cell death (Wang et al., 2019a). IFN-γ from these T cells decreases Slc3a2 and Slc7a11, components of the glutamate-cystine antiporter system x_c^-, on tumor cells (Wang et al., 2019a). This limits cystine uptake by tumor cells, reducing their capacity to antagonize ferroptosis. Cystine and cysteine depletion in combination with checkpoint blockade synergistically increase T cell antitumor immunity. MDSCs may limit T cell activation by restricting cysteine availability to T cells (Srivastava et al., 2010), as may DCs. This effect may be in part due to limitation of GSH synthesis and alteration of extracellular redox status.

Targeting Amino Acid Metabolism for Anti-Cancer Treatment

The dependence of cancer cells on amino acid uptake and metabolism implicates the specific targeting of these processes only in the relevant cell type as a means of cancer treatment. For example, a prodrug form of a glutamine antagonist, which is preferentially activated by enzymes in the tumor microenvironment, decreases glycolysis and OXPHOS in MC38 colon tumors *in vivo*, yet promotes these parameters in $CD8^+$ tumor-infiltrating lymphocytes (TILs) (Leone et al., 2019). TILs, but not MC38 tumor cells, are able to compensate for decreased glutamine metabolism by increasing flux of glycolytic metabolites into the TCA cycle. Overall, this enhances antitumor immunity. Recombinant human arginase 1 has been tested in the clinic in an attempt to deplete serum arginine supply to tumor cells (Qiu et al., 2015). In this setting, modulation of arginine supply could also limit anti-tumor T cell activity.

Differential transporter expression on cancer cells and tumor-associated immune cells may facilitate this specific targeting of amino acid metabolism. Increased expression of particular transporters on cancer cells can be used to target drugs specifically to these cells and may also imply an increased reliance of tumor cells on certain amino acids. For example, cancer cells frequently increase expression of Slc1a5, Slc38a2, Slc7a5, and Slc38a5 to increase glutamine uptake, and knockdown or small molecular targeting of these factors inhibits tumor cell growth in several different preclinical models (Kandasamy et al., 2018). Small molecule inhibitors of the system x_c^- antiporter component Slc7a11, such as sulfasalazine and erastin, may sensitize tumor cells to oxidative stress (Timmerman et al., 2013). It will be important to establish selectivity for the transporters of interest, to minimize off-target effects. Amino acid transporters can also be hijacked to specifically deliver drugs with amino acid moieties, or amino acid-like structures, to tumor cells. Nitrogen mustards are a class of DNA alkylating agents that kill tumor cells. Glycine conjugation to a nitrogen mustard compound allows its uptake by Slc7a5 and selective killing of cancer cells with high Slc7a5 expression (Hosoya et al., 2008).

Cancers exposed to high oxidative stress use amino acids to produce antioxidant molecules. Lung adenocarcinomas with nuclear factor erythroid 2-related factor (Nrf)2/ Kelch-like ECH-associated protein (Keap)1 mutations are highly dependent on exogenous non-essential amino acids, and diets lacking asparagine or both serine and glycine reduce Keap1 mutant tumor growth in mice (LeBoeuf et al., 2020). This implicates dietary interventions as potential anti-cancer strategies. Acute methionine restriction sensitizes tumor cells to chemotherapy and radiation treatment in mouse colorectal cancer and sarcoma models, and such acute methionine limitation was tolerated in mouse and a small human sample (Gao et al., 2019).

Open Questions and Future Directions

Amino acids influence immune cell function in many more ways beyond providing material for protein synthesis. Their roles in central energy metabolism, redox balance, epigenetic modification, and PTMs allow them to modulate immunity via multiple mechanisms (Figure 5) and, consequently, provide a variety of targets for therapeutic intervention (Box 2).

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Box 2. Therapeutic Implications of Targeting Amino Acid Metabolism

- Amino acid metabolism can be targeted at multiple points, by altering dietary supply, or by modulating transporters, metabolic enzymes, or storage.
- Inhibitors of the transporters Slc7a5 (Geier et al., 2013) and Slc1a5 (Schulte et al., 2018) antagonize cancer cell growth. This could spare amino acids to boost immune cell function in a tumor microenvironment, a phenomenon that occurs when glutamine metabolism is specifically blocked in cancer cells (Leone et al., 2019). Transporter inhibition could also target immune cells to dampen autoimmunity or hyperinflammation.
- Erastin and sulfasalazine inhibit the cystine-glutamate antiporter, which could limit T cell activation in EAE (Evonuk et al., 2015).
- Multiple compounds, including BPTES and CB-839, inhibit glutamine metabolizing enzymes, with potential benefits in EAE (Kono et al., 2018) and rheumatoid arthritis (Takahashi et al., 2017).
- Blocking GSH synthesis with buthionine sulfoximine could spare its component amino acids for tRNA thiolation and increased biosynthesis, possibly promoting immune cell proliferation and activation.
- Serine limitation dampens excessive macrophage activation in endotoxemia (Rodriguez et al., 2019), which may aid sepsis treatment.
- Halofuginone mimics amino acid starvation by pharmacologically activating GCN2. This compound reduces gut inflammation (Ravindran et al., 2016) and induces autophagy (Chen et al., 2017), which may increase longevity by opposing the age-related decline in immune cell function.
- Modulating amino acid supply to tumors in combination with checkpoint blockade is a promising anti-cancer strategy, as observed for cysteine/cystine depletion in a melanoma model (Wang et al., 2019a).

Initial acquisition of amino acids is an early point of control to manipulate amino acid metabolism, so much so that viruses have evolved to target amino acid transporters. HIV targets serine incorporators 3 and 5, and SNAT1 (Slc38a1), on T cells to restrict T cell mitogenesis and antiviral immunity (Matheson et al., 2015). In this respect, amino acid transporters are good possible drug targets, and dietary interventions could have similar effects. Both strategies may be beneficial to treat cancer and other pathologies (Box 2). Dietary interventions may avoid undesirable side effects associated with pharmacological treatments. Dietary methionine restriction limits autoimmunity, decreasing T_h cell activation and consequently delaying EAE onset and progression (Roy et al., 2020). This work clearly illustrates that limitation of just one nutrient can delay disease. Conversely, amino acid supplementation could boost anti-pathogen immunity. In particular, a number of clinical trials have been conducted with arginine supplementation, which enhances NK cell activity and antibody production in an older population vaccinated against *Streptococcus pneumoniae* (Moriguti et al., 2005).

The relevance of many novel amino acid metabolic pathways to immune cells is as yet unknown. Diverse immune cells are likely to respond differently to amino acid perturbations, depending on whether they express transporters and intracellular metabolic enzymes to process these amino acids. Amino acids outside the proteinogenic 20 remain to be examined. For example, taurine metabolism is modulated by LPS and IFN-γ, and its transporter is regulated in fetal T cells (Iruloh et al., 2007). Selenocysteine is an excellent antioxidant and occurs in enzymes including glutathione peroxidases. Selenoprotein deficiency reduces the number of mature T cells emerging from lymphoid tissues, decreases TCR-induced calcium flux (Verma et al., 2011), and antagonizes T cell proliferation due to decreased buffering of ROS (Shrimali et al., 2008), and roles for selenoproteins have been proposed in allergic airway inflammation (Hoffmann et al., 2007).

Different tissues and niches contain different amino acid repertoires (Behringer et al., 2019), a concept that is particularly relevant to migratory immune cells, which are likely to experience various microenvironments. Immune cells may elect not to indiscriminately use available amino acids for protein synthesis, but to selectively use them or store them. Lysosomes could release stored amino acids according to demand or limit them to induce autophagy as a protective mechanism when nutrients are scarce. Amino acids could also be stored in the form of small peptides such as glutathione, which could then be catabolized to release cysteine, glutamate, and glycine, which can in turn be metabolized to other amino acids. The intracellular amino acid pool is also regulated by transporter expression, but many amino acid transporters remain unidentified.

Amino acid-derived PTMs have been investigated in bacteria, including lysinylation (Kristian et al., 2003) and alanylation (Saar-Dover et al., 2012), while arginylation prevents neurodegeneration in mice (Wang et al., 2017a). However, many PTMs have not been investigated in an immune cell, or even a mammalian, context. Amino acid supply may dictate the type of PTM formed. For example, the autophagy inhibitor EP300 can be activated by acetylation or inhibited by spermidine. BCAAs provide acetyl groups by generating acetyl-CoA, while arginine provides spermidine. It would be intriguing to investigate whether the balance between BCAAs and arginine influences which modification dominates, or if such a phenomenon occurs for other PTMs.

Overall, targeting immune cell amino acid metabolism is a useful means of augmenting or antagonizing immune responses, and increased understanding of amino acid metabolism in immune cells is likely to be of great therapeutic benefit.

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DECLARATION OF INTERESTS

E.L.P. is an SAB member of Immunomet Therapeutics and a founder of Rheos Medicines.

REFERENCES

Abu-Remaileh, M., Wyant, G.A., Kim, C., Laqtom, N.N., Abbasi, M., Chan, S.H., Freinkman, E., and Sabatini, D.M. (2017). Lysosomal metabolomics reveals V-ATPase- and mTOR-dependent regulation of amino acid efflux from lysosomes. Science *358*, 807–813.

Akkaya, M., Traba, J., Roesler, A.S., Miozzo, P., Akkaya, B., Theall, B.P., Sohn, H., Pena, M., Smelkinson, M., Kabat, J., et al. (2018). Second signals rescue B cells from activation-induced mitochondrial dysfunction and death. Nat. Immunol. *19*, 871–884.

Allis, C.D., and Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. Nat. Rev. Genet. *17*, 487–500.

Almutairi, S.M., Ali, A.K., He, W., Yang, D.S., Ghorbani, P., Wang, L., Fullerton, M.D., and Lee, S.H. (2019). Interleukin-18 up-regulates amino acid transporters and facilitates amino acid-induced mTORC1 activation in natural killer cells. J. Biol. Chem. *294*, 4644–4655.

Alvarez, S.W., Sviderskiy, V.O., Terzi, E.M., Papagiannakopoulos, T., Moreira, A.L., Adams, S., Sabatini, D.M., Birsoy, K., and Possemato, R. (2017). NFS1 undergoes positive selection in lung tumours and protects cells from ferroptosis. Nature *551*, 639–643.

Angiari, S., Runtsch, M.C., Sutton, C.E., Palsson-McDermott, E.M., Kelly, B., Rana, N., Kane, H., Papadopoulou, G., Pearce, E.L., Mills, K.H.G., et al. (2020). Pharmacological activation of pyruvate kinase M2 inhibits CD4(+) T cell pathogenicity and suppresses autoimmunity. Cell Metab. *31*, 391–405.e8.

Araki, K., Turner, A.P., Shaffer, V.O., Gangappa, S., Keller, S.A., Bachmann, M.F., Larsen, C.P., and Ahmed, R. (2009a). mTOR regulates memory CD8 T-cell differentiation. Nature *460*, 108–112.

Araki, Y., Wang, Z., Zang, C., Wood, W.H., 3rd, Schones, D., Cui, K., Roh, T.Y., Lhotsky, B., Wersto, R.P., Peng, W., et al. (2009b). Genome-wide analysis of histone methylation reveals chromatin state-based regulation of gene transcription and function of memory CD8+ T cells. Immunity *30*, 912–925.

Araujo, L., Khim, P., Mkhikian, H., Mortales, C.L., and Demetriou, M. (2017). Glycolysis and glutaminolysis cooperatively control T cell function by limiting metabolite supply to N-glycosylation. eLife *6*, e21330.

Aregger, M., and Cowling, V.H. (2017). Regulation of mRNA capping in the cell cycle. RNA Biol. *14*, 11–14.

Arsenio, J., Kakaradov, B., Metz, P.J., Kim, S.H., Yeo, G.W., and Chang, J.T. (2014). Early specification of CD8+ T lymphocyte fates during adaptive immunity revealed by single-cell gene-expression analyses. Nat. Immunol. *15*, 365–372.

Arsenio, J., Metz, P.J., and Chang, J.T. (2015). Asymmetric cell division in T lymphocyte fate diversification. Trends Immunol. *36*, 670–683.

Balmer, M.L., Ma, E.H., Bantug, G.R., Grählert, J., Pfister, S., Glatter, T., Jauch, A., Dimeloe, S., Slack, E., Dehio, P., et al. (2016). Memory CD8(+) T cells require increased concentrations of acetate induced by stress for optimal function. Immunity *44*, 1312–1324.

Bar-Peled, L., Chantranupong, L., Cherniack, A.D., Chen, W.W., Ottina, K.A., Grabiner, B.C., Spear, E.D., Carter, S.L., Meyerson, M., and Sabatini, D.M. (2013). A Tumor suppressor complex with GAP activity for the Rag GTPases that signal amino acid sufficiency to mTORC1. Science *340*, 1100–1106.

Barski, A., Cuddapah, S., Kartashov, A.V., Liu, C., Imamichi, H., Yang, W., Peng, W., Lane, H.C., and Zhao, K. (2017). Rapid recall ability of memory T cells is encoded in their epigenome. Sci. Rep. *7*, 39785.

Battu, S., Afroz, S., Giddaluru, J., Naz, S., Huang, W., Khumukcham, S.S., Khan, R.A., Bhat, S.Y., Qureshi, I.A., Manavathi, B., et al. (2018). Amino acid starvation sensing dampens IL-1β production by activating riboclustering and autophagy. PLoS Biol. *16*, e2005317.

Baumann, T., Dunkel, A., Schmid, C., Schmitt, S., Hiltensperger, M., Lohr, K., Laketa, V., Donakonda, S., Ahting, U., Lorenz-Depiereux, B., et al. (2020). Regulatory myeloid cells paralyze T cells through cell-cell transfer of the metabolite methylglyoxal. Nat. Immunol. *21*, 555–566.

Behringer, S., Wingert, V., Oria, V., Schumann, A., Grünert, S., Cieslar-Pobuda, A., Kölker, S., Lederer, A.K., Jacobsen, D.W., Staerk, J., et al. (2019). Targeted metabolic profiling of methionine cycle metabolites and redox thiol pools in mammalian plasma, cells and urine. Metabolites *9*, 235.

Beier, U.H., Angelin, A., Akimova, T., Wang, L., Liu, Y., Xiao, H., Koike, M.A., Hancock, S.A., Bhatti, T.R., Han, R., et al. (2015). Essential role of mitochondrial energy metabolism in Foxp3^{+} T-regulatory cell function and allograft survival. FASEB J. *29*, 2315–2326.

Birsoy, K., Wang, T., Chen, W.W., Freinkman, E., Abu-Remaileh, M., and Sabatini, D.M. (2015). An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. Cell *162*, 540–551.

Briggs, K.J., Koivunen, P., Cao, S., Backus, K.M., Olenchock, B.A., Patel, H., Zhang, Q., Signoretti, S., Gerfen, G.J., Richardson, A.L., et al. (2016). Paracrine induction of HIF by glutamate in breast cancer: EglN1 senses cysteine. Cell *166*, 126–139.

Brosnan, J.T., and Brosnan, M.E. (2006). Branched-chain amino acids: enzyme and substrate regulation. J. Nutr. *136* (*Suppl*), 207S–211S.

Buck, M.D., Sowell, R.T., Kaech, S.M., and Pearce, E.L. (2017). Metabolic instruction of immunity. Cell *169*, 570–586.

Bulua, A.C., Simon, A., Maddipati, R., Pelletier, M., Park, H., Kim, K.Y., Sack, M.N., Kastner, D.L., and Siegel, R.M. (2011). Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). J. Exp. Med. *208*, 519–533.

Burton, A.S., Stern, J.C., Elsila, J.E., Glavin, D.P., and Dworkin, J.P. (2012). Understanding prebiotic chemistry through the analysis of extraterrestrial amino acids and nucleobases in meteorites. Chem. Soc. Rev. *41*, 5459–5472.

Cantor, J., Browne, C.D., Ruppert, R., Féral, C.C., Fässler, R., Rickert, R.C., and Ginsberg, M.H. (2009). CD98hc facilitates B cell proliferation and adaptive humoral immunity. Nat. Immunol. *10*, 412–419.

Cantor, J., Slepak, M., Ege, N., Chang, J.T., and Ginsberg, M.H. (2011). Loss of T cell CD98 H chain specifically ablates T cell clonal expansion and protects from autoimmunity. J. Immunol. *187*, 851–860.

Carr, E.L., Kelman, A., Wu, G.S., Gopaul, R., Senkevitch, E., Aghvanyan, A., Turay, A.M., and Frauwirth, K.A. (2010). Glutamine uptake and metabolism are coordinately regulated by ERK/MAPK during T lymphocyte activation. J. Immunol. *185*, 1037–1044.

Cerra, F.B., Mazuski, J.E., Chute, E., Nuwer, N., Teasley, K., Lysne, J., Shronts, E.P., and Konstantinides, F.N. (1984). Branched chain metabolic support. A prospective, randomized, double-blind trial in surgical stress. Ann. Surg. *199*, 286–291.

Chaneton, B., Hillmann, P., Zheng, L., Martin, A.C.L., Maddocks, O.D.K., Chokkathukalam, A., Coyle, J.E., Jankevics, A., Holding, F.P., Vousden, K.H., et al. (2012). Serine is a natural ligand and allosteric activator of pyruvate kinase M2. Nature *491*, 458–462.

Chang, J.T., Palanivel, V.R., Kinjyo, I., Schambach, F., Intlekofer, A.M., Banerjee, A., Longworth, S.A., Vinup, K.E., Mrass, P., Oliaro, J., et al. (2007). Asymmetric T lymphocyte division in the initiation of adaptive immune responses. Science *315*, 1687–1691.

Chang, C.H., Curtis, J.D., Maggi, L.B., Jr., Faubert, B., Villarino, A.V., O'Sullivan, D., Huang, S.C., van der Windt, G.J., Blagih, J., Qiu, J., et al. (2013). Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell *153*, 1239–1251.

Chen, H.L., Li, C.F., Grigorian, A., Tian, W., and Demetriou, M. (2009). T cell receptor signaling co-regulates multiple Golgi genes to enhance N-glycan branching. J. Biol. Chem. *284*, 32454–32461.

Chen, G.Q., Gong, R.H., Yang, D.J., Zhang, G., Lu, A.P., Yan, S.C., Lin, S.H., and Bian, Z.X. (2017). Halofuginone dually regulates autophagic flux through nutrient-sensing pathways in colorectal cancer. Cell Death Dis. *8*, e2789.

Cheng, S.C., Quintin, J., Cramer, R.A., Shepardson, K.M., Saeed, S., Kumar, V., Giamarellos-Bourboulis, E.J., Martens, J.H., Rao, N.A., Aghajanirefah, A., et al. (2014). mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. Science *345*, 1250684.

Chouchani, E.T., Pell, V.R., Gaude, E., Aksentijević, D., Sundier, S.Y., Robb, E.L., Logan, A., Nadtochiy, S.M., Ord, E.N.J., Smith, A.C., et al. (2014). Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature *515*, 431–435.

Combs, J.A., and DeNicola, G.M. (2019). The non-essential amino acid cysteine becomes essential for tumor proliferation and survival. Cancers (Basel) *11*, 678.

Cornish, G.H., Sinclair, L.V., and Cantrell, D.A. (2006). Differential regulation of T-cell growth by IL-2 and IL-15. Blood *108*, 600–608.

Crompton, J.G., Narayanan, M., Cuddapah, S., Roychoudhuri, R., Ji, Y., Yang, W., Patel, S.J., Sukumar, M., Palmer, D.C., Peng, W., et al. (2016). Lineage relationship of CD8(+) T cell subsets is revealed by progressive changes in the epigenetic landscape. Cell. Mol. Immunol. *13*, 502–513.

Crooks, D.R., Maio, N., Lane, A.N., Jarnik, M., Higashi, R.M., Haller, R.G., Yang, Y., Fan, T.W., Linehan, W.M., and Rouault, T.A. (2018). Acute loss of iron-sulfur clusters results in metabolic reprogramming and generation of lipid droplets in mammalian cells. J. Biol. Chem. *293*, 8297–8311.

Curthoys, N.P., and Watford, M. (1995). Regulation of glutaminase activity and glutamine metabolism. Annu. Rev. Nutr. *15*, 133–159.

D'Angelo, J.A., Dehlink, E., Platzer, B., Dwyer, P., Circu, M.L., Garay, J., Aw, T.Y., Fiebiger, E., and Dickinson, B.L. (2010). The cystine/glutamate antiporter regulates dendritic cell differentiation and antigen presentation. J. Immunol. *185*, 3217–3226.

Dalle-Donne, I., Rossi, R., Colombo, G., Giustarini, D., and Milzani, A. (2009). Protein S-glutathionylation: a regulatory device from bacteria to humans. Trends Biochem. Sci. *34*, 85–96.

DeBerardinis, R.J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S., and Thompson, C.B. (2007). Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc. Natl. Acad. Sci. USA *104*, 19345–19350.

Delgoffe, G.M., Pollizzi, K.N., Waickman, A.T., Heikamp, E., Meyers, D.J., Horton, M.R., Xiao, B., Worley, P.F., and Powell, J.D. (2011). The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. Nat. Immunol. *12*, 295–303.

Della Chiesa, M., Carlomagno, S., Frumento, G., Balsamo, M., Cantoni, C., Conte, R., Moretta, L., Moretta, A., and Vitale, M. (2006). The tryptophan catabolite L-kynurenine inhibits the surface expression of NKp46- and NKG2D-activating receptors and regulates NK-cell function. Blood *108*, 4118–4125.

Demetriou, M., Granovsky, M., Quaggin, S., and Dennis, J.W. (2001). Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. Nature *409*, 733–739.

Demotte, N., Stroobant, V., Courtoy, P.J., Van Der Smissen, P., Colau, D., Luescher, I.F., Hivroz, C., Nicaise, J., Squifflet, J.L., Mourad, M., et al. (2008). Restoring the association of the T cell receptor with CD8 reverses anergy in human tumor-infiltrating lymphocytes. Immunity *28*, 414–424.

Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., Patel, D.N., Bauer, A.J., Cantley, A.M., Yang, W.S., et al. (2012). Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell *149*, 1060–1072.

Do, M.H., Wang, X., Zhang, X., Chou, C., Nixon, B.G., Capistrano, K.J., Peng, M., Efeyan, A., Sabatini, D.M., and Li, M.O. (2020). Nutrient mTORC1 signaling underpins regulatory T cell control of immune tolerance. J. Exp. Med. *217*, e20190848.

Doi, M., Yamaoka, I., Nakayama, M., Mochizuki, S., Sugahara, K., and Yoshizawa, F. (2005a). Isoleucine, a blood glucose-lowering amino acid, increases glucose uptake in rat skeletal muscle in the absence of increases in AMP-activated protein kinase activity. J. Nutr. *135*, 2103–2108.

Doi, N., Kakukawa, K., Oishi, Y., and Yanagawa, H. (2005b). High solubility of random-sequence proteins consisting of five kinds of primitive amino acids. Protein Eng. Des. Sel. *18*, 279–284.

Drapier, J.C. (1997). Interplay between NO and [Fe-S] clusters: relevance to biological systems. Methods *11*, 319–329.

Durek, P., Nordström, K., Gasparoni, G., Salhab, A., Kressler, C., de Almeida, M., Bassler, K., Ulas, T., Schmidt, F., Xiong, J., et al.; DEEP Consortium (2016). Epigenomic profiling of human CD4$^+$ T cells supports a linear differentiation model and highlights molecular regulators of memory development. Immunity *45*, 1148–1161.

Elia, I., Broekaert, D., Christen, S., Boon, R., Radaelli, E., Orth, M.F., Verfaillie, C., Grünewald, T.G.P., and Fendt, S.M. (2017). Proline metabolism supports metastasis formation and could be inhibited to selectively target metastasizing cancer cells. Nat. Commun. *8*, 15267.

Elia, I., Rossi, M., Stegen, S., Broekaert, D., Doglioni, G., van Gorsel, M., Boon, R., Escalona-Noguero, C., Torrekens, S., Verfaillie, C., et al. (2019). Breast cancer cells rely on environmental pyruvate to shape the metastatic niche. Nature *568*, 117–121.

Ericksen, R.E., Lim, S.L., McDonnell, E., Shuen, W.H., Vadiveloo, M., White, P.J., Ding, Z., Kwok, R., Lee, P., Radda, G.K., et al. (2019). Loss of BCAA catabolism during carcinogenesis enhances mTORC1 activity and promotes tumor development and progression. Cell Metab. *29*, 1151–1165.e6.

Evonuk, K.S., Baker, B.J., Doyle, R.E., Moseley, C.E., Sestero, C.M., Johnston, B.P., De Sarno, P., Tang, A., Gembitsky, I., Hewett, S.J., et al. (2015). Inhibition of system Xc(-) transporter attenuates autoimmune inflammatory demyelination. J. Immunol. *195*, 450–463.

Fallarino, F., Grohmann, U., Vacca, C., Bianchi, R., Orabona, C., Spreca, A., Fioretti, M.C., and Puccetti, P. (2002). T cell apoptosis by tryptophan catabolism. Cell Death Differ. *9*, 1069–1077.

Fallarino, F., Grohmann, U., You, S., McGrath, B.C., Cavener, D.R., Vacca, C., Orabona, C., Bianchi, R., Belladonna, M.L., Volpi, C., et al. (2006). The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. J. Immunol. *176*, 6752–6761.

Fletcher, M., Ramirez, M.E., Sierra, R.A., Raber, P., Thevenot, P., Al-Khami, A.A., Sanchez-Pino, D., Hernandez, C., Wyczechowska, D.D., Ochoa, A.C., and Rodriguez, P.C. (2015). l-Arginine depletion blunts antitumor T-cell responses by inducing myeloid-derived suppressor cells. Cancer Res. *75*, 275–283.

Franchi-Gazzola, R., Visigalli, R., Bussolati, O., Dall'Asta, V., and Gazzola, G.C. (1999). Adaptive increase of amino acid transport system A requires ERK1/2 activation. J. Biol. Chem. *274*, 28922–28928.

Frenkel-Pinter, M., Haynes, J.W., C, M., Petrov, A.S., Burcar, B.T., Krishnamurthy, R., Hud, N.V., Leman, L.J., and Williams, L.D. (2019). Selective incorporation of proteinaceous over nonproteinaceous cationic amino acids in model prebiotic oligomerization reactions. Proc. Natl. Acad. Sci. USA *116*, 16338–16346.

Friberg, M., Jennings, R., Alsarraj, M., Dessureault, S., Cantor, A., Extermann, M., Mellor, A.L., Munn, D.H., and Antonia, S.J. (2002). Indoleamine 2,3-dioxygenase contributes to tumor cell evasion of T cell-mediated rejection. Int. J. Cancer *101*, 151–155.

Frumento, G., Rotondo, R., Tonetti, M., Damonte, G., Benatti, U., and Ferrara, G.B. (2002). Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. J. Exp. Med. *196*, 459–468.

Gao, X., Sanderson, S.M., Dai, Z., Reid, M.A., Cooper, D.E., Lu, M., Richie, J.P., Jr., Ciccarella, A., Calcagnotto, A., Mikhael, P.G., et al. (2019). Dietary methionine influences therapy in mouse cancer models and alters human metabolism. Nature *572*, 397–401.

Garcia-Manteiga, J.M., Mari, S., Godejohann, M., Spraul, M., Napoli, C., Cenci, S., Musco, G., and Sitia, R. (2011). Metabolomics of B to plasma cell differentiation. J. Proteome Res. *10*, 4165–4176.

Geier, E.G., Schlessinger, A., Fan, H., Gable, J.E., Irwin, J.J., Sali, A., and Giacomini, K.M. (2013). Structure-based ligand discovery for the large-neutral amino acid transporter 1, LAT-1. Proc. Natl. Acad. Sci. USA *110*, 5480–5485.

Geiger, R., Rieckmann, J.C., Wolf, T., Basso, C., Feng, Y., Fuhrer, T., Kogadeeva, M., Picotti, P., Meissner, F., Mann, M., et al. (2016). L-arginine modulates T cell metabolism and enhances survival and anti-tumor activity. Cell *167*, 829–842.e13.

Geltink, R.I.K., Kyle, R.L., and Pearce, E.L. (2018). Unraveling the complex interplay between T cell metabolism and function. Annu. Rev. Immunol. *36*, 461–488.

Gerriets, V.A., Kishton, R.J., Nichols, A.G., Macintyre, A.N., Inoue, M., Ilkayeva, O., Winter, P.S., Liu, X., Priyadharshini, B., Slawinska, M.E., et al. (2015). Metabolic programming and PDHK1 control CD4+ T cell subsets and inflammation. J. Clin. Invest. *125*, 194–207.

Goh, C.C., Roggerson, K.M., Lee, H.C., Golden-Mason, L., Rosen, H.R., and Hahn, Y.S. (2016). Hepatitis C virus-induced myeloid-derived suppressor cells suppress NK cell IFN-γ production by altering cellular metabolism via arginase-1. J. Immunol. *196*, 2283–2292.

Golks, A., Tran, T.T., Goetschy, J.F., and Guerini, D. (2007). Requirement for O-linked N-acetylglucosaminyltransferase in lymphocytes activation. EMBO J. *26*, 4368–4379.

Grigorian, A., Lee, S.U., Tian, W., Chen, I.J., Gao, G., Mendelsohn, R., Dennis, J.W., and Demetriou, M. (2007). Control of T cell-mediated autoimmunity by metabolite flux to N-glycan biosynthesis. J. Biol. Chem. *282*, 20027–20035.

Grigorian, A., Torossian, S., and Demetriou, M. (2009). T-cell growth, cell surface organization, and the galectin-glycoprotein lattice. Immunol. Rev. *230*, 232–246.

Gu, X., Orozco, J.M., Saxton, R.A., Condon, K.J., Liu, G.Y., Krawczyk, P.A., Scaria, S.M., Harper, J.W., Gygi, S.P., and Sabatini, D.M. (2017). SAMTOR is an *S*-adenosylmethionine sensor for the mTORC1 pathway. Science *358*, 813–818.

Hagemann, T., Lawrence, T., McNeish, I., Charles, K.A., Kulbe, H., Thompson, R.G., Robinson, S.C., and Balkwill, F.R. (2008). "Re-educating" tumor-associated macrophages by targeting NF-kappaB. J. Exp. Med. *205*, 1261–1268.

Hardbower, D.M., Asim, M., Luis, P.B., Singh, K., Barry, D.P., Yang, C., Steeves, M.A., Cleveland, J.L., Schneider, C., Piazuelo, M.B., et al. (2017). Ornithine decarboxylase regulates M1 macrophage activation and mucosal inflammation via histone modifications. Proc. Natl. Acad. Sci. USA *114*, E751–E760.

Hart, G.W., Housley, M.P., and Slawson, C. (2007). Cycling of O-linked beta-N-acetylglucosamine on nucleocytoplasmic proteins. Nature *446*, 1017–1022.

Hayashi, K., Jutabha, P., Endou, H., Sagara, H., and Anzai, N. (2013). LAT1 is a critical transporter of essential amino acids for immune reactions in activated human T cells. J. Immunol. *191*, 4080–4085.

He, M., Chiang, H.H., Luo, H., Zheng, Z., Qiao, Q., Wang, L., Tan, M., Ohkubo, R., Mu, W.C., Zhao, S., et al. (2020). An acetylation switch of the NLRP3 inflammasome regulates aging-associated chronic inflammation and insulin resistance. Cell Metab. *31*, 580–591.e5.

Henning, A.N., Roychoudhuri, R., and Restifo, N.P. (2018). Epigenetic control of $CD8^+$ T cell differentiation. Nat. Rev. Immunol. *18*, 340–356.

Hoffmann, P.R., Jourdan-Le Saux, C., Hoffmann, F.W., Chang, P.S., Bollt, O., He, Q., Tam, E.K., and Berry, M.J. (2007). A role for dietary selenium and selenoproteins in allergic airway inflammation. J. Immunol. *179*, 3258–3267.

Holmgaard, R.B., Zamarin, D., Munn, D.H., Wolchok, J.D., and Allison, J.P. (2013). Indoleamine 2,3-dioxygenase is a critical resistance mechanism in antitumor T cell immunotherapy targeting CTLA-4. J. Exp. Med. *210*, 1389–1402.

Hosios, A.M., Hecht, V.C., Danai, L.V., Johnson, M.O., Rathmell, J.C., Steinhauser, M.L., Manalis, S.R., and Vander Heiden, M.G. (2016). Amino acids rather than glucose account for the majority of cell mass in proliferating mammalian cells. Dev. Cell *36*, 540–549.

Hosoya, K., Kyoko, H., Toyooka, N., Kato, A., Orihashi, M., Tomi, M., and Tachikawa, M. (2008). Evaluation of amino acid-mustard transport as L-type amino acid transporter 1 (LAT1)-mediated alkylating agents. Biol. Pharm. Bull. *31*, 2126–2130.

Howden, A.J.M., Hukelmann, J.L., Brenes, A., Spinelli, L., Sinclair, L.V., Lamond, A.I., and Cantrell, D.A. (2019). Quantitative analysis of T cell proteomes and environmental sensors during T cell differentiation. Nat. Immunol. *20*, 1542–1554.

Huang, S.C., Everts, B., Ivanova, Y., O'Sullivan, D., Nascimento, M., Smith, A.M., Beatty, W., Love-Gregory, L., Lam, W.Y., O'Neill, C.M., et al. (2014). Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat. Immunol. *15*, 846–855.

Hyde, R., Cwiklinski, E.L., MacAulay, K., Taylor, P.M., and Hundal, H.S. (2007). Distinct sensor pathways in the hierarchical control of SNAT2, a putative amino acid transceptor, by amino acid availability. J. Biol. Chem. *282*, 19788–19798.

Ikeda, K., Kinoshita, M., Kayama, H., Nagamori, S., Kongpracha, P., Umemoto, E., Okumura, R., Kurakawa, T., Murakami, M., Mikami, N., et al. (2017). Slc3a2 mediates branched-chain amino-acid-dependent maintenance of regulatory T cells. Cell Rep. *21*, 1824–1838.

Iruloh, C.G., D'Souza, S.W., Speake, P.F., Crocker, I., Fergusson, W., Baker, P.N., Sibley, C.P., and Glazier, J.D. (2007). Taurine transporter in fetal T lymphocytes and platelets: differential expression and functional activity. Am. J. Physiol. Cell Physiol. *292*, C332–C341.

Jain, M., Nilsson, R., Sharma, S., Madhusudhan, N., Kitami, T., Souza, A.L., Kafri, R., Kirschner, M.W., Clish, C.B., and Mootha, V.K. (2012). Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science *336*, 1040–1044.

Jennewein, M.F., and Alter, G. (2017). The immunoregulatory roles of antibody glycosylation. Trends Immunol. *38*, 358–372.

Jensen, H., Potempa, M., Gotthardt, D., and Lanier, L.L. (2017). Cutting edge: IL-2-induced expression of the amino acid transporters SLC1A5 and CD98 is a prerequisite for NKG2D-mediated activation of human NK cells. J. Immunol. *199*, 1967–1972.

Jha, A.K., Huang, S.C., Sergushichev, A., Lampropoulou, V., Ivanova, Y., Loginicheva, E., Chmielewski, K., Stewart, K.M., Ashall, J., Everts, B., et al. (2015). Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. Immunity *42*, 419–430.

Jiang, S., Yan, W., Wang, S.E., and Baltimore, D. (2018). Let-7 suppresses B cell activation through restricting the availability of necessary nutrients. Cell Metab. *27*, 393–403.e4.

Johnson, M.O., Wolf, M.M., Madden, M.Z., Andrejeva, G., Sugiura, A., Contreras, D.C., Maseda, D., Liberti, M.V., Paz, K., Kishton, R.J., et al. (2018). Distinct regulation of Th17 and Th1 cell differentiation by glutaminase-dependent metabolism. Cell *175*, 1780–1795.e19.

Jose, D.G., and Good, R.A. (1973). Quantitative effects of nutritional essential amino acid deficiency upon immune responses to tumors in mice. J. Exp. Med. *137*, 1–9.

Kakazu, E., Kanno, N., Ueno, Y., and Shimosegawa, T. (2007). Extracellular branched-chain amino acids, especially valine, regulate maturation and function of monocyte-derived dendritic cells. J. Immunol. *179*, 7137–7146.

Kamiński, M.M., Sauer, S.W., Kamiński, M., Opp, S., Ruppert, T., Grigaravičius, P., Grudnik, P., Gröne, H.J., Krammer, P.H., and Gülow, K. (2012). T cell activation is driven by an ADP-dependent glucokinase linking enhanced glycolysis with mitochondrial reactive oxygen species generation. Cell Rep. *2*, 1300–1315.

Kandasamy, P., Gyimesi, G., Kanai, Y., and Hediger, M.A. (2018). Amino acid transporters revisited: New views in health and disease. Trends Biochem. Sci. *43*, 752–789.

Kedersha, N., Chen, S., Gilks, N., Li, W., Miller, I.J., Stahl, J., and Anderson, P. (2002). Evidence that ternary complex (eIF2-GTP-tRNA(i)(Met))-deficient preinitiation complexes are core constituents of mammalian stress granules. Mol. Biol. Cell *13*, 195–210.

Kibe, R., Kurihara, S., Sakai, Y., Suzuki, H., Ooga, T., Sawaki, E., Muramatsu, K., Nakamura, A., Yamashita, A., Kitada, Y., et al. (2014). Upregulation of colonic luminal polyamines produced by intestinal microbiota delays senescence in mice. Sci. Rep. *4*, 4548.

Kiechl, S., Pechlaner, R., Willeit, P., Notdurfter, M., Paulweber, B., Willeit, K., Werner, P., Ruckenstuhl, C., Iglseder, B., Weger, S., et al. (2018). Higher spermidine intake is linked to lower mortality: a prospective population-based study. Am. J. Clin. Nutr. *108*, 371–380.

Kobayashi, T., Shimabukuro-Demoto, S., Yoshida-Sugitani, R., Furuyama-Tanaka, K., Karyu, H., Sugiura, Y., Shimizu, Y., Hosaka, T., Goto, M., Kato, N., et al. (2014). The histidine transporter SLC15A4 coordinates mTOR-dependent inflammatory responses and pathogenic antibody production. Immunity *41*, 375–388.

Komiya, T., and Huang, C.H. (2018). Updates in the clinical development of epacadostat and other indoleamine 2,3-dioxygenase 1 inhibitors (IDO1) for human cancers. Front. Oncol. *8*, 423.

Kono, M., Yoshida, N., Maeda, K., and Tsokos, G.C. (2018). Transcriptional factor ICER promotes glutaminolysis and the generation of Th17 cells. Proc. Natl. Acad. Sci. USA *115*, 2478–2483.

Kouzarides, T. (2007). Chromatin modifications and their function. Cell *128*, 693–705.

Krawczyk, C.M., Holowka, T., Sun, J., Blagih, J., Amiel, E., DeBerardinis, R.J., Cross, J.R., Jung, E., Thompson, C.B., Jones, R.G., and Pearce, E.J. (2010). Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. Blood *115*, 4742–4749.

Kristian, S.A., Dürr, M., Van Strijp, J.A., Neumeister, B., and Peschel, A. (2003). MprF-mediated lysinylation of phospholipids in Staphylococcus aureus leads to protection against oxygen-independent neutrophil killing. Infect. Immun. *71*, 546–549.

Kumar, A., Pyaram, K., Yarosz, E.L., Hong, H., Lyssiotis, C.A., Giri, S., and Chang, C.H. (2019). Enhanced oxidative phosphorylation in NKT cells is essential for their survival and function. Proc. Natl. Acad. Sci. USA *116*, 7439–7448.

Kurniawan, H., Franchina, D.G., Guerra, L., Bonetti, L., -Baguet, L.S., Grusdat, M., Schlicker, L., Hunewald, O., Dostert, C., Merz, M.P., et al. (2020). Glutathione restricts serine metabolism to preserve regulatory T cell function. Cell Metab. *31*, 920–936.e7.

Lairson, L.L., Henrissat, B., Davies, G.J., and Withers, S.G. (2008). Glycosyltransferases: structures, functions, and mechanisms. Annu. Rev. Biochem. *77*, 521–555.

Lam, W.Y., Becker, A.M., Kennerly, K.M., Wong, R., Curtis, J.D., Llufrio, E.M., McCommis, K.S., Fahrmann, J., Pizzato, H.A., Nunley, R.M., et al. (2016). Mitochondrial pyruvate import promotes long-term survival of antibody-secreting plasma cells. Immunity *45*, 60–73.

Lam, W.Y., Jash, A., Yao, C.H., D'Souza, L., Wong, R., Nunley, R.M., Meares, G.P., Patti, G.J., and Bhattacharya, D. (2018). Metabolic and transcriptional modules independently diversify plasma cell lifespan and function. Cell Rep. *24*, 2479–2492.e6.

Lamas, B., Vergnaud-Gauduchon, J., Goncalves-Mendes, N., Perche, O., Rossary, A., Vasson, M.P., and Farges, M.C. (2012). Altered functions of natural killer cells in response to L-arginine availability. Cell. Immunol. *280*, 182–190.

Lane, A.N., and Fan, T.W. (2015). Regulation of mammalian nucleotide metabolism and biosynthesis. Nucleic Acids Res. *43*, 2466–2485.

Lau, K.S., Partridge, E.A., Grigorian, A., Silvescu, C.I., Reinhold, V.N., Demetriou, M., and Dennis, J.W. (2007). Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation. Cell *129*, 123–134.

Laxman, S., Sutter, B.M., Wu, X., Kumar, S., Guo, X., Trudgian, D.C., Mirzaei, H., and Tu, B.P. (2013). Sulfur amino acids regulate translational capacity and metabolic homeostasis through modulation of tRNA thiolation. Cell *154*, 416–429.

LeBoeuf, S.E., Wu, W.L., Karakousi, T.R., Karadal, B., Jackson, S.R., Davidson, S.M., Wong, K.K., Koralov, S.B., Sayin, V.I., and Papagiannakopoulos, T. (2020). Activation of oxidative stress response in cancer generates a druggable dependency on exogenous non-essential amino acids. Cell Metab. *31*, 339–350.e4.

Lee, G.K., Park, H.J., Macleod, M., Chandler, P., Munn, D.H., and Mellor, A.L. (2002). Tryptophan deprivation sensitizes activated T cells to apoptosis prior to cell division. Immunology *107*, 452–460.

Leone, R.D., Zhao, L., Englert, J.M., Sun, I.M., Oh, M.H., Sun, I.H., Arwood, M.L., Bettencourt, I.A., Patel, C.H., Wen, J., et al. (2019). Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion. Science *366*, 1013–1021.

Levring, T.B., Hansen, A.K., Nielsen, B.L., Kongsbak, M., von Essen, M.R., Woetmann, A., Odum, N., Bonefeld, C.M., and Geisler, C. (2012). Activated human CD4+ T cells express transporters for both cysteine and cystine. Sci. Rep. *2*, 266.

Levring, T.B., Kongsbak, M., Rode, A.K., Woetmann, A., Ødum, N., Bonefeld, C.M., and Geisler, C. (2015). Human CD4+ T cells require exogenous cystine for glutathione and DNA synthesis. Oncotarget *6*, 21853–21864.

Li, H.B., Tong, J., Zhu, S., Batista, P.J., Duffy, E.E., Zhao, J., Bailis, W., Cao, G., Kroehling, L., Chen, Y., et al. (2017). m^6A mRNA methylation controls T cell homeostasis by targeting the IL-7/STAT5/SOCS pathways. Nature *548*, 338–342.

Lian, G., Gnanaprakasam, J.R., Wang, T., Wu, R., Chen, X., Liu, L., Shen, Y., Yang, M., Yang, J., Chen, Y., et al. (2018). Glutathione de novo synthesis but not recycling process coordinates with glutamine catabolism to control redox homeostasis and directs murine T cell differentiation. eLife *7*, e36158.

Lill, R. (2009). Function and biogenesis of iron-sulphur proteins. Nature *460*, 831–838.

Liu, Y.C., Li, F., Handler, J., Huang, C.R., Xiang, Y., Neretti, N., Sedivy, J.M., Zeller, K.I., and Dang, C.V. (2008). Global regulation of nucleotide biosynthetic genes by c-Myc. PLoS One *3*, e2722.

Liu, P.S., Wang, H., Li, X., Chao, T., Teav, T., Christen, S., Di Conza, G., Cheng, W.C., Chou, C.H., Vavakova, M., et al. (2017). α-ketoglutarate orchestrates macrophage activation through metabolic and epigenetic reprogramming. Nat. Immunol. *18*, 985–994.

Loftus, R.M., Assmann, N., Kedia-Mehta, N., O'Brien, K.L., Garcia, A., Gillespie, C., Hukelmann, J.L., Oefner, P.J., Lamond, A.I., Gardiner, C.M., et al. (2018). Amino acid-dependent cMyc expression is essential for NK cell metabolic and functional responses in mice. Nat. Commun. *9*, 2341.

Longo, L.M., and Blaber, M. (2012). Protein design at the interface of the prebiotic and biotic worlds. Arch. Biochem. Biophys. *526*, 16–21.

Longo, L.M., Tenorio, C.A., Kumru, O.S., Middaugh, C.R., and Blaber, M. (2015). A single aromatic core mutation converts a designed "primitive" protein from halophile to mesophile folding. Protein Sci. *24*, 27–37.

Lu, S.C. (2000). *S*-Adenosylmethionine. Int. J. Biochem. Cell Biol. *32*, 391–395.

Lu, S.C. (2009). Regulation of glutathione synthesis. Mol. Aspects Med. *30*, 42–59.

Lund, P.J., Elias, J.E., and Davis, M.M. (2016). Global analysis of O-GlcNAc glycoproteins in activated human T cells. J. Immunol. *197*, 3086–3098.

Ma, E.H., Bantug, G., Griss, T., Condotta, S., Johnson, R.M., Samborska, B., Mainolfi, N., Suri, V., Guak, H., Balmer, M.L., et al. (2017). Serine is an essential metabolite for effector T cell expansion. Cell Metab. *25*, 482.

Madeo, F., Carmona-Gutierrez, D., Kepp, O., and Kroemer, G. (2018). Spermidine delays aging in humans. Aging (Albany N.Y.) *10*, 2209–2211.

Mak, T.W., Grusdat, M., Duncan, G.S., Dostert, C., Nonnenmacher, Y., Cox, M., Binsfeld, C., Hao, Z., Brüstle, A., Itsumi, M., et al. (2017). Glutathione primes T cell metabolism for inflammation. Immunity *46*, 1089–1090.

Marchingo, J.M., Sinclair, L.V., Howden, A.J., and Cantrell, D.A. (2020). Quantitative analysis of how Myc controls T cell proteomes and metabolic pathways during T cell activation. eLife *9*, e53725.

Masle-Farquhar, E., Bröer, A., Yabas, M., Enders, A., and Bröer, S. (2017). ASCT2 (SLC1A5)-deficient mice have normal B-cell development, proliferation, and antibody production. Front. Immunol. *8*, 549.

Matheson, N.J., Sumner, J., Wals, K., Rapiteanu, R., Weekes, M.P., Vigan, R., Weinelt, J., Schindler, M., Antrobus, R., Costa, A.S., et al. (2015). Cell surface proteomic map of HIV infection reveals antagonism of amino acid metabolism by Vpu and Nef. Cell Host Microbe *18*, 409–423.

Matsushita, M., Freigang, S., Schneider, C., Conrad, M., Bornkamm, G.W., and Kopf, M. (2015). T cell lipid peroxidation induces ferroptosis and prevents immunity to infection. J. Exp. Med. *212*, 555–568.

Mehrmohamadi, M., Liu, X., Shestov, A.A., and Locasale, J.W. (2014). Characterization of the usage of the serine metabolic network in human cancer. Cell Rep. *9*, 1507–1519.

Mejia, N.R., and MacKenzie, R.E. (1985). NAD-dependent methylenetetrahydrofolate dehydrogenase is expressed by immortal cells. J. Biol. Chem. *260*, 14616–14620.

Mills, E.L., Ryan, D.G., Prag, H.A., Dikovskaya, D., Menon, D., Zaslona, Z., Jedrychowski, M.P., Costa, A.S.H., Higgins, M., Hams, E., et al. (2018). Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. Nature *556*, 113–117.

Minton, D.R., Nam, M., McLaughlin, D.J., Shin, J., Bayraktar, E.C., Alvarez, S.W., Sviderskiy, V.O., Papagiannakopoulos, T., Sabatini, D.M., Birsoy, K., and Possemato, R. (2018). Serine catabolism by SHMT2 is required for proper mitochondrial translation initiation and maintenance of formylmethionyl-tRNAs. Mol. Cell *69*, 610–621.e5.

Mondanelli, G., Bianchi, R., Pallotta, M.T., Orabona, C., Albini, E., Iacono, A., Belladonna, M.L., Vacca, C., Fallarino, F., Macchiarulo, A., et al. (2017). A relay pathway between arginine and tryptophan metabolism confers immunosuppressive properties on dendritic cells. Immunity *46*, 233–244.

Moriguti, J.C., Ferriolli, E., Donadi, E.A., and Marchini, J.S. (2005). Effects of arginine supplementation on the humoral and innate immune response of older people. Eur. J. Clin. Nutr. *59*, 1362–1366.

Mullican, S.E., Gaddis, C.A., Alenghat, T., Nair, M.G., Giacomin, P.R., Everett, L.J., Feng, D., Steger, D.J., Schug, J., Artis, D., and Lazar, M.A. (2011). Histone deacetylase 3 is an epigenomic brake in macrophage alternative activation. Genes Dev. *25*, 2480–2488.

Munn, D.H., Shafizadeh, E., Attwood, J.T., Bondarev, I., Pashine, A., and Mellor, A.L. (1999). Inhibition of T cell proliferation by macrophage tryptophan catabolism. J. Exp. Med. *189*, 1363–1372.

Munn, D.H., Sharma, M.D., Hou, D., Baban, B., Lee, J.R., Antonia, S.J., Messina, J.L., Chandler, P., Koni, P.A., and Mellor, A.L. (2004). Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. J. Clin. Invest. *114*, 280–290.

Nakai, Y., Umeda, N., Suzuki, T., Nakai, M., Hayashi, H., Watanabe, K., and Kagamiyama, H. (2004). Yeast Nfs1p is involved in thio-modification of both mitochondrial and cytoplasmic tRNAs. J. Biol. Chem. *279*, 12363–12368.

Nakaya, M., Xiao, Y., Zhou, X., Chang, J.H., Chang, M., Cheng, X., Blonska, M., Lin, X., and Sun, S.C. (2014). Inflammatory T cell responses rely on amino acid transporter ASCT2 facilitation of glutamine uptake and mTORC1 kinase activation. Immunity *40*, 692–705.

Neinast, M.D., Jang, C., Hui, S., Murashige, D.S., Chu, Q., Morscher, R.J., Li, X., Zhan, L., White, E., Anthony, T.G., et al. (2019). Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. Cell Metab. *29*, 417–429.e4.

Netea, M.G., Joosten, L.A., Latz, E., Mills, K.H., Natoli, G., Stunnenberg, H.G., O'Neill, L.A., and Xavier, R.J. (2016). Trained immunity: a program of innate immune memory in health and disease. Science *352*, aaf1098.

Newsholme, P., Curi, R., Gordon, S., and Newsholme, E.A. (1986). Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. Biochem. J. *239*, 121–125.

Newsholme, P., Curi, R., Pithon Curi, T.C., Murphy, C.J., Garcia, C., and Pires de Melo, M. (1999). Glutamine metabolism by lymphocytes, macrophages, and neutrophils: its importance in health and disease. J. Nutr. Biochem. *10*, 316–324.

Nilsson, R., Jain, M., Madhusudhan, N., Sheppard, N.G., Strittmatter, L., Kampf, C., Huang, J., Asplund, A., and Mootha, V.K. (2014). Metabolic enzyme expression highlights a key role for MTHFD2 and the mitochondrial folate pathway in cancer. Nat. Commun. *5*, 3128.

Nishitani, S., Takehana, K., Fujitani, S., and Sonaka, I. (2005). Branched-chain amino acids improve glucose metabolism in rats with liver cirrhosis. Am. J. Physiol. Gastrointest. Liver Physiol. *288*, G1292–G1300.

Norian, L.A., Rodriguez, P.C., O'Mara, L.A., Zabaleta, J., Ochoa, A.C., Cella, M., and Allen, P.M. (2009). Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. Cancer Res. *69*, 3086–3094.

Nusinzon, I., and Horvath, C.M. (2006). Positive and negative regulation of the innate antiviral response and beta interferon gene expression by deacetylation. Mol. Cell. Biol. *26*, 3106–3113.

O'Neill, L.A., Kishton, R.J., and Rathmell, J. (2016). A guide to immunometabolism for immunologists. Nat. Rev. Immunol. *16*, 553–565.

Oberlies, J., Watzl, C., Giese, T., Luckner, C., Kropf, P., Müller, I., Ho, A.D., and Munder, M. (2009). Regulation of NK cell function by human granulocyte arginase. J. Immunol. *182*, 5259–5267.

Opitz, C.A., Litzenburger, U.M., Sahm, F., Ott, M., Tritschler, I., Trump, S., Schumacher, T., Jestaedt, L., Schrenk, D., Weller, M., et al. (2011). An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. Nature *478*, 197–203.

Pacheco, R., Ciruela, F., Casadó, V., Mallol, J., Gallart, T., Lluis, C., and Franco, R. (2004). Group I metabotropic glutamate receptors mediate a dual role of glutamate in T cell activation. J. Biol. Chem. *279*, 33352–33358.

Pacheco, R., Oliva, H., Martinez-Navío, J.M., Climent, N., Ciruela, F., Gatell, J.M., Gallart, T., Mallol, J., Lluis, C., and Franco, R. (2006). Glutamate released by dendritic cells as a novel modulator of T cell activation. J. Immunol. *177*, 6695–6704.

Pacheco, R., Gallart, T., Lluis, C., and Franco, R. (2007). Role of glutamate on T-cell mediated immunity. J. Neuroimmunol. *185*, 9–19.

Palmieri, E.M., Gonzalez-Cotto, M., Baseler, W.A., Davies, L.C., Ghesquière, B., Maio, N., Rice, C.M., Rouault, T.A., Cassel, T., Higashi, R.M., et al. (2020). Nitric oxide orchestrates metabolic rewiring in M1 macrophages by targeting aconitase 2 and pyruvate dehydrogenase. Nat. Commun. *11*, 698.

Palsson-McDermott, E.M., Curtis, A.M., Goel, G., Lauterbach, M.A.R., Sheedy, F.J., Gleeson, L.E., van den Bosch, M.W.M., Quinn, S.R., Domingo-Fernandez, R., Johnston, D.G.W., et al. (2015). Pyruvate kinase M2 regulates Hif-1α activity and IL-1β induction and is a critical determinant of the Warburg effect in LPS-activated macrophages. Cell Metab. *21*, 347.

Papathanassiu, A.E., Ko, J.H., Imprialou, M., Bagnati, M., Srivastava, P.K., Vu, H.A., Cucchi, D., McAdoo, S.P., Ananieva, E.A., Mauro, C., and Behmoaras, J. (2017). BCAT1 controls metabolic reprogramming in activated human macrophages and is associated with inflammatory diseases. Nat. Commun. *8*, 16040.

Park, M.H., Cooper, H.L., and Folk, J.E. (1981). Identification of hypusine, an unusual amino acid, in a protein from human lymphocytes and of spermidine as its biosynthetic precursor. Proc. Natl. Acad. Sci. USA *78*, 2869–2873.

Pearce, E.L., Walsh, M.C., Cejas, P.J., Harms, G.M., Shen, H., Wang, L.S., Jones, R.G., and Choi, Y. (2009). Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature *460*, 103–107.

Pesce, J.T., Ramalingam, T.R., Mentink-Kane, M.M., Wilson, M.S., El Kasmi, K.C., Smith, A.M., Thompson, R.W., Cheever, A.W., Murray, P.J., and Wynn, T.A. (2009). Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. PLoS Pathog. *5*, e1000371.

Petro, T.M., and Bhattacharjee, J.K. (1981). Effect of dietary essential amino acid limitations upon the susceptibility to Salmonella typhimurium and the effect upon humoral and cellular immune responses in mice. Infect. Immun. *32*, 251–259.

Phelps, S.S., Malkiewicz, A., Agris, P.F., and Joseph, S. (2004). Modified nucleotides in tRNA(Lys) and tRNA(Val) are important for translocation. J. Mol. Biol. *338*, 439–444.

Pietrocola, F., Pol, J., Vacchelli, E., Rao, S., Enot, D.P., Baracco, E.E., Levesque, S., Castoldi, F., Jacquelot, N., Yamazaki, T., et al. (2016). Caloric restriction mimetics enhance anticancer immunosurveillance. Cancer Cell *30*, 147–160.

Pollizzi, K.N., Patel, C.H., Sun, I.H., Oh, M.H., Waickman, A.T., Wen, J., Delgoffe, G.M., and Powell, J.D. (2015). mTORC1 and mTORC2 selectively regulate CD8$^+$ T cell differentiation. J. Clin. Invest. *125*, 2090–2108.

Pollizzi, K.N., Sun, I.H., Patel, C.H., Lo, Y.C., Oh, M.H., Waickman, A.T., Tam, A.J., Blosser, R.L., Wen, J., Delgoffe, G.M., and Powell, J.D. (2016). Asymmetric inheritance of mTORC1 kinase activity during division dictates CD8(+) T cell differentiation. Nat. Immunol. *17*, 704–711.

Pucciarelli, S., Moreschini, B., Micozzi, D., De Fronzo, G.S., Carpi, F.M., Polzonetti, V., Vincenzetti, S., Mignini, F., and Napolioni, V. (2012). Spermidine and spermine are enriched in whole blood of nona/centenarians. Rejuvenation Res. *15*, 590–595.

Puleston, D.J., Zhang, H., Powell, T.J., Lipina, E., Sims, S., Panse, I., Watson, A.S., Cerundolo, V., Townsend, A.R., Klenerman, P., and Simon, A.K. (2014). Autophagy is a critical regulator of memory CD8(+) T cell formation. eLife *3*, e03706.

Puleston, D.J., Buck, M.D., Klein Geltink, R.I., Kyle, R.L., Caputa, G., O'Sullivan, D., Cameron, A.M., Castoldi, A., Musa, Y., Kabat, A.M., et al. (2019). Polyamines and eIF5A hypusination modulate mitochondrial respiration and macrophage activation. Cell Metab. *30*, 352–363.e8.

Qiu, F., Huang, J., and Sui, M. (2015). Targeting arginine metabolism pathway to treat arginine-dependent cancers. Cancer Lett. *364*, 1–7.

Quéméneur, L., Gerland, L.M., Flacher, M., Ffrench, M., Revillard, J.P., and Genestier, L. (2003). Differential control of cell cycle, proliferation, and survival of primary T lymphocytes by purine and pyrimidine nucleotides. J. Immunol. *170*, 4986–4995.

Quintin, J., Saeed, S., Martens, J.H.A., Giamarellos-Bourboulis, E.J., Ifrim, D.C., Logie, C., Jacobs, L., Jansen, T., Kullberg, B.J., Wijmenga, C., et al. (2012). Candida albicans infection affords protection against reinfection via functional reprogramming of monocytes. Cell Host Microbe *12*, 223–232.

Rao, R.R., Li, Q., Odunsi, K., and Shrikant, P.A. (2010). The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-bet and Eomesodermin. Immunity *32*, 67–78.

Rath, M., Müller, I., Kropf, P., Closs, E.I., and Munder, M. (2014). Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. Front. Immunol. *5*, 532.

Ravindran, R., Loebbermann, J., Nakaya, H.I., Khan, N., Ma, H., Gama, L., Machiah, D.K., Lawson, B., Hakimpour, P., Wang, Y.C., et al. (2016). The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. Nature *531*, 523–527.

Reiner, S.L., and Adams, W.C. (2014). Lymphocyte fate specification as a deterministic but highly plastic process. Nat. Rev. Immunol. *14*, 699–704.

Rieckmann, J.C., Geiger, R., Hornburg, D., Wolf, T., Kveler, K., Jarrossay, D., Sallusto, F., Shen-Orr, S.S., Lanzavecchia, A., Mann, M., and Meissner, F. (2017). Social network architecture of human immune cells unveiled by quantitative proteomics. Nat. Immunol. *18*, 583–593.

Rodriguez, P.C., Quiceno, D.G., Zabaleta, J., Ortiz, B., Zea, A.H., Piazuelo, M.B., Delgado, A., Correa, P., Brayer, J., Sotomayor, E.M., et al. (2004). Arginase I production in the tumor microenvironment by mature myeloid cells inhibits T-cell receptor expression and antigen-specific T-cell responses. Cancer Res. *64*, 5839–5849.

Rodriguez, A.E., Ducker, G.S., Billingham, L.K., Martinez, C.A., Mainolfi, N., Suri, V., Friedman, A., Manfredi, M.G., Weinberg, S.E., Rabinowitz, J.D., and Chandel, N.S. (2019). Serine metabolism supports macrophage IL-1β production. Cell Metab. *29*, 1003–1011.e4.

Roger, T., Lugrin, J., Le Roy, D., Goy, G., Mombelli, M., Koessler, T., Ding, X.C., Chanson, A.L., Reymond, M.K., Miconnet, I., et al. (2011). Histone deacetylase inhibitors impair innate immune responses to Toll-like receptor agonists and to infection. Blood *117*, 1205–1217.

Ron-Harel, N., Ghergurovich, J.M., Notarangelo, G., LaFleur, M.W., Tsubosaka, Y., Sharpe, A.H., Rabinowitz, J.D., and Haigis, M.C. (2019). T cell activation depends on extracellular alanine. Cell Rep. *28*, 3011–3021.e4.

Roy, D.G., Chen, J., Mamane, V., Ma, E.H., Muhire, B.M., Sheldon, R.D., Shorstova, T., Koning, R., Johnson, R.M., Esaulova, E., et al. (2020). Methionine metabolism shapes T helper cell responses through regulation of epigenetic reprogramming. Cell Metab. *31*, 250–266.e9.

Russ, B.E., Olshanksy, M., Smallwood, H.S., Li, J., Denton, A.E., Prier, J.E., Stock, A.T., Croom, H.A., Cullen, J.G., Nguyen, M.L., et al. (2014). Distinct epigenetic signatures delineate transcriptional programs during virus-specific CD8(+) T cell differentiation. Immunity *41*, 853–865.

Saar-Dover, R., Bitler, A., Nezer, R., Shmuel-Galia, L., Firon, A., Shimoni, E., Trieu-Cuot, P., and Shai, Y. (2012). D-alanylation of lipoteichoic acids confers resistance to cationic peptides in group B streptococcus by increasing the cell wall density. PLoS Pathog. *8*, e1002891.

Saeed, S., Quintin, J., Kerstens, H.H., Rao, N.A., Aghajanirefah, A., Matarese, F., Cheng, S.C., Ratter, J., Berentsen, K., van der Ent, M.A., et al. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science *345*, 1251086.

Sagné, C., Agulhon, C., Ravassard, P., Darmon, M., Hamon, M., El Mestikawy, S., Gasnier, B., and Giros, B. (2001). Identification and characterization of a lysosomal transporter for small neutral amino acids. Proc. Natl. Acad. Sci. USA *98*, 7206–7211.

Saini, P., Eyler, D.E., Green, R., and Dever, T.E. (2009). Hypusine-containing protein eIF5A promotes translation elongation. Nature *459*, 118–121.

Saxton, R.A., and Sabatini, D.M. (2017). mTOR signaling in growth, metabolism, and disease. Cell *169*, 361–371.

Schuller, A.P., Wu, C.C., Dever, T.E., Buskirk, A.R., and Green, R. (2017). eIF5A functions globally in translation elongation and termination. Mol. Cell *66*, 194–205.e5.

Schuller-Levis, G.B., and Park, E. (2003). Taurine: new implications for an old amino acid. FEMS Microbiol. Lett. *226*, 195–202.

Schulte, M.L., Fu, A., Zhao, P., Li, J., Geng, L., Smith, S.T., Kondo, J., Coffey, R.J., Johnson, M.O., Rathmell, J.C., et al. (2018). Pharmacological blockade of ASCT2-dependent glutamine transport leads to antitumor efficacy in preclinical models. Nat. Med. *24*, 194–202.

Sena, L.A., and Chandel, N.S. (2012). Physiological roles of mitochondrial reactive oxygen species. Mol. Cell *48*, 158–167.

Sena, L.A., Li, S., Jairaman, A., Prakriya, M., Ezponda, T., Hildeman, D.A., Wang, C.R., Schumacker, P.T., Licht, J.D., Perlman, H., et al. (2013). Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity *38*, 225–236.

Shi, W., Liao, Y., Willis, S.N., Taubenheim, N., Inouye, M., Tarlinton, D.M., Smyth, G.K., Hodgkin, P.D., Nutt, S.L., and Corcoran, L.M. (2015). Transcriptional profiling of mouse B cell terminal differentiation defines a signature for antibody-secreting plasma cells. Nat. Immunol. *16*, 663–673.

Shi, H., Chapman, N.M., Wen, J., Guy, C., Long, L., Dhungana, Y., Rankin, S., Pelletier, S., Vogel, P., Wang, H., et al. (2019). Amino acids license kinase mTORC1 activity and Treg cell function via small G proteins Rag and Rheb. Immunity *51*, 1012–1027.e7.

Shirai, T., Nazarewicz, R.R., Wallis, B.B., Yanes, R.E., Watanabe, R., Hilhorst, M., Tian, L., Harrison, D.G., Giacomini, J.C., Assimes, T.L., et al. (2016). The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. J. Exp. Med. *213*, 337–354.

Shrimali, R.K., Irons, R.D., Carlson, B.A., Sano, Y., Gladyshev, V.N., Park, J.M., and Hatfield, D.L. (2008). Selenoproteins mediate T cell immunity through an antioxidant mechanism. J. Biol. Chem. *283*, 20181–20185.

Sies, H., Berndt, C., and Jones, D.P. (2017). Oxidative stress. Annu. Rev. Biochem. *86*, 715–748.

Sigoillot, F.D., Berkowski, J.A., Sigoillot, S.M., Kotsis, D.H., and Guy, H.I. (2003). Cell cycle-dependent regulation of pyrimidine biosynthesis. J. Biol. Chem. *278*, 3403–3409.

Sinclair, L.V., Rolf, J., Emslie, E., Shi, Y.B., Taylor, P.M., and Cantrell, D.A. (2013). Control of amino-acid transport by antigen receptors coordinates the metabolic reprogramming essential for T cell differentiation. Nat. Immunol. *14*, 500–508.

Sinclair, L.V., Neyens, D., Ramsay, G., Taylor, P.M., and Cantrell, D.A. (2018). Single cell analysis of kynurenine and system L amino acid transport in T cells. Nat. Commun. *9*, 1981.

Sinclair, L.V., Howden, A.J., Brenes, A., Spinelli, L., Hukelmann, J.L., Macintyre, A.N., Liu, X., Thomson, S., Taylor, P.M., Rathmell, J.C., et al. (2019). Antigen receptor control of methionine metabolism in T cells. eLife *8*, e44210.

Siska, P.J., Kim, B., Ji, X., Hoeksema, M.D., Massion, P.P., Beckermann, K.E., Wu, J., Chi, J.T., Hong, J., and Rathmell, J.C. (2016). Fluorescence-based measurement of cystine uptake through xCT shows requirement for ROS detoxification in activated lymphocytes. J. Immunol. Methods *438*, 51–58.

Son, S.M., Park, S.J., Lee, H., Siddiqi, F., Lee, J.E., Menzies, F.M., and Rubinsztein, D.C. (2019). Leucine signals to mTORC1 via its metabolite acetyl-coenzyme A. Cell Metab. *29*, 192–201.e7.

Srivastava, M.K., Sinha, P., Clements, V.K., Rodriguez, P., and Ostrand-Rosenberg, S. (2010). Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer Res. *70*, 68–77.

Stachlewitz, R.F., Li, X., Smith, S., Bunzendahl, H., Graves, L.M., and Thurman, R.G. (2000). Glycine inhibits growth of T lymphocytes by an IL-2-independent mechanism. J. Immunol. *164*, 176–182.

Stipanuk, M.H. (1986). Metabolism of sulfur-containing amino acids. Annu. Rev. Nutr. *6*, 179–209.

Stover, P., and Schirch, V. (1990). Serine hydroxymethyltransferase catalyzes the hydrolysis of 5,10-methenyltetrahydrofolate to 5-formyltetrahydrofolate. J. Biol. Chem. *265*, 14227–14233.

Sturgill, J.L., Mathews, J., Scherle, P., and Conrad, D.H. (2011). Glutamate signaling through the kainate receptor enhances human immunoglobulin production. J. Neuroimmunol. *233*, 80–89.

Sullivan, L.B., Gui, D.Y., Hosios, A.M., Bush, L.N., Freinkman, E., and Vander Heiden, M.G. (2015). Supporting aspartate biosynthesis is an essential function of respiration in proliferating cells. Cell *162*, 552–563.

Swamy, M., Pathak, S., Grzes, K.M., Damerow, S., Sinclair, L.V., van Aalten, D.M., and Cantrell, D.A. (2016). Glucose and glutamine fuel protein O-GlcNAcylation to control T cell self-renewal and malignancy. Nat. Immunol. *17*, 712–720.

Takahashi, S., Saegusa, J., Sendo, S., Okano, T., Akashi, K., Irino, Y., and Morinobu, A. (2017). Glutaminase 1 plays a key role in the cell growth of fibroblast-like synoviocytes in rheumatoid arthritis. Arthritis Res. Ther. *19*, 76.

Tani, H., Ohnishi, S., Shitara, H., Mito, T., Yamaguchi, M., Yonekawa, H., Hashizume, O., Ishikawa, K., Nakada, K., and Hayashi, J.I. (2018). Mice deficient in the Shmt2 gene have mitochondrial respiration defects and are embryonic lethal. Sci. Rep. *8*, 425.

Taniuchi, S., Miyake, M., Tsugawa, K., Oyadomari, M., and Oyadomari, S. (2016). Integrated stress response of vertebrates is regulated by four eIF2α kinases. Sci. Rep. *6*, 32886.

Tannahill, G.M., Curtis, A.M., Adamik, J., Palsson-McDermott, E.M., McGettrick, A.F., Goel, G., Frezza, C., Bernard, N.J., Kelly, B., Foley, N.H., et al. (2013). Succinate is an inflammatory signal that induces IL-1β through HIF-1α. Nature *496*, 238–242.

Tellier, J., Shi, W., Minnich, M., Liao, Y., Crawford, S., Smyth, G.K., Kallies, A., Busslinger, M., and Nutt, S.L. (2016). Blimp-1 controls plasma cell function through the regulation of immunoglobulin secretion and the unfolded protein response. Nat. Immunol. *17*, 323–330.

Timmerman, L.A., Holton, T., Yuneva, M., Louie, R.J., Padró, M., Daemen, A., Hu, M., Chan, D.A., Ethier, S.P., van 't Veer, L.J., et al. (2013). Glutamine sensitivity analysis identifies the xCT antiporter as a common triple-negative breast tumor therapeutic target. Cancer Cell *24*, 450–465.

Toney, M.D. (2014). Aspartate aminotransferase: an old dog teaches new tricks. Arch. Biochem. Biophys. *544*, 119–127.

Tönjes, M., Barbus, S., Park, Y.J., Wang, W., Schlotter, M., Lindroth, A.M., Pleier, S.V., Bai, A.H.C., Karra, D., Piro, R.M., et al. (2013). BCAT1 promotes cell proliferation through amino acid catabolism in gliomas carrying wild-type IDH1. Nat. Med. *19*, 901–908.

Tsou, P., Katayama, H., Ostrin, E.J., and Hanash, S.M. (2016). The emerging role of B cells in tumor immunity. Cancer Res. *76*, 5597–5601.

Tsukishiro, T., Shimizu, Y., Higuchi, K., and Watanabe, A. (2000). Effect of branched-chain amino acids on the composition and cytolytic activity of liver-associated lymphocytes in rats. J. Gastroenterol. Hepatol. *15*, 849–859.

van der Windt, G.J., Everts, B., Chang, C.H., Curtis, J.D., Freitas, T.C., Amiel, E., Pearce, E.J., and Pearce, E.L. (2012). Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity *36*, 68–78.

Varshney, D., Lombardi, O., Schweikert, G., Dunn, S., Suska, O., and Cowling, V.H. (2018). mRNA cap methyltransferase, RNMT-RAM, promotes RNA Pol II-dependent transcription. Cell Rep. *23*, 1530–1542.

Vats, D., Mukundan, L., Odegaard, J.I., Zhang, L., Smith, K.L., Morel, C.R., Wagner, R.A., Greaves, D.R., Murray, P.J., and Chawla, A. (2006). Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. Cell Metab. *4*, 13–24.

Vené, R., Delfino, L., Castellani, P., Balza, E., Bertolotti, M., Sitia, R., and Rubartelli, A. (2010). Redox remodeling allows and controls B-cell activation and differentiation. Antioxid. Redox Signal. *13*, 1145–1155.

Verbist, K.C., Guy, C.S., Milasta, S., Liedmann, S., Kamiński, M.M., Wang, R., and Green, D.R. (2016). Metabolic maintenance of cell asymmetry following division in activated T lymphocytes. Nature *532*, 389–393.

Verma, S., Hoffmann, F.W., Kumar, M., Huang, Z., Roe, K., Nguyen-Wu, E., Hashimoto, A.S., and Hoffmann, P.R. (2011). Selenoprotein K knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses. J. Immunol. *186*, 2127–2137.

Wallace, C., and Keast, D. (1992). Glutamine and macrophage function. Metabolism *41*, 1016–1020.

Wang, R., Dillon, C.P., Shi, L.Z., Milasta, S., Carter, R., Finkelstein, D., McCormick, L.L., Fitzgerald, P., Chi, H., Munger, J., and Green, D.R. (2011). The transcription factor Myc controls metabolic reprogramming upon T lymphocyte activation. Immunity *35*, 871–882.

Wang, J., Han, X., Leu, N.A., Sterling, S., Kurosaka, S., Fina, M., Lee, V.M., Dong, D.W., Yates, J.R., 3rd, and Kashina, A. (2017a). Protein arginylation targets alpha synuclein, facilitates normal brain health, and prevents neurodegeneration. Sci. Rep. *7*, 11323.

Wang, Y., Liu, J., Jin, X., Zhang, D., Li, D., Hao, F., Feng, Y., Gu, S., Meng, F., Tian, M., et al. (2017b). *O*-GlcNAcylation destabilizes the active tetrameric PKM2 to promote the Warburg effect. Proc. Natl. Acad. Sci. USA *114*, 13732–13737.

Wang, W., Green, M., Choi, J.E., Gijón, M., Kennedy, P.D., Johnson, J.K., Liao, P., Lang, X., Kryczek, I., Sell, A., et al. (2019a). $CD8^+$ T cells regulate tumour ferroptosis during cancer immunotherapy. Nature *569*, 270–274.

Wang, Z., Yip, L.Y., Lee, J.H.J., Wu, Z., Chew, H.Y., Chong, P.K.W., Teo, C.C., Ang, H.Y., Peh, K.L.E., Yuan, J., et al. (2019b). Methionine is a metabolic dependency of tumor-initiating cells. Nat. Med. *25*, 825–837.

Watanabe, R., Shirai, T., Namkoong, H., Zhang, H., Berry, G.J., Wallis, B.B., Schaefgen, B., Harrison, D.G., Tremmel, J.A., Giacomini, J.C., et al. (2017). Pyruvate controls the checkpoint inhibitor PD-L1 and suppresses T cell immunity. J. Clin. Invest. *127*, 2725–2738.

Wculek, S.K., Khouili, S.C., Priego, E., Heras-Murillo, I., and Sancho, D. (2019). Metabolic control of dendritic cell functions: digesting information. Front. Immunol. *10*, 775.

West, A.P., Brodsky, I.E., Rahner, C., Woo, D.K., Erdjument-Bromage, H., Tempst, P., Walsh, M.C., Choi, Y., Shadel, G.S., and Ghosh, S. (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. Nature *472*, 476–480.

White, M.T., Verity, R., Griffin, J.T., Asante, K.P., Owusu-Agyei, S., Greenwood, B., Drakeley, C., Gesase, S., Lusingu, J., Ansong, D., et al. (2015). Immunogenicity of the RTS,S/AS01 malaria vaccine and implications for duration of vaccine efficacy: secondary analysis of data from a phase 3 randomised controlled trial. Lancet Infect. Dis. *15*, 1450–1458.

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. Amino Acids *37*, 1–17.

Wu, G. (2013). Functional amino acids in nutrition and health. Amino Acids *45*, 407–411.

Wyant, G.A., Abu-Remaileh, M., Wolfson, R.L., Chen, W.W., Freinkman, E., Danai, L.V., Vander Heiden, M.G., and Sabatini, D.M. (2017). mTORC1 activator SLC38A9 is required to efflux essential amino acids from lysosomes and use protein as a nutrient. Cell *171*, 642–654.e12.

Xiao, M., Yang, H., Xu, W., Ma, S., Lin, H., Zhu, H., Liu, L., Liu, Y., Yang, C., Xu, Y., et al. (2012). Inhibition of α-KG-dependent histone and DNA demethylases by fumarate and succinate that are accumulated in mutations of FH and SDH tumor suppressors. Genes Dev. *26*, 1326–1338.

Yan, Z., Garg, S.K., Kipnis, J., and Banerjee, R. (2009). Extracellular redox modulation by regulatory T cells. Nat. Chem. Biol. *5*, 721–723.

Yang, X., Wang, Z., Li, X., Liu, B., Liu, M., Liu, L., Chen, S., Ren, M., Wang, Y., Yu, M., et al. (2018). SHMT2 desuccinylation by SIRT5 drives cancer cell proliferation. Cancer Res. *78*, 372–386.

Yang, L., Garcia Canaveras, J.C., Chen, Z., Wang, L., Liang, L., Jang, C., Mayr, J.A., Zhang, Z., Ghergurovich, J.M., Zhan, L., et al. (2020). Serine catabolism feeds NADH when respiration is impaired. Cell Metab. *31*, 809–821.e6.

Yaqoob, P., and Calder, P.C. (1997). Glutamine requirement of proliferating T lymphocytes. Nutrition *13*, 646–651.

Yarian, C., Townsend, H., Czestkowski, W., Sochacka, E., Malkiewicz, A.J., Guenther, R., Miskiewicz, A., and Agris, P.F. (2002). Accurate translation of the genetic code depends on tRNA modified nucleosides. J. Biol. Chem. *277*, 16391–16395.

Yeramian, A., Martin, L., Arpa, L., Bertran, J., Soler, C., McLeod, C., Modolell, M., Palacín, M., Lloberas, J., and Celada, A. (2006a). Macrophages require distinct arginine catabolism and transport systems for proliferation and for activation. Eur. J. Immunol. *36*, 1516–1526.

Yeramian, A., Martin, L., Serrat, N., Arpa, L., Soler, C., Bertran, J., McLeod, C., Palacín, M., Modolell, M., Lloberas, J., and Celada, A. (2006b). Arginine transport via cationic amino acid transporter 2 plays a critical regulatory role in classical or alternative activation of macrophages. J. Immunol. *176*, 5918–5924.

Yeung, F., Hoberg, J.E., Ramsey, C.S., Keller, M.D., Jones, D.R., Frye, R.A., and Mayo, M.W. (2004). Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. *23*, 2369–2380.

Yoon, B.R., Oh, Y.J., Kang, S.W., Lee, E.B., and Lee, W.W. (2018). Role of SLC7A5 in metabolic reprogramming of human monocyte/macrophage immune responses. Front. Immunol. *9*, 53.

Yoshida, R., and Hayaishi, O. (1978). Induction of pulmonary indoleamine 2,3-dioxygenase by intraperitoneal injection of bacterial lipopolysaccharide. Proc. Natl. Acad. Sci. USA *75*, 3998–4000.

Yu, X., and Long, Y.C. (2016). Crosstalk between cystine and glutathione is critical for the regulation of amino acid signaling pathways and ferroptosis. Sci. Rep. *6*, 30033.

Yurdagul, A., Jr., Subramanian, M., Wang, X., Crown, S.B., Ilkayeva, O.R., Darville, L., Kolluru, G.K., Rymond, C.C., Gerlach, B.D., Zheng, Z., et al. (2020). Macrophage metabolism of apoptotic cell-derived arginine promotes continual efferocytosis and resolution of injury. Cell Metab. *31*, 518–533.e10.

Zhang, D.D., and Hannink, M. (2003). Distinct cysteine residues in Keap1 are required for Keap1-dependent ubiquitination of Nrf2 and for stabilization of Nrf2 by chemopreventive agents and oxidative stress. Mol. Cell. Biol. *23*, 8137–8151.

Zhu, J., Berisa, M., Schwörer, S., Qin, W., Cross, J.R., and Thompson, C.B. (2019). Transsulfuration activity can support cell growth upon extracellular cysteine limitation. Cell Metab. *30*, 865–876.e5.