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Matched and Mismatched Metabolic Fuels in Lymphocyte Function

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Abstract

Immunological function requires metabolic support to suit the needs of lymphocytes at a variety of distinct differentiation and activation states. It is now evident that the signaling pathways that drive lymphocyte survival and activity can directly control cellular metabolism. This linkage provides a mechanism by which activation and specific signaling pathways provide a supply of appropriate and required nutrients to support cell functions in a pro-active supply rather than consumption-based metabolic model. In this way, the metabolism and fuel choices of lymphocytes are guided to specifically match the anticipated needs. If the fuel choice or metabolic pathways of lymphocytes are dysregulated, however, metabolic checkpoints can become activated to disrupt immunological function. These changes are now shown in several immunological diseases and may open new opportunities to selectively enhance or suppress specific immune functions through targeting of glucose, lipid, or amino acid metabolism.

Keywords

Lymphocyte; glycolysis; mitochondria; apoptosis

1. SUPPLY-BASED METABOLISM IN IMMUNITY

The immune system has a critical role to protect organisms from pathogens yet refrain from excess inflammation or autoimmunity. To accomplish this goal, a variety of distinct cell types with specific functions and physiological requirements must each perform in concert. In their normal basal state, innate immunological cells and lymphocytes that drive adaptive immunity are in quiescent surveillance for their target. However, infection triggers both rapid innate and slower antigen-specific adaptive immune cells into action to eliminate the invading agent. A successful immune response requires significant new synthesis of cytokines, antibodies, and other effector proteins as well as the induction of rapid cell proliferation and clonal expansion. After the response, cells must return to quiescence and adaptive memory cells are preserved while the remaining cells undergo cell death.

This transition from rest to highly active and back to rest, places very specific demands on the cells. In particular, immunological cells undergo a rapid and dramatic metabolic reprogramming to switch from that of a quiescent cell, requiring generation of ATP and

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replacement biosynthesis, to a rapidly growing cell that must increase cell mass and produce effector proteins. This increase in mass requires a sharp rise in the synthesis of new proteins, lipids, and nucleotides. At the end of an immune response, adaptive memory cells shift back to favor energy efficiency. These changes are not insignificant, as lymphocytes can rapidly switch from a quiescent state to grow to double their mass and divide as often as every four to six hours [1]. It is now clear that fuel choice and these metabolic shifts are highly regulated and critical in immune cell function and fate. Because inappropriately matched cell metabolism can lead to cell death or potentially alter cellular differentiation, understanding the mechanisms and roles of metabolism in immunity may open new opportunities for metabolic modulation of immune diseases.

It has always been evident that cells must have energy to support their basal and essential functions. It had been long assumed in immunity, however, that the inherent allosteric regulation and product inhibition on enzyme activity were the key regulatory elements in lymphocyte metabolism. In this model, the consumption of nutrients and metabolites would relieve the allosteric inhibition of relevant pathways to allow a subsequent increase in activity to support cell demands. This consumption-based model predicts increased flux through pathways that are the most active and certainly contributes to metabolic regulation in immunity. However, it also implies that key metabolic pathways function at perpetually limiting levels that lag cellular demand. Delays and additional metabolic stresses would likely ensure, thus hindering immune function. In contrast, a supply-driven model mediated by increased metabolic activity directly as a consequence of cell stimulation would provide an anticipatory source of energy and biosynthetic precursors. A central component of a supply model is that cell-extrinsic signals that drive activation and immunity would directly promote specific metabolic pathways and programs that link specific nutrients to specific cell needs and functions.

A key to a nutrient supply-driven metabolic model is that immunologically activating signals drive specific nutrient uptake and metabolic pathways, but which pathways? There are a variety of potential fuels for energy and cell growth, each with specific benefits and roles. The picture has now emerged that cell metabolism must match the demands of each cell type, and failure of this can disrupt immunity. Thus, fuel choice is under tight regulation and distinct fuels support distinct metabolic outcomes that are critical for proper immune function. This review will address the metabolic profile and fuel regulation of different types of immune cells and how fuel choice can dictate cell function and immunity.

2. FUELS AND METABOLIC PATHWAYS

Glucose, amino acids, and lipids are the major metabolic fuels for lymphocytes. Each is systemically available in levels controlled by classic metabolic tissues and should not be limiting in the serum under normal conditions. Glucose is highly regulated through the diet and gluconeogenesis in the liver to maintain blood glucose levels between 3–5 mM at nearly all times. Amino acids and lipids can vary more, but are also generally well controlled. Malnutrition or undernutrition and starvation are well described as immunosuppressive conditions [2] and can lead to lower blood levels of amino acids and lipids. Blood glucose is maintained, except in more prolonged cases, through gluconeogenesis and the conversion of lipid stores into glucose. In contrast to starvation, diet-induced obesity can lead to diabetes with hyperlipidemia and hyperglycemia, and is associated with a basal inflammatory state [3, 4]. Oxygen is also an important component of mitochondrial and oxidative metabolism of glucose, lipids, and amino acids. It can become limiting in tumors, or damaged or poorly vascularized tissues, such as lymph nodes, which can be hypoxic [5].

The general availability of these primary fuels supports distinct metabolic pathways important for lymphocyte proliferation and energy production. The key point of regulation, therefore, is the decision through which fuel and metabolic pathways cells employ. Glucose can flux through glycolysis or the pentose phosphate pathway (PPP). Glycolysis allows for the anaerobic generation of ATP, yielding NADH, ATP, and pyruvate. Intermediates can be removed from the pathway to support the biosynthesis of nucleic acids or amino acids, such as serine. Pyruvate can then be either converted to lactate for export, or transported into the mitochondria and converted by pyruvate dehydrogenase into acetyl CoA to support the tricarboxylic acid cycle (TCA) and electron transport. The PPP plays a key role to provide ribose for nucleic acid synthesis as well as NADPH reducing potential essential for lipid synthesis and control of reactive oxygen species. Lipid metabolism can be complex as there are a wide variety of potential lipid species, but synthesis of lipids generally builds from glucose-derived malonyl CoA. Lipid oxidation is highly energy efficient and requires transport of fatty acids into the mitochondria via the Carnitine-Palmitoyl Transferase 1a (CPT1a), where, beta-oxidation produces FADH₂, NADH, and acetyl CoA. Lipid synthesis and oxidation are tightly linked and malonyl CoA can inhibit CPT1a to allow an allosteric inhibition of lipid oxidation as precursors accumulate. Amino acid metabolism varies for each amino acid, but glutamine is a predominant fuel that is converted to glutamate and then to α -ketoglutarate to enter the TCA cycle. This pathway plays a particularly important role to replenish the TCA cycle in growing cells through anapleurosis, as the TCA cycle intermediate, citrate, can be rapidly removed from the mitochondria to generate malonyl CoA for lipid synthesis.

The TCA cycle can serve several purposes and plays roles to maximize both cell growth and energy generation. In contrast to glycolysis, which is highly ATP-inefficient but allows robust generation of intermediates for biosynthesis, the TCA cycle is highly energy efficient. Predominantly, the TCA cycle leads to the production of NADH to provide potential for oxidative phosphorylation through the electron transport chain and ATP production. Lipids, in particular, are highly energy-rich and yield over one hundred ATP per palmitate. TCA intermediates are also used to produce malonyl CoA for lipid synthesis as well as some amino acids. Through regulation of the balance of these metabolic pathways and promoting specific energy or biosynthetic pathways, lymphocytes can support their functional needs at each stage of activation and immunity. However, if the proper fuel is not available to meet the cellular demands for energy or biosynthesis, cells face metabolic checkpoints that can disrupt their function or lead to their death.

3. T CELL METABOLISM: CELL SURVIVAL AND PROLIFERATION

The metabolism of T cells is highly dynamic and specific to distinct states of activation and differentiation. The challenge for proper immunity, in particular, is the need of cells to balance the use of fuels to support cell growth and effector function versus the need to use energy efficient fuels for high rates of ATP production. These two metabolic demands are balanced by fuel selection depending on the state of the T cell.

3.1 Naïve T cells

After thymic differentiation, mature naïve T cells migrate to peripheral lymphoid organs where they remain in the G0 phase of the cell cycle and maintain surveillance for their specific antigen. Intravital two-photon microscopy studies have examined the motions and activities of resting T cells [6]. These studies have shown that resting T cells are anything but resting. Rather, they are in constant migration through the densely packed cells of the lymph nodes and spleen. Although antigenically unstimulated, T cells neither grow nor proliferate at appreciable rates, cell migration involves continuous rearrangement of the actin cytoskeleton and ATP hydrolysis. Thus, resting T cells have a clear metabolic demand

for energy, with little need for biosynthesis. Consistent with this metabolic demand, resting T cells utilize a largely oxidative metabolism. Resting T cells, therefore, have a balanced use of glucose, amino acids, and lipids that are metabolized and oxidized in the mitochondria for maximal ATP generation [7].

One key feature of naïve T cell physiology is the reliance on homeostatic growth signals to maintain survival and metabolism *in vivo*. Primarily, T cells require signals through the T cell receptor (TCR) and the cytokine interleukin-7 receptor (IL-7R) [8]. If deprived of these signals, the T cell lifespan is greatly shortened. Importantly, TCR and IL-7R-induced signals are also critical to maintain basal T cell metabolism. T cells that are neglected and deprived of growth signals internalize and degrade the cell surface glucose transporter Glut1 and undergo cellular atrophy that leads to reduced rates of glucose metabolism and the induction of pro-apoptotic Bcl-2 family proteins [9–13]. As nutrient uptake decreases, T cells activate the self-digestive process of autophagy and proteolytic degradation [12, 14]. This atrophy and metabolic stress has been demonstrated *in vivo*. Inhibition of chemokine receptor signaling prevents T cells from migrating into T cell zones in secondary lymphoid tissues where IL-7 is available [15, 16]. As a consequence, T cells undergo atrophy¹² when they fail *in vivo* to reach necessary signals [15]. In another model, a conditional IL-7R transgene was expressed in IL-7R^{-/-} animals to rescue T cell IL-7R expression [17]. Upon transgene deletion *in vivo*, peripheral T cells lost IL-7R expression and atrophied, with reduced basal rates of glycolysis and shortened lifespan. The decreased glucose metabolism of neglected T cells can directly contribute to T cell apoptosis, because maintenance of glucose uptake with expression of a Glut1 transgene can delay their death and leads to increased T cell numbers [18–20].

3.2 T cell activation and glucose metabolism

T cell activation and co-stimulation leads to a dramatic metabolic reprogramming. T cells switch from a primarily oxidative metabolism to glycolysis, with significant rates of lactate production, to support the demands of rapid cell growth and proliferation. While resting cells oxidize glucose, glutamine and lipids for maximal ATP generation, proliferating cells sacrifice energy efficiency for robust biosynthesis. To accomplish this, T cells rapidly activate a metabolic program that closely resembles that of many cancer cells [21], with high rates of glucose uptake and glycolysis. In addition, glutamine oxidation increases to maintain TCA flux and provide a source of ATP and biosynthetic precursors. In contrast, lipid oxidation decreases, giving way to a lipid synthesis to build new membranes.

These changes in metabolism are coordinated by cell signaling events through the TCR and CD28 co-stimulation. CD28 co-stimulation both augments TCR signals and activates the phosphatidylinositol 3-kinase (PI3K)/Akt/mTORC1 pathway to strongly promote glucose uptake and glycolysis. The Akt/mTORC1 pathway can promote the upregulation and cell surface trafficking of Glut1, the activity of glycolytic enzymes, as well as the induction of SREBP1 to stimulate lipid synthesis [22, 23]. The induction of cMyc also plays a key role to promote the transcription of both glycolytic and glutaminolytic genes [24]. cMyc deletion blocked T cell growth and proliferation *in vitro* and *in vivo*, impairing glycolytic flux but not fatty acid oxidation or oxygen consumption [24]. In parallel, T cells induce cMyc-dependent glutamine catabolism to promote anaplerosis through production of α -ketoglutarate in the TCA cycle and nucleotide biosynthesis. This is coordinated with cMyc-dependent glucose catabolism to together support the biosynthesis of amino acids, nucleotides, and lipids. In addition, expression of the nuclear hormone receptor Estrogen Related Receptor- α (ERR α) (NR3B1) increases upon T cell activation [25] and can promote the expression of TCA and electron transport genes, essential for efficient mitochondrial metabolism. Co-stimulation through CD28 is critical, as if this signal is not provided or unavailable, PI3K signaling and ERR α induction are reduced and T cells fail to

upregulate glucose uptake or glycolysis. Together, these changes promote up to a forty-fold increase in glycolytic flux after one day of stimulation [18, 19, 24]. Interestingly, although Hypoxia Inducible Factor 1 alpha (HIF-1 α) contributes to aerobic glycolysis in many tumor cells, Wang et al. showed that this transcription factor is not necessary for T cell proliferation or glycolysis during T cell activation [24] and instead has a specific role in Th17 cells [26, 27]. Co-stimulation through CD28 is critical, as if this signal is not provided or unavailable, PI3K signaling and ERRA induction are reduced and T cells fail to upregulate glucose uptake or glycolysis [25, 28].

As glucose and glutamine metabolism increase in activated T cells, lipid metabolism switches from oxidation to synthesis. The regulation of this pathway is likely through both signaling and substrate-mediated inhibition mechanisms. The PI3K/Akt pathway can suppress the expression and activity of CPT1a, reducing lipid oxidation [29]. In addition, production of the lipid biosynthesis precursor malonyl-CoA from TCA-derived citrate further inhibits CPT1a and fatty acid oxidation [30]. To stimulate lipid biosynthesis, Akt and mTOR activate SREBP-2, a transcription factor that broadly controls lipid synthesis [23, 31].

The fuel choice switch of activated T cells from oxidation of lipids to glycolysis and glutaminolysis plays a critical role in T cell growth and activation. Bental and Deutsch [7] concluded that even though glutamine oxidation increases upon lymphocyte stimulation, ATP is mainly produced through aerobic glycolysis. Furthermore, they showed that 67% of the lactate comes from glucose. In support of these findings, glucose limitation can sharply suppress T cell proliferation and cytokine production [18, 24, 32–34]. Even if other carbon sources are available, glucose is required for T cell survival and function as well as for the production of IFN γ [18, 32]. Conversely, transgenic overexpression of Glut1 to increase basal and induced T cell glucose uptake decreased the requirement of T cells for CD28-mediated co-stimulation and increased both T cell proliferation and the production of IL-2 and IFN γ [18]. Over time, this has a significant impact on immune homeostasis, as Glut1 transgenic animals accumulate large numbers of memory phenotype CD4 and CD8 T cells and hyper-gammaglobulinemia with immune complex deposition in the kidney [18, 19]. Together, these results suggest that while lipids are spared, glucose and glutamine are critical fuels to drive proliferation of activated T cells.

3.3 OXPHOS and T cell activation

Even though aerobic glycolysis is a clear outcome of lymphocyte activation, the regulation and role of mitochondrial oxidative phosphorylation (OXPHOS) is less certain in lymphocyte activation. Although metabolism of stimulated T cells shifts to predominantly favor glycolysis, mitochondrial oxidative metabolism continues and can increase [24]. The fuel for OXPHOS is likely not lipid, as CPT1a and lipid oxidation decrease [24, 29], but rather glucose and glutamine. Previously, O'Rourke et al. showed that 10% of the glucose in activated lymphocytes [35] was driven to the mitochondria and entirely oxidized, which could support approximately 65% of the ATP generated by the cell. Roos et al. [36] also showed that 15% of the total ATP generation came from aerobic glycolysis, with the rest from OXPHOS. Despite this evidence for a major role of glucose to support OXPHOS, glutamine also contributes. After entry into the TCA cycle as α -ketoglutarate, glutamine-derived carbons can exit the mitochondria as malate or citrate, which can then be used for lipid synthesis or to generate lactate and produce NADPH by malic enzyme and replenish cytosolic NAD $^{+}$ by lactate dehydrogenase. Glutamine oxidation accounted for 30% of the lactate produced by activated cells and 75% of the lactate produced by naïve T cells [7]. It is important to note, however, that many earlier studies relied on mixed lymphocyte populations and lectin stimulation rather than antigen-specific activation of purely naïve T

cells. It will be important to validate these metabolic findings to confirm which pathways are the most critical for T cell effector function.

4. T CELL METABOLISM IN CELL FATE AND FUNCTION

To provide immunity against a wide variety of threats, CD4⁺ T cells have the ability to differentiate into several subsets and to become long-lasting memory cells, each with a unique role in immunity. T cell differentiation is determined by the cytokine environment and drives the immune response appropriate for the immunological threat at hand. Th1 effector T cells aid in viral responses, Th2 in humoral and antibody responses, while Th17 cells are involved with mucosal immunity [37]. In addition to these effector populations (Teff), a naïve T cell can also differentiate into a regulatory T cell (Treg). Tregs serve to suppress Teff proliferation and function as a means to prevent excessive inflammation. Central memory T cells are very long-lived and can allow for rapid secondary responses if exposed to the same or similar pathogen. The balance of these subsets is critical and alterations or dysregulation of this balance lies at the root of many immunological diseases.

4.1 CD4⁺ T cell subsets

It has become clear that each CD4⁺ T cell subset has a unique metabolic pattern adapted to its function. Teff have high levels of Glut1 and high rates of glucose uptake and glycolysis [19]. Th17 cells are particularly dependent on glucose, as differentiation into Th17 fails when glycolysis is inhibited [19, 26, 27]. Moreover, HIF-1 α , a protein that regulates glycolysis and is induced under hypoxia, enhances Th17 development [26, 27]. Simultaneously, HIF-1 α promotes the degradation of the transcription factor forkhead box P3 (FoxP3), the transcription factor characteristic of T regulatory cells, impairing Treg development [26]. In contrast, Treg express much lower levels of Glut1 and have lower rates of glucose uptake and glycolysis. Instead, rates of lipid oxidation are higher in Treg than in effector T cells [19, 27]. Consistent with this metabolic distinction, glucose deprivation or the glycolytic inhibitor 2-deoxyglucose prevent effector T cell generation yet promote the specification of Treg both *in vitro* and *in vivo* [19, 27]. Regulation of glucose and lipid metabolism by mTORC1 may play a key role in this difference between effector and regulatory T cells, as mTOR promotes glycolysis and is selectively essential for effector but not for regulatory T cells. T cell specific mTOR knockout mice and mice treated with the mTOR inhibitor rapamycin fail to generate effector T cells and have increased Treg generation [38, 39].

Complicating Treg metabolism is emerging evidence that the PDHK1 inhibitor DCA can promote FoxP3 expression and Treg generation [40]. PDHK1 inhibition inhibits lactate production and instead drives glucose oxidation in the mitochondria. These results suggest that Tregs do not have an obligatory reliance on a particular fuel source such as lipids, but rather require mitochondrial oxidation of either lipids or glucose. Why this type of metabolism may be favored is unclear. Tregs do need to be able to go into hostile environments and suppress Teff cells and it may be to their advantage to have a distinct and energy efficient metabolic phenotype. Further, the high efficiency of oxidative metabolism may increase the survival and provide a greater degree of metabolic flexibility and adaptability.

One additional explanation for the distinct metabolism of Treg is the potential of Treg to selectively target the metabolism of effector T cells as a means of suppression. Tregs express a surface enzyme that degrades ATP into AMP called NTPDase 1 or CD39. FoxP3 drives NTPDase 1 transcription, and enzymatic activity can increase when associated with the T cell receptor. In effect, Treg remove ATP from the environment to suppress the pro-inflammatory effects of extracellular ATP on effector T cells [41]. In addition, effector T

cell proliferation is enhanced by glutathione, which is synthesized by dendritic cells (DC), and important to help control reactive oxygen species (ROS). Treg interfere with GSH metabolism in DCs by impairing cysteine production and uptake that is essential for GSH metabolism [42]. Thus Treg can selectively impair metabolic pathways in surrounding cells. The roles of specific metabolites and metabolic pathways in Treg and Teff function remain largely uncertain yet may allow modulation of the immune response and provide new ways to treat inflammatory disorders.

4.2 Memory T cells

At the end of an immune response, when pathogens are cleared and TCR stimulation and inflammatory cytokines are reduced, a majority of the effector T cells die by apoptosis. However, some survive and persist as memory cells. This transition back from stimulated to a long-lived quiescent cell also must involve additional metabolic reprogramming, as cells no longer require active biosynthesis and instead need an efficient supply of ATP. Consistent with this need to adapt to new metabolic demands, memory T cells have been shown to switch from glycolysis to oxidative metabolism. Initially described in TRAF6^{-/-} T cells, where gene expression analyses pointed to increased expression of lipid oxidation genes, inhibition of glycolysis and stimulation of lipid oxidation at the peak of an immune response was then shown to enhance T cell memory formation [43, 44]. The role of TRAF6 and its mechanism of metabolic regulation remain unclear, but the role of lipid oxidation in memory T cell generation was supported in studies in which T cell metabolism was modulated with the mTORC1 inhibitor rapamycin, or the AMPK activator, metformin [43, 44]. Each of these treatments decreases glycolysis and lactate production to instead favor oxidative metabolism generally, and lipid oxidation in particular.

More recently, Van der Windt et al. described how the survival of memory CD8 T cells was enhanced by greater mitochondrial spare respiratory capacity (SRC) and regulation of lipid oxidation through CPT1a [45]. SRC represents the extent to which cells can increase oxygen consumption beyond the basal rate of respiration, with adequate nutrients, when placed under stress. SRC is expressed as a proportion of respiration under basal and stressed conditions and does not measure direct rates of OXPHOS, but rather the ability of cells to respond and rapidly increase respiration and, therefore, ATP production. SRC can be mediated both by increased rates of electron transport as well as by increased density or number of mitochondria per cell. The source of memory T cell oxidative metabolism and SRC is largely mediated through lipid oxidation, a process regulated in part through expression of CPT1a. Indeed, Van Der Windt et al found that CPT1a levels are elevated in memory T cells and that the modulation of CPT1a influences both SRC and memory generation [45]. It is intriguing to speculate that the metabolic state of the mitochondria may not only influence the long-term survival of immune cells but also modify the ability of T cells to react to new stimuli and provide an immediate energy supply to fuel rapid re-activation of memory T cells upon subsequent antigen encounter.

5.B CELL METABOLISM

B cell metabolism has been less widely investigated than T cell metabolism. Given the important role of B cells in autoimmunity and inflammation, this is certainly an area deserving attention. We can speculate that the metabolic characteristics of the different subsets of B cells may be similar to those of T cells. Although yet to be demonstrated experimentally, the diversity of metabolic phenotypes of T cells suggests that B cells and their different functional stages and types may also be distinct. Indeed there is emerging evidence showing that there are metabolic differences between different types of B cells. Before antigen encounter, B cells pass through multiple phases of differentiation and selection. In the bone marrow, hematopoietic stem cells differentiate into pro-B cells, which

undergo rearrangement of the immunoglobulin genes and differentiate into pre-B cells. Once B cells express IgM they subsequently undergo the first of successive negative selection or receptor editing. B cells that survive these checkpoints migrate to the spleen where they pass T1 and T2 stages until they become naive long-lived B cells, which will encounter antigen and eventually become effectors and plasma cells capable of secreting different immunoglobulins [46].

The different stages of B cell development have been shown to have particular metabolic phenotypes. Interestingly, each pre-B cell stage was reported to have a different sensitivity to the glucose analogue 2-deoxyglucose (2-DG) [47], suggesting different dependencies on glucose as B cells develop. B220+CD43⁻ early pre-B cells and IgM⁺ immature B cells are more sensitive to low concentrations of 2-DG than the other B cell subtypes. Interestingly, pre-B cells were resistant to 2-DG, suggesting that they are less dependent on glucose metabolism than immature B cells. Pre-B cells also have much lower levels of Glut1 and Pfkfb3, which are responsible for the transport glucose and the conversion of Fructose 6-P to fructose 1,6-bi-P, respectively. This correlates with diminished levels of ATP synthase and malate dehydrogenase, fundamental in the TCA cycle and OXPHOS [47]. The fuel switching through B cell development is not clear, but modulation of lipid oxidation may provide an alternate source of energy for pre-B cells.

Once stimulated, naïve B cells undergo a proliferative burst that is accompanied by an increase in glycolysis and lactate production [48] similar to what occurs in T cell activation (Figure 1). In addition to glucose, amino acids such as glutamine, phenylalanine, tryptophan, and tyrosine are rapidly consumed during the proliferation phase and glutamine is necessary for both cell proliferation and differentiation into plasma cells [49]. Thus, in addition to increased expression of glycolytic genes such as glyceraldehyde-2-phosphate dehydrogenase (GAPDH) and α -enolase, amino acid metabolizing enzymes such as ornithine aminotransferase, phosphoglycerate dehydrogenase, and phosphoserine aminotransferase are induced. This suggests that B cells, like T cells, depend on both glucose and amino acid metabolism for activation and proliferation [50].

Ultimately, B cells can differentiate into antibody-secreting plasma cells (Figure 1). The immunoglobulin synthesis and secretion capacity of plasma cells are extremely high, while proliferation is limited. Thus plasma cells require ATP and a selective ability to synthesize large amounts of protein. Increased mitochondrial metabolism is suited to fit this mixed metabolic demand, with high efficiency generation of ATP, as well as amino acid precursors. Consistent with this model, the metabolism of plasma cells has been characterized by a reduced use of glucose with amino acid production due to the oxidative consumption of glutamine [48]. To support this metabolic program, the transcription factor XBP1 promotes an increase in mass and function of the endoplasmic reticulum and mitochondria [51]. In addition, STAT3 activation by IL-21 is important for the generation of antibody secreting cells [52] and STAT3 has been proposed to regulate mitochondrial electron transport [53]. Although further studies are needed, it appears that B cell metabolism is matched with the function of that particular B cell subtype. Proliferating B cells rely on glucose metabolism through aerobic glycolysis whereas long-lived plasma and memory B cells preferentially oxidize amino acids. The role and regulation of lipid metabolism, however, has not been well studied in B cells and requires further investigation.

6. METABOLIC CHECKPOINTS TO ENHANCED OR MISMATCHED METABOLISM

The selective fuel usage of distinct lymphocyte subsets and differentiation stages is, in large part, to support specific cell functions. In general, glycolytic metabolism correlates with

rapid cell growth, while oxidative metabolism better supports efficient energy production and can allow cells to adapt to stress. While these outcomes are not mutually exclusive, failure of lymphocytes to upregulate the appropriate metabolic program or access the appropriate fuels can lead to metabolic checkpoints that disrupt cell proliferation or survival. Activated lymphocytes require high rates of glucose metabolism and inhibition of this pathway can suppress T cell proliferation and cytokine production [18, 19, 27, 32, 33] as well as disrupt effector T cell differentiation [19, 27]. Conversely, inappropriately elevated metabolism can enhance immunity [18, 19, 45]. This is the case for over-nutrition, which is associated with an inflammatory state [3, 4]. However, the immunological phenotype of obesity is complicated by hormonal and multiple cell-extrinsic processes.

Direct modification of lymphocyte intrinsic metabolic pathways has proven useful to test the association between altered metabolism and changes in cell fate. Indeed, we have shown that transgenic expression of Glut1 specifically in T cells promotes an increased rate of glycolysis that enhances T cell activation [18, 19]. Glut1 transgenic T cells responded with increased rates of cell growth, more rapid proliferation, and enhanced cytokine production. With age, transgenic mice accumulate activated and memory CD4⁺ CD44^{high} T cells in the spleen, lymph nodes, and liver and show signals typical of autoimmunity, including anti-dsDNA antibodies and immunoglobulin deposition in the kidneys [18, 19]. Importantly, increased glucose uptake selectively impacted activated and effector T cells that normally become reliant on glucose. While naïve resting T cells remained phenotypically normal, T cell activation was enhanced and effector T cell populations, including Th1, Th2, and Th17, were increased. Treg, which rely on lipid and oxidative metabolism [19, 27], were unaltered by increased Glut1 expression [18, 19]. In a second example, modulation of CPT1a selectively impacted the generation of memory CD8 T cells [45]. Increased CPT1a enhanced CD8 T cell lipid oxidation and enhanced CD8 memory. Conversely, inhibition of CPT1a could suppress CD8 memory. Thus, direct modification of metabolic pathways shows that fuels and metabolic pathways can be limiting, as altered flux can enhance or suppress immunity in a fuel and cell-type specific manner.

Because metabolism can modify lymphocyte function and fate, inappropriately matched metabolism and fuel selection may lead to metabolic checkpoints that disrupt immunity. This potential, however, may provide important opportunities to modulate immunity. While poorly understood, three main outcomes of mismatched or insufficient fuel usage have been described. First, inhibition of appropriate metabolism may lead to altered or inhibited differentiation and T cell specification. Effector T cell activation and differentiation into Th1, Th2, and Th17 can be prevented when glucose is limiting [18, 19, 25, 27]. However, even these T cells that fail to activate are impacted and can instead become rendered anergic [34]. This is likely due to the activation of AMPK to inhibit mTORC1, a process closely associated with T cell anergy [54]. In parallel, because Treg utilize lipids rather than glucose [19, 27] and are not dependent on mTOR [39], inhibition of glucose metabolism does not prevent generation of Treg [19, 27]. Similarly, HIF-1 α is important to promote glycolysis in Th17 cells and HIF-1 α deficiency prevents Th17 and instead leads to Treg differentiation [26, 27]. The second chief outcome of inappropriate fuel usage can be inhibition of proliferation or cellular senescence. In particular, AMPK activation and mitochondrial dysfunction have been shown to lead to p53 phosphorylation and the induction of p21 and cell senescence [55, 56]. This pathway, however, has not been well examined in lymphocytes.

The third and potentially most significant impact of metabolic checkpoints is the induction of cell death (Figure 2). Metabolic stress initially leads to autophagy as an alternate intracellular source of nutrients. This process, however, is self-limiting and ultimately cells undergo apoptosis [57]. Cells initiate the apoptotic program when they receive the

appropriate signal or when under significant stress, such as may be induced by a metabolic checkpoint. The intrinsic pathway of apoptosis is tightly controlled by the equilibrium of Bcl-2 family proteins. The Bcl-2 family is composed of pro-apoptotic members, such as Bax and Bak, and anti-apoptotic members, including Bcl-2, Bcl-xL, and Mcl-1. Together with the BH3-only proteins such as Bim, Bid, Puma, Noxa, and others, these proteins interact at the mitochondria to regulate mitochondrial outer membrane permeability (MOMP) and to promote the release of cytochrome c. Once initiated, MOMP occurs quickly and has two fundamental outcomes for the fate of the cell [58]. The primary response is activation of the apoptosome by cytochrome c, Apaf-1, and caspase 9, which leads to caspase activation and the morphology of apoptosis. A secondary impact is that damaged mitochondria are no longer functional and can instead produce high levels of ROS. In this state, it is unlikely that cells will survive regardless of caspase activity, but higher levels of glycolysis and expression of GAPDH may allow some cells to avoid cell death and recover from mitochondrial dysfunction [59].

Data now show clear associations between metabolism and apoptosis. In some instances, Bcl-2 family proteins are directly linked to metabolic machinery. The BH3-only protein Bad, for example, forms a complex that includes the glycolytic enzyme glucokinase (hexokinase IV) that plays a key role in glucose sensing and metabolism [60, 61]. The BH3-domain of Bad mediates both of these functions and is essential for its regulation of glucose metabolism as well as the ability of Bad to promote cell death. The capacity of Bad to bind to anti-apoptotic proteins like Bcl-2 or Bcl-xL is impaired when it is phosphorylated by pro-survival kinases in the presence of glucose. The BH3-only protein Noxa also can form complexes with glycolytic proteins and may both promote glycolysis when glucose is abundant and also stimulate apoptosis when glucose is limiting [62]. Conversely, the interaction of Bax and Bak on the outer mitochondrial membrane with the Voltage Dependent Anion Channel (VDAC) can modulate apoptosis, with hexokinase proposed to prevent the pro-apoptotic association of Bax and VDAC [63] while Bak association with VDAC2 can suppress apoptosis [64, 65].

In addition to direct physical associations, metabolic stress signaling pathways regulate Bcl-2 family proteins to promote the death of cells with mismatched metabolic fuel. Noxa and Mcl1 are closely associated and T cell activation leads to an increase in Noxa expression and association with Mcl1 [66]. If glucose becomes limiting to activated T cells, Mcl1 protein levels decrease, thus reducing the anti-apoptotic protection from Noxa and other pro-apoptotic Bcl-2 family proteins. This regulation of Mcl1 appears to occur largely through decreased protein translation rather than proteolytic degradation [9, 67]. Metabolic stress can activate AMPK, which in turn can inactivate the mTORC1 pathway to decrease protein translation [9, 67]. While protein translation is broadly suppressed under this condition, Mcl1 may be particularly sensitive given its short half-life [68]. As Noxa titrates the dwindling amount of Mcl1, lesser amounts of anti-apoptotic protein capacity are available to suppress the pro-apoptotic functions of other pro-apoptotic Bcl-2 family members. In particular, Bim and Puma can each associate with Mcl1 and also induce Bax activation [69]. We have found that Bim and Puma are each induced in activated T cells upon inhibition of glycolysis and play important roles to initiate cell death [9, 10, 70]. Mechanistically, the regulation of Bim and Puma is not certain, but may involve ER stress [71] and AMPK activation of the tumor suppressor p53 [10, 55, 70]. Importantly, Bim and Puma-deficient cells are protected from apoptosis for prolonged periods even in the absence of glucose [9, 10].

Even if Bcl-2 proteins fail to induce apoptosis, fuel mismatch can still lead to cell death. Autophagy is a metabolic stress response that can supply nutrients [72, 73], but metabolic stress will ultimately promote necrosis as cells become unable to match their demands for

ATP [74]. Prior to this, however, even Bak and Bax-deficient cells can undergo caspase-dependent cell death with glucose starvation [75]. In this case, cell death is delayed but still occurs through an atypical activation of caspase 8, which then stimulates further caspase activity and apoptosis. The regulation of this pathway is uncertain, but clearly shows that metabolic stress has multiple pathways to lead to apoptosis. Together, the hierarchy of the stress response appears to be first through the regulation of Noxa, Bim, Puma, and Mcl1, then atypical caspase 8 activation, followed by necrosis after a prolonged period using autophagy as an alternate energy source.

7. LYMPHOCYTE METABOLISM IN DISEASE

The requirement that lymphocyte fuel usage match their demands opens the potential that altered metabolism may both promote diseases as well as provide a means to target specific immunological functions. The potential of targeting the inflammation associated with obesity is a particularly promising avenue to alleviate disease [4], but many more classical immunological diseases are also potentially amenable to such approaches. The success of anti-metabolites as standard immunosuppressive treatments [76] demonstrates that broad metabolic targeting using these and other metabolic interventions can be effective in immunological diseases (Table 1). It is now important to identify the metabolic phenotype and fuel usage of lymphocytes in atopic and inflammatory diseases to better understand how these pathways may be targeted to specifically initiate metabolic checkpoints in immunological diseases. Several inflammatory diseases have been examined from a metabolic point of view and provide insight to opportunities for metabolic approaches for treatment.

7.1 Asthma

Asthma is caused by inflammation in the respiratory tract that leads to constriction and tissue remodeling. While generally well controlled by conventional therapies, a significant number of patients with severe asthma fail to respond and lymphocyte metabolism may provide a fundamental new pathway to target. Indeed, human and mouse T cells from asthma subjects produce higher amounts of lactate than T cells from healthy individuals [40] indicating high levels of glycolysis. In humans with asthma, lactate levels in the serum correlate with the severity of the disease and *in vitro* lactate production correlates with T cell proliferation. The correlation between asthma severity, lactate levels, and glycolysis is corroborated by the increased expression of glycolytic enzymes and proteins such as pyruvate dehydrogenase kinase 1 (PDHK1), 6-phosphofructo-2-kinase/fructose-2-bisphosphatase 3 (PFKFB3), and fructose 1,6-bisphosphatase 1 (FBP1) [40]. PDHK1 is a potentially important enzyme target to modify T cell metabolism that acts to inhibit the pyruvate dehydrogenase (PDH) enzyme complex. As a result, pyruvate is instead converted to lactate. Inhibition of PDHK1 can relieve suppression of PDH activity to promote the oxidation of pyruvate and is a key regulator of the fate of glucose and glycolysis. PDHK1 is active in highly glycolytic cells, such as highly proliferative cells and in many tumor cells. In asthma as well as in a model of arthritis, inhibition of PDHK1 with dichloroacetate has been shown to protect against inflammation and disease [40, 77].

7.2 GVHD

Allograft rejections and Graft vs. Host disease (GVHD) are important health concerns for solid organ transplants and the treatment of hematopoietic tumors. Interestingly, unlike acute activation of T cells that induces glycolysis, alloreactivity that promotes GVHD has been associated with increased oxidative metabolism and oxygen consumption [78]. In this case, alloreactive T cells can rapidly proliferate, but also preferentially increase their oxygen consumption and mitochondrial activity. In contrast, glucose uptake and metabolism appear

to be only modestly upregulated in GVHD. The precise fuel to support the oxidative metabolism is not clear, but may include lipids. Why alloreactive T cells utilize a distinct fuel and metabolic program is also uncertain, but chronic stimulation in an MHC mismatched environment or the robust strength of signal in alloreactivity may activate an alternate metabolic pathway. Nevertheless, this distinct metabolic program offers a new direction to selectively target the metabolism of GVHD-associated T cells. In particular, targeting mitochondrial metabolism may selectively impact GVHD-associated T cells. Treating mice suffering from GVHD with Bz-423, an inhibitor of the mitochondrial F(1)F(0) adenosine triphosphate synthase (F(1)F(0)-ATPase) increased superoxide production and caused cell death specifically to the proliferating donor CD4⁺ T cells [78]. Other cell types, however, were protected from Bz-423 possibly due to an increased capacity to modify their fuel choice and metabolic pathways to handle ROS stress or reduced ROS production. As a consequence, Bz-423 treatment improved the survival of different GVHD mouse models and specifically targeted the alloreactive proliferative cells [78].

7.3 Systemic Lupus Erythematosus

Systemic Lupus Erythematosus (SLE) is a chronic, remitting, relapsing and inflammatory autoimmune disorder caused by the production of autoantibodies. While autoantibodies against nuclear antigens are the most characteristic of SLE, a wide variety of other antibody specificities are generated. Anti-metabolites, such as methotrexate, leflunomide, and azathioprine, that block folate, pyrimidine, and purine metabolism, respectively, have been broadly effective to treat SLE. There are a number of reasons why these drugs may be effective, and the activation of direct metabolic checkpoints may be involved. Studies of the metabolism of peripheral lymphocytes from SLE patients, showed that, much like in alloreactive cells from GVHD, SLE-associated T cells had active and even hyperpolarized mitochondria⁷⁸ that was associated with increased ROS production [79, 80]. Interestingly, this hyperpolarized mitochondrial state may be due in part to metabolic stress and compensation, as SLE-associated T cells were also found to have lower levels and capacity to increase ATP despite potentially more active oxidative mitochondrial metabolism [81]. Interestingly, treatment of the SLE model *Lymphoproliferation (lpr)* mutant mice with Bz-423 also helped to alleviate disease and to reduce glomerulonephritis [82]. These data suggest a common metabolism of allo- and auto-reactive T cells in which chronic stimulation results in oxidative metabolism and reduced ability to handle ROS stress.

8. CONCLUSION

The remarkable plasticity of the immune system to defend against an infinite array of pathogens while minimizing damage to normal tissue requires a complex signaling network that coordinates lymphocyte function and cellular physiology. The unique functional requirements of specific stages of lymphocyte differentiation and activity each require distinct metabolic pathways and support. These can be broadly divided into growth or energy promoting pathways, but there are many nuances and these pathways are not mutually exclusive. Importantly, control of lymphocyte metabolism does not appear to be through a consumption model, in which lymphocyte are able to use nutrients and replenish as they require. Rather, a supply-based system driven by activating and specific signals is critical to drive the pathways that lymphocytes will then use for their function and survival. While this approach provides an anticipatory source of energy or biosynthesis, it also implies that fuel choice is dictated in part by extracellular cues. Failure to obtain the appropriate fuels or activate the anticipated metabolic pathways may lead to metabolic stress and checkpoints that can prevent the immune response or open new susceptibilities that could be exploited in immunosuppression. On the other hand, exposure to high fuels levels, such as occurs in obesity, may lead to an excessive lymphocyte activation, prevention of anergy, and altered immune responses.

The primary fuels for lymphocytes are glucose, lipids, and amino acids and the balance of these pathways is critical in both normal immunity and inflammatory disease. Given the emerging data concerning lymphocyte metabolism in diseases such as asthma, GVHD, and SLE, it is clear that lymphocytes can use each of these fuels in disease-specific fashion. The key differences in metabolic fuel choice between normal or acute lymphocyte activation and that of immunologic diseases may be chronic versus acute activation. However, in each case the metabolic phenotype may provide new opportunities for therapy. Further studies of lymphocyte metabolism in normal and diseased states will certainly provide new insight and potentially new therapeutic approaches to treat disease.

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Highlights

- Lymphocytes balance glucose, lipid, and amino acid metabolism for energy and growth
- Metabolic fuel choices are controlled by activation and differentiation signals
- Distinct lymphocyte subsets and types have specific metabolic programs
- Targeting metabolism can initiate checkpoints to stop growth or to kill cells
- Metabolic approaches to direct lymphocyte fate will be useful in immune disease

Figure 1. B cell metabolism in activation

A naïve B cell utilizes a variety of fuels for energy, including glucose (Glc), amino acids (aac) and fatty acids (FA). These fuels are metabolized in the mitochondria to provide energy for survival and migration. Upon stimulation, a B cell increases the uptake of amino acids, primarily glutamine, and glucose. These are used to provide biosynthetic precursors for cell division. Instead of being oxidized in the mitochondria, glucose is converted to lactate in the cytoplasm in a process known as aerobic glycolysis. Fatty acid oxidation is decreased and the lipids are instead used to build new membranes. Once a B cell differentiates into a plasma cell, the metabolic phenotype becomes more about generating ATP energy. The cell metabolizes glutamine for energy and the rate of glycolysis is decreased.

Figure 2. Metabolic checkpoints and apoptosis

Activation, metabolism, and cell death are interconnected. Upon stimulation, lymphocytes activate pro-survival and proliferative pathways in conjunction with an increase in glucose metabolism. Under low glucose conditions, the pathways that drive activation and proliferation are inhibited, and if the signal persists, pro-apoptotic genes will be induced. On the other hand, under low or no stimuli, metabolic genes are downregulated, leading cells to a low metabolic state and eventually energy. In both cases, the metabolic state of the cell can influence cell fate. For instance, low glucose will lead to an insufficient response, while a high glucose environment may cause an excessive response under low stimulation.

Table 1

Metabolic therapies used in immunological diseases.

Compound	Description	Use	References
2dg (2-deoxyglucose)	Glucose analogue that blocks hexokinase.	Phase I/II antitumoral clinical trials	[83]
DCA (Dichloroacetate)	PDHK inhibitor. It activates PDH and reduces lactate production.	Treatment of lacticidosis, inhibits tumor growth, and inflammation in arthritis murine model.	[77], [84]
Rapamycin (Sirolimus)	Macrolide that inhibits mTor.	Mainly used against transplant rejection. Induces Treg differentiation.	[38]
Bz-423	1,4-benzodiazepine. Inhibits F1F0-ATPase, generating O_2^- and apoptosis.	GVHD and Lupus by killing active lymphocytes.	[85],[78]
thiazolidinedione	PPAR γ agonist.	Antidiabetic. Multiple sclerosis.	[86]
statins	Inhibit HMG-CoA	Lower cholesterol. Used in clinical trials on MS.	[87]
methotrexate	Antifolate drug, inhibiting purine and pyrimidine synthesis (DNA).	Cancer chemotherapy, autoimmune diseases.	
leflunomide	Inhibits dihydroorotate dehydrogenase, pyrimidine synthesis	Rheumatoid arthritis and other Autoimmune diseases	[88]
azathioprine	Purine analogue. Inhibits DNA synthesis.	Transplant rejection and autoimmune diseases	