Revving the Engine: Signal Transduction Fuels T Cell Activation

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For initiation of an immune response, resting T cells must reprogram their metabolism. Continuing the “From the Field” series (see Editorial [2007] 26, 131), Jones and Thompson draw attention to the importance of metabolism during T cell activation and consider how this process is regulated by receptor-mediated signal transduction.

Introduction
Stimulation through the T cell receptor (TCR) and costimulatory receptors triggers a switch in T cell differentiation from a quiescent (resting) state to an activated state characterized by rapid rates of growth and proliferation and the acquisition of effector function. The decision to proliferate—to increase biomass and replicate the genome in order to create two daughter cells—presents a substantial bioenergetic challenge. A T cell must substantially increase ATP production and acquire or synthesize the substrates (lipids, proteins, and nucleic acids) needed to support growth at a sufficient rate to meet the demands of proliferation. Without sufficient support for their increased bioenergetic needs, activated T cells die by apoptosis. Recent evidence indicates that this increase in cellular metabolism is not a homeostatic response to the increased consumption of ATP and macromolecules upon activation. Rather, specific signal transduction pathways direct nutrient uptake and metabolism during T cell activation. How the intracellular metabolism of acquired nutrients is redirected from mitochondrial oxidative phosphorylation (OXPHOS) to synthetic metabolic pathways during T cell growth and proliferation is poorly understood.

T Cell Activation Triggers a Metabolic Conversion
Quiescent (naive and memory) and activated T cells display different metabolic signatures (Table 1). Resting T cells have fewer energetic and biosynthetically demanding processes relative to the activated state and devote the majority of energy metabolism toward housekeeping functions. They display a lower rate of nutrient uptake and derive most of their ATP from catabolic processes that fuel OXPHOS (Krauss et al., 2001). In the absence of intracellular signals for maintaining homeostatic processes, quiescent T cells undergo progressive atrophy (Rathmell et al., 2000). (For a more extensive review of the metabolism of quiescent lymphocytes, please see Fox et al. [2005]). Upon stimulation, T cells undergo conversion from a resting state to one of active growth. Anabolic processes such as protein and lipid synthesis are increased, whereas catabolic processes including β-oxidation are actively suppressed. One hallmark of this metabolic conversion is that, despite adequate oxygen to support complete oxidation of glucose, cellular ATP production switches from OXPHOS to high throughput glycolysis. As will be discussed later, this switch to glycolytic energy production, known as aerobic glycolysis, is important for both energy production and biosynthetic programs during T cell activation.

Effective T cell activation requires two signals: an antigen-specific signal delivered by the TCR and a costimulatory signal, delivered by surface receptors such as CD28. Antigen-specific signals via the TCR drive a program of transcription and translation. This program provides the instructional code for proliferation. Initiation of this program commits the T cell to an genetically demanding process that can only proceed if there are sufficient intracellular resources. Signal transduction pathways downstream of costimulatory receptors such as CD28 actively upregulate pathways of nutrient uptake, glycolytic ATP production, and macromolecular synthesis to facilitate the metabolic conversion of T cells from quiescence to proliferation (Figure 1). From a metabolic perspective, costimulation can be viewed as a fuel signal—ensuring that nutrient uptake and energy production meets or exceeds the bioenergetic demand of proliferation.

Glycolysis: A Source of Energy and Metabolic Intermediates during Cell Growth
Glucose is a critical metabolite for proliferating cells. It serves as the primary substrate for ATP generation, is an intermediate in glycosylation reactions, and is an essential carbon source for the synthesis of other macromolecules. T cell activation triggers a rapid increase in glucose uptake and glycolytic metabolism (Roos and Loos, 1973). T cell activation also triggers the rapid transcription of genes encoding glycolytic enzymes including glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase, aldolase, and enolase. Maximal glycolytic metabolism requires both a TCR signal and costimulation via CD28 (Frauwirth et al., 2002). TCR activation along with CD28 costimulation triggers the activity of phosphatidylinositol 3′-kinase (PI3K), which, through conversion of the membrane

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lipid PIP₂ to PIP₃, leads to the activation of the serine-threonine kinase Akt. The lipid phosphatase PTEN antagonizes Akt activation by dephosphorylating PIP₃. Akt promotes increased cellular glucose metabolism by stimulating the localization of the glucose transporter Glut1 to the plasma membrane, thus facilitating increased glucose uptake (Frauwirth et al., 2002; Rathmell et al., 2003). In addition, Akt increases glycolytic metabolism by stimulating the activity of hexokinase and phosphofructokinase, both rate-limiting enzymes of the glycolytic pathway. The effects of Akt on glucose metabolism in lymphocytes are antagonized by the inhibitory receptor CTLA-4, suggesting that antagonists of T cell activation may function in part by disrupting glucose metabolism. Other costimulatory molecules including OX40 and 4-1BB can also activate the PI3K-Akt pathway and contribute to the regulation of nutrient uptake and metabolism in activated T cells. In addition, Notch signaling has been reported to stimulate glycolytic metabolism in thymocytes through activation of PI3K-Akt (Ciolfani and Zuñiga-Pflucker, 2005). CD19 in B cells and DAP10 in NK cells appear to initiate a similar metabolic signaling pathway to that initiated in T cells by CD28. Signaling downstream of cytokine receptors can augment aerobic glycolysis in proliferating lymphocytes by stimulating the PI3K-Akt pathway or by enhancing Jak-Stat-dependent regulation of glycolytic genes (Fox et al., 2005).

Each molecule of glucose processed by glycolysis yields two molecules each of ATP, NADH, and pyruvate. In proliferating lymphocytes, the majority of the pyruvate produced is converted to lactate, which allows the recycling of NADH back to NAD⁺ to maintain glycolytic flux. Although the energetic yield of aerobic glycolysis is much smaller than OXPHOS, under conditions of unlimited glucose supply, glycolysis produces ATP substantially faster. Therefore, stimulating glucose uptake and glycolysis is the most direct way a cell can increase its energy production to meet the demands of anabolic growth.

In addition to providing energy for proliferating T cells, glycolysis also generates metabolic intermediates critical for cell growth. Metabolism of glucose through the pentose phosphate shunt generates both ribose-5-phosphate, a key intermediate in nucleotide biosynthesis, and NADPH, which supplies reductive power for both nucleotide and fatty acid biosynthesis. Glycolytic intermediates also contribute to protein and lipid synthesis. 3-phosphoglycerate provides the carbon backbone for the production of the amino acids serine and glycine. Glycerol moieties for phospholipid synthesis are derived from dihydroxyacetone phosphate. Finally, the glycolytic end product pyruvate can be imported into the mitochondria where it can be converted into substrates for the production of additional amino acids or for fatty acid synthesis. Pyruvate metabolism in the mitochondria also maintains mitochondrial membrane potential, thus suppressing apoptosis in proliferating lymphocytes.

### The Metabolic Signatures of Resting and Activated T Cells

<table>
<thead>
<tr>
<th>Metabolic Signature</th>
<th>Quiescent T Cells</th>
<th>Activated T Cells</th>
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<tbody>
<tr>
<td>Primary Source of ATP Generation</td>
<td>OXPHOS</td>
<td>Glycolysis</td>
</tr>
<tr>
<td>Nutrient Uptake</td>
<td>Low</td>
<td>High rate of glucose and amino acid uptake</td>
</tr>
<tr>
<td>Glycolytic Rate</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Biosynthesis</td>
<td>Homeostatic</td>
<td>Increased lipid, protein, and nucleic acid synthesis</td>
</tr>
<tr>
<td>Catabolic Processes</td>
<td>Interchangeable degradation of glucose, amino acids, and lipids for ATP generation</td>
<td>Suppression of β-oxidation; Net amino acid synthesis</td>
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<td>Growth Profile</td>
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<td>Exponential growth and proliferation</td>
</tr>
</tbody>
</table>

Quiescent (resting) and activated T cells display distinct metabolic signatures on the basis of their bioenergetic and biosynthetic requirements. Naïve T cells derive the majority of their ATP through catabolic metabolism of intracellular contents (glucose, amino acids, and lipids) and oxidative phosphorylation (OXPHOS) and maintain basal rates of nutrient uptake, glycolysis, and biosynthesis. Activated T cells switch to an anabolic metabolic profile characterized by increased rates of nutrient uptake, glycolysis, and biosynthesis and suppression of catabolic pathways such as β-oxidation.

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**Commentary**

T cell activation is accompanied by increased rates of protein synthesis that is essential for supporting cell growth and effector function. A key regulator of protein synthesis in T cells is the mammalian Target of Rapamycin (mTOR) (Wullschleger et al., 2006). mTOR modulates the rate of protein synthesis by regulating both the availability of amino acids and the process of cap-dependent translation. Phosphorylation by mTOR promotes the initiation of cap-dependent translation by altering the activity of components of the translational machinery including the translational inhibitor 4E-BP1, the translation initiation factor EIF2B, and ribosomal p70 S6 kinase.
Rapamycin, which is a potent inhibitor of mTOR, suppresses cap-dependent protein translation. Rapamycin is an immunosuppressant and functions to suppress lymphocyte proliferation in part by inducing a G1 cell-cycle arrest (Kay et al., 1991).

As with glucose metabolism, protein metabolism in proliferating T cells is regulated by TCR and CD28 signals through the activation of the PI3K-Akt pathway. Akt regulates mTOR function by phosphorylating TSC2. This phosphorylation disrupts the ability of the TSC1-TSC2 complex to inhibit Rheb, a GTP-binding protein that serves as an mTOR activator (Inoki et al., 2002). T cells with enhanced Akt activity display increased cell size because of an enhancement of mTOR-dependent growth (Rathmell et al., 2003). The oncogenic kinases of the PIM family also function to couple cytokine-mediated signals to translational control. PIM2 enhances protein translation through concomitant inhibition of 4E-BP1 and activation of the initiation factor eIF4E (Hammerman et al., 2005). In mice, combined deficiency of the Akt1, Pim1, and Pim2 genes leads to decreased resting T cell size, and the inhibition of both the PI3K-Akt-mTOR and PIM pathways leads to dramatic defects in T cell growth and proliferation. Thus, the PI3K-Akt-mTOR and PIM pathways function as redundant but essential mediators of TCR- and CD28-dependent translation control.

Amino acid availability represents an additional regulatory step for protein translation. mTOR signaling also promotes increased amino acid uptake by regulating the surface expression of amino acid transporters. The activation of nutrient transporters on the cell surface depends on ongoing signal transduction. When receptor signaling is interrupted, the turnover of nutrient transporters rapidly decreases the rate of nutrient uptake and terminates growth (Edinger et al., 2003).

Antigen-presenting cells (APCs) can also affect T cell activation by controlling extracellular amino acid availability.
availability. Macrophages and dendritic cells (DCs) can limit the extracellular availability of the essential amino acid tryptophan through degradation by the enzyme indoleamine 2,3-dioxygenase (IDO). Tryptophan catabolism can limit T cell responsiveness by inducing anergy (Munn et al., 2005). CTLA-4, a regulator of T cell anergy that both negatively regulates CD28-dependent glucose metabolism and stimulates IDO activity in DCs, promotes T cell tolerance in part through its effects on tryptophan catabolism (Grohmann et al., 2002). The regulation of cellular metabolism in APCs may represent an additional control point for T cell activation.

The Mitochondria as a Biosynthetic Hub
The primary site for energy generation in vegetative cells is the mitochondrion. Enzymes of the tricarboxylic acid (TCA)-cycle resident in the mitochondrial matrix facilitate the complete oxidation of pyruvate for ATP production through electron-transport-coupled OXPHOS. Pyruvate generated from glycolysis is converted to acetyl-CoA in the mitochondrion. The resulting acetyl-CoA then enters the TCA cycle by condensing with oxaloacetic acid (OAA) to form citrate. Citrate can be sequentially processed back to OAA by enzymes of the TCA cycle, generating reducing equivalents (NADH and FADH$_2$) and CO$_2$ in the process. The electrons from NADH and FADH$_2$ can be donated to complex I and II of the electron transport chain for ATP production. Complete oxidation of glucose by the TCA cycle results in maximal ATP production. In proliferating cells such as lymphocytes, the mitochondrion takes up a new role as a biosynthetic hub, converting metabolites such as pyruvate and glutamine into intermediates utilized by other biosynthetic pathways.

Despite the potential ability to take up lipids from the extracellular environment, the lipids used for membrane synthesis during lymphoid growth are generated de novo from glucose with TCA-cycle intermediates (Bauer et al., 2005). Glucose-derived citrate, generated from the condensation of acetyl-CoA with OAA, is exported from the mitochondrion to the cytosol and converted back to acetyl-CoA by ATP citrate lyase (ACL). Shunting of glucose through this “truncated TCA cycle” generates a source of acetyl-CoA for the biosynthesis of lipids including membrane phospholipids and cholesterol. In addition, this cytosolic pool of citrate-derived acetyl-CoA is essential for the production of sphingomyelin, a required component of lipid rafts, and isoprenoid moieties including farnesyl and geranyl-geranyl groups that modify raft-associated signaling proteins. Akt positively regulates this process by phosphorylating ACL (Berwick et al., 2002). Inhibition of ACL results in decreased glucose-dependent lipid synthesis and impaired cell proliferation (Hatzivassiliou et al., 2005).

The amino acids aspartate, asparagine, arginine, glutamate, and proline needed for increased translation are also derived from TCA-cycle intermediates. One consequence of using TCA-cycle intermediates for biosynthesis is that the OAA needed as an acceptor for pyruvate-derived acetyl-CoA must be regenerated so that the integrity of the TCA cycle could be maintained. This can be achieved through the stepwise oxidation of glutamine in proliferating cells. Glutamine is deaminated in the mitochondria for generation of α-ketoglutarate, which when metabolized through the TCA cycle serves to regenerate the OAA consumed by biosynthesis. In addition, glutamine acts as an amine donor in purine biosynthesis and in production of nonessential amino acids. This may explain why glutamine is an essential metabolite for T cell proliferation (Griffiths and Keast, 1990).

Mitochondria Directly Contribute to T Cell Activation
Initial TCR signal transduction in a quiescent cell leads to enhanced mitochondrial ATP production as a result of Ca$^{2+}$-dependent stimulation of TCA-cycle activity. Inositol 1,4,5-triphosphate (IP$_3$) generated after TCR ligation promotes the release of Ca$^{2+}$ from endoplasmic reticulum (ER) stores. Ca$^{2+}$ released from the ER is rapidly taken up by mitochondria, resulting in the stimulation of Ca$^{2+}$-dependent TCA-cycle enzymes that in turn generate an increased rate of oxidative phosphorylation (Krauss et al., 2001). This burst of ATP fuels the initial steps of receptor-mediated signal transduction. However, when TCR signaling is sustained, depletion of ER Ca$^{2+}$ stores can lead to a sustained influx of extracellular Ca$^{2+}$. Under these conditions, Ca$^{2+}$-dependent stimulation of TCA-cycle dehydrogenases in glycolytically-converted lymphocytes results in an elevation of mitochondrial NADH to a level greater than that needed to sustain mitochondrial respiration (Jones et al., 2007). One consequence of a persistently saturated electron transport chain is an increase in the production of reactive oxygen species (ROS). Although ROS is toxic in many biological settings, there is evidence that its generation may play a critical role in shaping the T cell response (Chaudhri et al., 1986), potentially by stimulating nucleotide biosynthesis and S phase entry. Thus, mitochondrial-dependent ROS may act as an important second messenger that indicates when sufficient energetic conditions exist to support T cell proliferation.

Intracellular Sensors of Bioenergetics Can Suppress T Cell Activation
Recent advances in understanding how growth and proliferation are regulated have led to the identification of metabolic control pathways that regulate many aspects of anabolic and catabolic metabolism. Although many of these pathways have been studied extensively in a metabolic context (diabetes, vascular injury, and exercise), their significance to T lymphocyte biology remains unknown. One such pathway is the energy-sensing pathway mediated by the AMP-activated protein kinase (AMPK). AMPK is an ancient regulator of anabolic and catabolic metabolism found in all eukaryotes (Carling, 2004). Under conditions of energetic stress, AMPK is activated by the serine-threonine kinase LKB1 in a reaction dependent on the cellular ratio of AMP to ATP. The net result of AMPK signaling is the engagement of ATP-generating pathways and inhibition of ATP-consuming processes.
AMPK plays multiple roles in the metabolic control of proliferating cells including the regulation of protein translation through stimulation of TSC1-TSC2 complex-mediated inhibition of mTOR (Inoki et al., 2003) and through modulation of the cellular rates of glycolysis and β-oxidation (Buzzai et al., 2005). AMPK was recently demonstrated to be activated by TCR-dependent Ca<sup>2+</sup> signals (Tamas et al., 2006), although the impact of receptor-mediated AMPK activation on T cell function remains unknown.

Recent reports have implicated the G<sub>S</sub>-S cell-cycle checkpoint regulator p53 in coordinating control of the cell cycle with changes in cellular metabolism. Bioenergetic stress stimulates p53 activity via AMPK, which can induce p53-dependent proliferative arrest in response to ATP limitation (Jones et al., 2005). p53 activity favors the production of ATP by OXPHOS through the transcripational regulation of the fructose-2,6-bisphosphatase TP53-induced glycolysis and apoptosis regulator (TIGAR) and the synthesis of cytochrome c oxidase (SCO2) subunit of complex IV of the electron transport chain (Bensaad et al., 2006; Matoba et al., 2006). TIGAR negatively regulates glycolysis by degrading fructose-2,6-bisphosphate, an allosteric activator of the glycolytic enzyme 6-phosphofructo-1-kinase (PFK-1). SCO2 is required for assembly of the cytochrome C oxidase (COX) complex. Loss of p53-dependent regulation of SCO2 cripples the mitochondrial respiratory chain and promotes a switch in ATP production from OXPHOS to glycolysis. Thus, in addition to having well-documented roles in cell-cycle arrest and cell viability, p53 may regulate the metabolic switch from aerobic glycolysis to OXPHOS and thus facilitate the return of a lymphocyte to an inert or anergic state.

**Final Thoughts**

Recent advances in understanding the crosstalk between signal transduction and metabolic pathways have led to better understanding of how T lymphocytes regulate their bioenergetics for meeting the challenge of proliferation. Future work will focus on the molecular mechanisms by which T cells regulate biosynthetic pathways. A number of questions remain. For example, why is glucose such a critical metabolite for T cells? Proliferating lymphocytes consume 10-fold more glucose than any other amino acid. As a result, immunologists routinely supplement their culture medium with additional glucose. The basis for this additional requirement for a nonessential amino acid is poorly understood. Another question is how do lymphocytes convert from blastogenesis (cell growth) to proliferation (division) after activation? One possibility is through cytochrome-mediated stimulation of pathways that activate ribose-sugar production and nucleotide biosynthesis. Alternatively, mitochondrial-dependent ROS production triggered by Ca<sup>2+</sup> signals may act as the molecular switch that regulates this conversion. Finally, does the stimulatory receptor-dependent regulation of glucose uptake contribute directly to the regulation of T cell activation? For example, does the resulting glucose-dependent synthesis of sphingomyelin, an integral component of lipid rafts, and isoprenoid moieties (including farnesyl and geranyl-geranyl groups) used for modifying raft-associated signaling proteins contribute to the stable formation of the immune synapse and signaling lipid rafts that sustain T cell progression through the cell cycle? How these metabolic issues impact on T cell biology is poorly understood.

Another unanswered question is whether metabolic factors influence T cell fate. For example, does inadequate nutrient uptake or metabolism contribute to the induction of anergy or influence the development or survival of regulatory T cells? What is the influence of internal metabolic regulators such as AMPK and p53 on T cell biology? Are transcription factors, such NFAT and Foxp3, that control T cell differentiation influenced by bioenergetic factors? The answers to these questions could help in the identification of key metabolic pathways required for supporting T cell growth, proliferation, and survival and lead to new therapeutic strategies for the treatment of cancer and immune disorders such as autoimmunity and transplant rejection.


