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Amino Acids Fuel T Cell-Mediated Inflammation

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Reprogramming cellular metabolism helps support T cell growth and effector function upon activation. In this issue of *Immunity*, Nakaya et al. (2014) report that the glutamine transporter ASCT2 regulates T cell metabolism and mTOR kinase signaling to shape inflammatory T helper cell responses.

T lymphocytes are central effectors of the adaptive immune system, and their proper function is critical to mediate long-lasting immunity to foreign pathogens. Upon activation by antigens, naive $CD4^+$ T cells expand and differentiate into specific T helper (Th) cell populations, including Th1, Th2, and Th17 cells, each with specific effector functions tailored toward the given pathogen. Although proinflammatory Th responses are important for mediating immunity to foreign pathogens, unchecked or misdirected Th cell responses can also promote inflammation and autoimmunity. One of the fundamental programs that helps drive T cell activation is the regulation of cellular metabolism, the series of biochemical reactions that mediate cellular energy production and biosynthesis. The predominant metabolic program of activated $CD4^+$ Th cells is a shift to aerobic glycolysis (also known as the "Warburg Effect"), a progrowth metabolic program that generates both ATP and macromolecules required for T cell proliferation (MacIver et al., 2013). Amino acids (AAs) are also key nutrients for T cells, because they can serve as both a fuel source and a pool of biosynthetic precursors for protein and nucleic acid biosynthesis. Of particular relevance to T cell biology is the nonessential amino acid (NEAA) glutamine, which is rapidly taken up by T cells upon activation (Carr et al., 2010; Wang et al., 2011). However, the importance of glutamine to $CD4^+$ T cell-mediated immune responses in vivo has been difficult to study. Here, Nakaya et al. (2014) demonstrate that the alanine, serine, and cysteine (ASC) system AA transporter 2 (ASCT2) is a key regulator of glutamine uptake in $CD4^+$ Th cells and influences the development of proinflammatory Th1 and Th17 responses in vitro and in vivo.

ASCT2 is a sodium-dependent, neutral amino acid transporter encoded by *SLC1A5* that mediates the cotransport of Na^+ along with glutamine (or other neutral AAs such as alanine, cysteine, serine, and threonine) with high affinity (Figure 1). However, in addition to ASCT2, there are a number of AA transporters expressed by activated T cells, including the sodium-coupled neutral AA transporters SNAT1 and SNAT2 (Carr et al., 2010; Sinclair et al., 2013), which are capable of transporting glutamine. By using gene-targeted mice lacking ASCT2 expression ($Asct2^{-/-}$ mice), Nakaya et al. (2014) demonstrated that glutamine uptake by T cells, both short-term (30 min) and long-term (>20 hr) postactivation, were markedly reduced in $CD4^+$ T cells from $Asct2^{-/-}$ mice. These data indicate that ASCT2 is a major regulator of glutamine transport in T lymphocytes. Activated $Asct2^{-/-}$ T cells also displayed reduced rates of glucose uptake, lactate production (an indicator of aerobic glycolysis), and oxygen consumption (a measurement of oxidative phosphorylation [OXPHOS]), suggesting that the metabolic program normally triggered by TCR stimulation was attenuated in T cells lacking ASCT2. The authors attributed these observations to reduced expression of the glucose transporter Glut1 and transcription factor c-Myc, both key components of metabolic reprogramming in T cells (MacIver et al., 2013), in $Asct2^{-/-}$ T cells. Addition of exogenous glutamine restored the glycolytic defect of ASCT2-deficient T cells, suggesting that glutamine availability can directly impact other metabolic programs required for T cell proliferation.

Mechanistically, the authors identified the CARMA1-BCL10-MALT1 (CBM) signalosome, a signaling complex that activates nuclear factor-κB (NF-κB) signaling downstream of the TCR (Thome, 2004), as a regulator of TCR-mediated glutamine uptake and ASCT2 expression in T cells (Figure 1). Regulation of glutamine uptake by the CBM complex appeared to be independent from CBM-dependent NF-κB activation, as IKKβ-deficient T cells displayed no impairment in glutamine

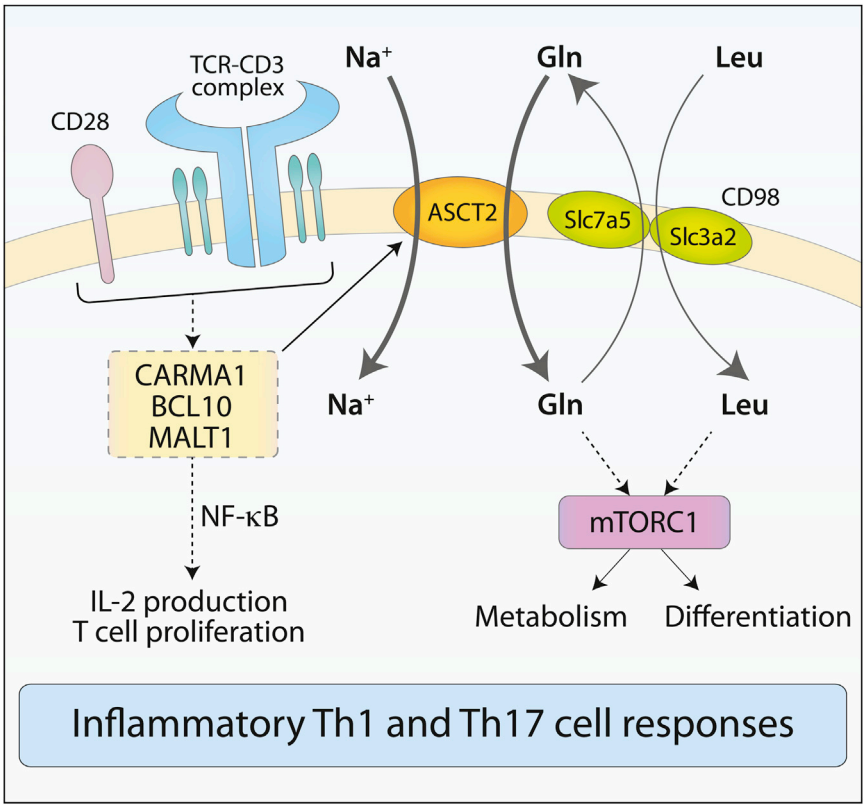

Figure 1. Regulation of CD4$^+$ T Helper Cell Function by ASCT2-Mediated Amino Acid Transport

TCR-dependent glutamine (Gln) import by the Na$^+$-dependent neutral amino acid transporter ASCT2 (orange) promotes the development of proinflammatory CD4$^+$ Th1 and Th17 cell responses. ASCT2 cotransports extracellular Na$^+$ and Gln, with the latter used to import leucine (Leu) via the Slc7a5-Slc3a2 antiporter (green). The Slc7a5-Slc3a2 complex is also known as CD98. Leu promotes the activation of mTORC1 (purple), which regulates both T cell metabolism and Th1 and Th17 cell differentiation. Glutamine might also exert effects on mTORC1 activation through indirect mechanisms. ASCT2 expression is regulated downstream of TCR and CD28 signaling by the CARMA1-BCL10-MALT1 (CBM) complex (yellow). In the absence of ASCT2, Gln and Leu uptake by CD4$^+$ T cells is reduced, leading to decreased mTORC1 activity and attenuated CD4$^+$ Th1 and Th17 responses. T cell proliferation and IL-2 production are unaffected in *Asct2*$^{-/-}$ T cells.

transport. CARMA1 was shown to regulate both the expression of ASCT2 mRNA and colocalization of ASCT2 with the TCR. CARMA1 immunoprecipitated with ASCT2 in overexpression studies in HEK293 cells, suggesting a possible interaction between CBM-ASCT2.

To explore the role of ASCT2 in CD4$^+$ T cell function, Nakaya et al. examined the impact of ASCT2 loss on peripheral lymphocyte homeostasis. While peripheral T and B cell numbers were comparable between young (6- to 7-week-old) *Asct2*$^{+/+}$ and *Asct2*$^{-/-}$ mice, the authors observed a progressive decline in CD4$^+$ T cell numbers in aged (>5 months) *Asct2*$^{-/-}$ mice, with noticeable changes in the number and frequency of CD4$^+$CD44^{hi}CD62^{lo} T effector memory (Tem) cells. The authors observed a drop in the number of Th1 (interferon-γ [IFN-γ]-producing) and Th17 (IL-17-producing) cells in the CD4$^+$ Tem cell population, whereas the frequency of CD4$^+$Foxp3$^+$ T regulatory (Treg) cells was unaffected by loss of ASCT2. Loss of ASCT2 did not alter rates of T cell proliferation or IL-2 production in response to anti-CD3 and anti-CD28 stimulation in vitro (surprising, given the reduction in glucose metabolism and OXPHOS in *Asct2*$^{-/-}$ T cells). However, the authors observed a reduction in the ability of naive *Asct2*$^{-/-}$ CD4$^+$ T cells to differentiate into Th1 and Th17 cells, but not Th2 or Treg cells, in vitro.

The authors next asked whether ASCT2 deficiency could affect Th1 or Th17 responses in vivo by using three well-established experimental models: (1) Th1 and Th17 cell-mediated colitis following adoptive transfer of naive CD4$^+$ CD45RB^{hi} T cells into *Rag1*$^{-/-}$ mice, (2) Th1 cell responses following infection with the Gram-negative bacterium *L. monocytogenes*, and (3) Th1 and Th17-mediated induction of experimental autoimmune encephalomyelitis (EAE) following immunization with myelin oligodendrocyte glycoprotein (MOG) peptide. The results from each experimental model were clear: lack of ASCT2 resulted in reduced numbers of IFN-γ- and/or IL-17A-producing CD4$^+$ T cells in vivo. Results from the EAE experiments were particularly striking, with *Asct2*$^{-/-}$ mice displaying reduced infiltration of IFN-γ$^+$ and IL-17$^+$ T cells in the central nervous system (CNS) and overall reduced severity of EAE disease. These results highlight a central role for ASCT2 in establishing proper Th1- and Th17-cell-mediated immune responses.

How does an amino acid transporter exert such a dramatic effect on T cell-mediated immune responses? The authors observed that TCR-dependent activation of mammalian target of rapamycin complex 1 (mTORC1) signaling was defective in T cells lacking ASCT2. mTORC1 is a serine/threonine kinase complex that couples growth factor signals, nutrient availability, and cellular energy status to the regulation of cell growth and metabolism in lymphocytes and plays a prominent role in the differentiation of CD4$^+$ effector T cells (Zeng and Chi, 2013). CD4$^+$ T cells deficient for the small GTPase Rheb, which regulates mTORC1 activity in T cells (Delgoffe et al., 2011), display defects in Th1 and Th17 (but not Th2) cell differentiation similar to that observed with *Asct2*$^{-/-}$ mice.

What links ASCT2-mediated glutamine uptake to mTORC1 activation in T cells? One possible explanation is that ASCT2 might work in concert with other AA transporters to facilitate AA-dependent mTORC1 activation. One such complex is the Slc7a5-Slc3a2 antiporter, also known as CD98, which imports branched amino acids (such as leucine) while exporting glutamine. In this model, ASCT2 is required to generate a pool of intracellular glutamine, which in turn drives CD98-dependent leucine import and leucine-dependent mTORC1 activation (Nicklin et al., 2009) (Figure 1).

Alternatively, glutamine might affect mTORC1 activity through glutamine metabolism in the tricarboxylic acid (TCA) cycle (Durán et al., 2012).

Reduced glutamine import in ASCT2-deficient T cells would reduce intracellular leucine concentrations, resulting in reduced mTORC1 signaling and attenuation of proinflammatory $CD4^+$ T cell responses (Figure 1). Indeed, TCR-stimulated leucine uptake was dramatically reduced in *Asct2*$^{-/-}$ T cells; moreover, defects in both mTORC1 activation and Th17 differentiation could be rescued in ASCT2-deficient T cells by the administration of exogenous leucine. Interestingly, Cantrell and colleagues recently reported similar defects in T cell metabolism, mTORC1 activation, and Th1 and Th17 cell differentiation in mice harboring a T cell-specific deletion of *SLC7A5* (Sinclair et al., 2013). Together these studies provide insight into the influence of AAs on shaping T cell-mediated immune responses, independent of their role in protein biosynthesis. It is important to note that glutamine is not the sole AA transported by ASCT2, and whether deficiency in the transport of other AAs contributes to the phenotype of *Asct2*$^{-/-}$ T cells remains to be determined.

The work of Nakaya and colleagues raises important questions regarding the impact of nutrient availability on T cell function. As T cells travel from sites of immune priming in secondary lymphoid organs to distant sites of infection, they are likely to experience different metabolic microenvironments that might impact their function. TCR-dependent control of nutrient transporters such as ASCT2 might be one way that $CD4^+$ T cells acquire sufficient nutrient levels to maintain mTORC1 activity and T cell effector function. Understanding the mechanisms that coordinate—and regulate—proinflammatory $CD4^+$ Th cell responses might uncover new mechanisms for modulating T cell responses, with applications ranging from new treatments for autoimmune disease to improving the effectiveness of cancer immunotherapy. The work by Nakaya et al. (2014) indicates that the management of metabolic resources, including the import of specific amino acids, is a critical determinant for the proper development and function of proinflammatory $CD4^+$ T cell responses.

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IL-10 and Macrophages Orchestrate Gut Homeostasis

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Macrophages in the gut originate from blood precursors and have distinct functional properties. In this issue of *Immunity*, Zigmond et al. (2014) and Shouval et al. (2014) report that interleukin-10 drives macrophages to express homeostatic tolerogenic functions, preventing colitis.

Constant exposure to food and environmental antigens and a tremendous variety of commensal bacteria make the intestine a vulnerable setting. Intestinal immune cells are engaged in a robust balanced immune response, aimed at controlling pathogen invasion while preventing unintended harmful tissue injuries. Key players in the maintenance of gut defense and homeostasis are intestinal mononuclear phagocytes, of which monocyte-derived macrophages constitute the most abundant population (Zigmond and Jung, 2013). Failure in the macrophage-mediated shaping of gut immune responses might result in deregulated excessive inflammatory responses, as those observed in inflammatory bowel disorders. The immune networks governing this balance in the steady-state intestinal lamina propria have recently begun to be unraveled, with the important clinical endpoint to identify therapeutic targets for gut chronic inflammatory diseases. However, the local molecular mediators driving the education of intestinal macrophages into immunomodulating cells have not been fully identified.

This issue of *Immunity* features two original articles (Zigmond et al., 2014;