Sugar addiction

Sugar addiction, an Achille's heel of auto-immune diseases?

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In this issue of *Cell Metabolism*, Hochrein *et al.* identify a metabolic checkpoint controlling the transcriptional programming of effector CD4$^+$ T cells. The authors show that GLUT3-mediated glucose import and ACLY-dependent acetyl-CoA generation control histone acetylation and, hence, the epigenetic imprinting of effector gene expression in differentiated effector CD4$^+$ T cells. These findings suggest a novel therapeutic for inflammation-associated diseases.

Auto-immune diseases, such as multiple sclerosis (MS) and rheumatoid arthritis, are often debilitating and largely treated by systemic immune suppression. However, this is can be accompanied by a range of severe side effects that include susceptibility to infections. In a new publication in *Cell Metabolism*, a novel metabolic checkpoint in effector T cells has been identified which might provide an option for more specific therapeutic targeting (Hochrein *et al.* 2022).

To efficiently fend off infections, the immune response should respond in a pathogen-specific way. Immune responses for different types of pathogens are guided by the expansion and differentiation of specific $CD4^+$ T helper cells. For instance, the differentiation of naïve CD4 T cells into so-called T helper 17 (Th17) cells is essential for protection against extracellular bacterial and fungal infections (Korn *et al*., 2009). Th17 cells produce the cytokine interleukin-17 (IL-17) which induces the release of chemokines from epithelial cells within inflamed tissues. This leads to the rapid influx of pro-inflammatory neutrophils and monocytes to the site of infection, driving the clearance of invading pathogens. However, sustained tissue inflammation can initiate the onset of autoimmune diseases. A better understanding of the molecular mechanisms underlying Th17 cell differentiation and the maintenance of Th17 cell effector functions are critical for developing novel immunotherapeutic strategies for the treatment of inflammation-associated diseases and autoimmunity. Identifying a novel metabolic checkpoint for Th17 cells, Hochrein *et al.* may have uncovered a possible Achille's heal of inflammatory diseases.

Activation of naïve $CD4^+$ T cells results in clonal expansion and differentiation into distinct helper T (Th) cell subsets. Within days, these antigen-specific $CD4^+$ T cells dominate the overall immune response. To sustain this rapid proliferation, T cells undergo a fundamental switch from mitochondrial respiration to aerobic glycolytic metabolism. T cell receptor (TCR) signaling, in partnership with CD28-mediated co-stimulation, increases the expression of the SLC2A family of glucose transporters (GLUTs), ensuring sufficient glucose supply within activated cells. In this context, T cell-specific GLUT1-deficiency has previously been shown to impair activation, proliferation, and differentiation into T effector (Teff) cells (Macintyre *et al.*, 2014). Concomitant to metabolic changes, epigenome remodeling is essential for T cell differentiation and functional reprogramming. Histone acetylation results in a more accessible chromatin structure permissive to the transcriptional machinery. A rate-limiting step in this process is the availability of acetyl-CoA, which is the sole source of acetyl groups in eukaryotic cells. For instance, reducing intracellular acetyl-CoA levels by deleting lactate dehydrogenase A (LDHA) in T cells leads to reduced histone acetylation at the IFNγ enhancer, and protects from lethal autoinflammation in animal models of disease (Peng et *al.*, 2016). Such findings have uncovered a direct, mechanistic link between acetyl-CoA supply and gene expression in $CD4^+$ T-cells.

Hochrein *et al.* focused their investigation on the function of GLUT3, a high-affinity glucose transporter. GLUT3 is upregulated in T cells after activation, however, its role has not previously been evaluated. In Th17 cells, GLUT3 expression was found to be impacted by a complex interplay of factors including TCR-engagement, co-stimulation, cytokine signaling, and hypoxia. To assess its functional role, animals were generated with a T cell-specific GLUT3-deficiency. GLUT3-deficiency resulted in impaired glucose uptake in Th17 cells, coupled with reduced basal and activation-induced glycolysis. Importantly, these effects did not affect Th17 cell proliferation, or the expression of activation markers, demonstrating a clear difference to GLUT1-deficiency (Macintyre *et al.*, 2014). While the authors found only minor effects of GLUT3-deficiency on the expression of Th17 signature transcription factor RORγt, effector cytokine expression was markedly reduced in GLUT3-deficient Th17 cells. Metabolic analyses identified acetyl-CoA to be among the most downregulated metabolites in GLUT3-deficient T cells. This correlated with a reduction of citrate, with the lack of

acetyl-CoA being primarily cytosolic-nuclear rather than mitochondrial. To consolidate the link between citrate and GLUT3-driven acetyl-CoA production two approaches were taken. Mice were generated with a T cell-specific deletion in ATP-citrate lyase (ACLY), and wild-type mice were treated with a competitive inhibitor of ACLY (2-hydroxycitrate; 2-HC). While animals showed no obvious sign of immune dysregulation, total acetyl-CoA levels were reduced by about half in Th17 cells derived from ACLY-deficient, or 2-HC treated mice. This directly correlated with a reduction in effector cytokine expression. FACS-based analysis revealed lower histone H3 acetylation in GLUT3-deficient T cells, with reduced incorporation of glucose-derived [^{14}C] carbons in histones on both ACLY and GLUT3-deficient Th17 cells. To focus on the locus specificity of these observations, a ChIP-seq approach was employed. While no obvious differences were observed in H3 acetylation within the loci of housekeeping or activation marker genes, acetylation peaks around the promoter regions of effector cytokines, such as *Il2*, *Il17a,* or *Il17f* were reduced in GLUT3-deficient cells. Loss of GLUT3 also had a wider effect on gene expression compared to ACLY deficiency, suggesting a broader role of GLUT3. These observations demonstrate that in Th17 cells GLUT3-dependent acetyl-CoA supply can regulate epigenetic remodeling in a locus-dependent manner controlling effector cell functions.

The most pronounced effects of this novel metabolic checkpoint on CD4$^+$ T cell subsets were in Th17 cells. This is possibly because the signature transcription factor for Th17, RORγt, has no imprinting auto-feedback loop. In contrast to RORγt, the signature transcription factors of other CD4$^+$ T cell populations, such as T-bet (Th1), Gata3 (Th2), or Foxp3 (Treg), all bind to the promoter region of their genes, reinforcing their own expression and driving the differentiation program they regulate. Consequently, the phenotype of these T cell populations is very stable, while Th17 cells can lose their phenotype and differentiate into Th1 cells once they have entered the site of inflammation. In line with the pronounced effects in Th17 cells, the authors also find the strongest *in vivo* effects in Experimental Autoimmune Encephalomyelitis (EAE), a well-established Th17-mediated mouse model for MS. Mice with a T cell-specific deficiency of GLUT3 or ACLY, as well as mice treated with 2-HC, showed a dramatic reduction of IL-17 expression in Teff cells, and protection from EAE symptoms. Furthermore, GLUT3-deficiency also attenuated immune responses to *Citrobacter rodentium* infection with reduced IL-17A expression and Th17 numbers. Since the ACLY inhibitor 2-HC has already been used in a variety of therapeutic studies, these findings warrant an assessment of this compound in settings of inflammatory diseases.

While the findings by Hochrein *et al.* implicate both GLUT3 and ACLY as important metabolic regulators of T effector cell function, they also raise several questions that require further evaluation. In particular, the differences between GLUT1- and GLUT3-deficiency raises the question of how such a difference can be explained mechanistically. How can the metabolic fate of glucose be determined by its transporter? Could differences between GLUT1 and GLUT3 be due to the differences in glucose-affinity, or perhaps differential localization allowing glucose channeling into specific metabolic routes? The spatial distribution of GLUT3 during T cell activation is unknown but it is possible that metabolic compartmentalization could underlie the differences between GLUT1- and GLUT3-deficiency.

Acetyl-CoA can be generated from acetate, citrate, and pyruvate, and in general, this occurs within the subcellular compartment where it is needed. While not evaluated in this study, ACLY is present in the nucleus where it can generate localized acetyl-CoA (Sivanand *et al.,* 2018). It is plausible that locus-specific association of ACLY, for example at the *Il17a* promoter, could mediate the GLUT3-driven epigenetic responses observed. The generation of acetyl-CoA from citrate may therefore provide an additional level of specificity in terms of epigenetic regulation of effector T cells.

The specificity of GLUT3-action in terms of other lymphocyte subsets remains unclear. While the authors demonstrate that Th1 cells also have reduced glucose uptake, no effect on Th1 signature cytokine production was observed. This suggests that GLUT3-mediated glucose import *per se* is not sufficient to drive chromatin remodeling, and additional cellular context must also be relevant. Differentiation of immunosuppressive Treg cells was also inhibited by GLUT3 inactivation, although the mechanism underlying this was not evaluated. These data further support the possibility that targeting specific glucose transporters is a relevant approach to target T cell subsets driving inflammatory diseases.

Irrespective of these questions, the work presented by Hochrein *et al.* identifies GLUT3 and citrate-derived acetyl-CoA generation as a requirement for Th17 cell function. This enhanced understanding of the metabolic requirements for Teff cell function may provide a unique opportunity for the treatment of often debilitating inflammatory diseases.

References

Hochrein SM, Wu H, Eckstein M, Arrigoni L, Herman JS, Schumacher S, Gerecke C, Rosenfeldt M, Grün D, Kleuser B, Gasteiger G, Kastenmüller W, Ghesquière B, Van den Bossche J, E. Abel D and Vaeth M. The glucose transporter GLUT3 controls T helper 17 cell responses through glycolytic-epigenetic reprogramming. *Cell Metabolism*, in press.

Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol.* 2009; 27:485-517.

Macintyre AN, Gerriets VA, Nichols AG, Michalek RD, Rudolph MC, Deoliveira D, Anderson SM, Abel ED, Chen BJ, Hale LP, Rathmell JC. The glucose transporter Glut1 is selectively essential for CD4 T cell activation and effector function. *Cell Metab.* 2014 Jul 1;20(1):61-72.

Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. *Science* 2016 Oct 28;354(6311):481-484.

Sivanand S, Viney I, Wellen KE. Spatiotemporal Control of Acetyl-CoA Metabolism in Chromatin Regulation..*Trends Biochem Sci.* 2018 Jan;43(1):61-74.