2. Sakaguchi, S., Yamaguchi, T., Nomura, T. & Ono, M. *Cell* **133**, 775–787 (2008).
3. Kwon, H.-K., Chen, H.-M., Mathis, D. & Benoist, C. *Nat. Immunol.* **18,** 1238–1248 (2017).
4. Calo, E. & Wysocka, J. *Mol. Cell* **49**, 825–837 (2013).
5. Jeong, K.W. *et al. Nat. Struct. Mol. Biol.* **18**, 1358–1365 (2011).
6. Samstein, R.M. *et al. Cell* **151**, 153–166 (2012).
7. Arvey, A. *et al. Nat. Immunol.* **15**, 580–587 (2014).
8. DuPage, M. *et al. Immunity* **42**, 227–238 (2015).
9. Wu, Y. *et al. Cell* **126**, 375–387 (2006).
10. Ono, M. *et al. Nature* **446**, 685–689 (2007).
11. Mahmud, S.A. *et al. Nat. Immunol.* **15**, 473–481 (2014).
12. Li, H. *et al. Nature* **442**, 91–95 (2006).

Cytokine-driven role of Srebps in killer cell metabolism

Camille Guillerey & Mark J Smyth

A new and unexpected role is identified for Srebps in controlling NK cell activity that is independent of their role in lipid biosynthesis.

Recent research has highlighted the importance of immune cell metabolism in shaping the immune response. Following activation, immune cells proliferate rapidly and/or produce large amounts of effector molecules, and these functional changes require matching adjustments in cellular metabolism to provide sufficient energy and biosynthetic precursors[1]. Sterol-regulatory-element-binding proteins (Srebps) are master regulators of cellular lipid metabolism that are reportedly involved in the regulation of macrophages and T cell function[2,3]. In this issue of *Nature Immunology*, Assmann *et al.* identify a new and unexpected role for Srebps in controlling natural killer (NK) cell activity that is independent of their role in lipid biosynthesis[4]. They demonstrate that in cytokine-activated NK cells, Srebps induce a metabolic configuration in which glucose is metabolized through the citrate–malate shuttle and thereby allow NK cell growth, proliferation and function. Notably, Srebp transcription factors are found to be essential for the protective activity of cytokine-activated NK cells when adoptively transferred into tumor-bearing mice. The discovery of this alternative metabolic pathway in NK cells opens up new avenues for the clinical manipulation of these cells.

The proper development of an immune response requires immune cells to switch from a naive state to an effector or memory state. Transition between these states is associated with profound metabolic reprogramming to match the cells' function, lifespan and bioenergetic requirements. For instance, naive and memory T cells have a quiescent metabolism characterized by a low level of nutrient uptake, and they use glycolysis coupled to oxidative phosphorylation (OxPhos) as their main source of ATP[1]. In contrast, effector T cells rely mainly on aerobic glycolysis, in which pyruvate, the final product of the glycolysis, is transformed into lactate and exported from the cell. Aerobic glycolysis provides biosynthetic precursors for the synthesis of plasma-membrane and cellular organelles and thereby supports robust cellular growth and proliferation.

NK cells are innate lymphocytes that have an essential role in the early elimination of infected or transformed cells[5,6]. Similarly to T cells, NK cells undergo dramatic metabolic reprogramming upon activation. Following cytokine or microbial stimulation, NK cells increase their rate of glycolysis and OxPhos[7,8]. Published work has revealed that the metabolic checkpoint kinase mTORC1 is essential for attainment of the elevated glycolytic state required for the effector functions of NK cells[7,8]. However, until now, other pathways that might control the metabolic reprogramming of NK cells have remained largely unexplored.

In this study, Assmann *et al.* consider the role of *de novo* lipid-biosynthesis pathways and Srebp transcription factors in NK cells[4]. Srebps are well known to control the transcription of genes encoding products involved in cholesterol and fatty-acid metabolism[3]. In newly activated CD8$^+$ T cells, Srebps enable clonal expansion by promoting the rapid upregulation of lipid-biosynthetic pathways required for membrane synthesis[2]. Surprisingly, Assmann *et al.* find that although Srebps are required for the activation of NK cells, this process does not require lipid biosynthesis[4]. Instead, their work identifies a new role for Srebps in controlling the citrate–malate shuttle that directs the cytosolic transfer of mitochondrial citrate generated from the tricarboxylic acid cycle (the Krebs cycle) (**Fig. 1**). The authors show that this metabolic pathway allows NK cells to attain the high levels of glycolysis and OxPhos required for their effector functions. This finding is a completely unexpected discovery, as this is the first time that Srebp transcription factors have been found to regulate glucose metabolism.

This study identifies distinct roles for Srebp transcription factors in T cells and NK cells: the lipid-biosynthesis program induced by Srebp is necessary for the proliferation of CD8$^+$ T cells but not for that of NK cells, while Srebp-mediated control of the citrate–malate shuttle has a prominent role in the NK cell response to cytokines[4]. Actually, inhibition of the citrate–malate shuttle profoundly perturbs glucose metabolism in NK cells but not in interleukin 2 (IL-2)-stimulated CD8$^+$ T cells. However, these observations do not exclude the possibility that CD8$^+$ T cells might use the citrate–malate shuttle in response to other stimuli, such as stimulation of the T cell antigen receptor. Intriguingly, *de novo* lipid-synthesis pathways are found to be dispensable for the activation of NK cells. This finding represents another major difference between T cells and NK cells and raises the question of the source of the fatty acids used for the generation of new plasma-membrane and cellular components in proliferating NK cells.

There are several reasons for possible use of the citrate–malate shuttle by activated NK cells. An important function of this shuttle is electron transfer from the cytosol to mitochondrial NADH to fuel OxPhos and ATP synthesis and thus to provide enough energy for cellular activity. Indeed, the authors report altered OxPhos rates following inhibition of the citrate–malate shuttle in NK cells, which leads to decreased production of interferon-γ and granzyme B[4]. An additional function of the citrate–malate shuttle is the generation of cytosolic acetyl-CoA that constitutes a substrate for acetylation reactions. A possibility is that acetyl-CoA might allow histone-acetylation reactions that would favor the transcription of genes encoding effector molecules such as interferon-γ[9]. In this context, the histone-acetylation status might confer to NK cells temporary memory of a first stimulus and might endow them with increased responsiveness. Finally, another important role of the citrate–malate shuttle

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might be the generation of cytosolic NAD^+ that would support sustained glycolysis.

Consistent with a fast innate immune response, early NK cell responses that occur 4 hours after cytokine- or receptor-mediated activation are not associated with dramatic modifications of metabolism[10]. Actually, metabolic reprogramming of NK cells is observed only after prolonged cytokine stimulation over 18 hours. Because they are delayed, such changes might reflect the adaptive functions of NK cells[5]. One hypothesis would be that NK cells that undergo metabolic reprogramming may become memory NK cells—that is, long-lived NK cells able to mount robust recall response. Still, the elevated rates of glycolysis induced by cytokines in NK cells are not in agreement with a memory phenotype, since long-lived cells such as memory T cells are metabolically quiescent[1]. In contrast, elevated rates of glycolysis and OxPhos seem more likely to support NK cell priming—the process by which naive NK cells acquire full effector function in response to environmental factors such as cytokines. Primed NK cells have enhanced killing ability and secrete larger amounts of cytokines upon target-cell recognition relative to that of naive NK cells, and such functional features are probably supported by enhanced glucose metabolism.

The manipulation of NK cells has recently gained interest, since these cells hold great therapeutic potential for the treatment of patients with cancer[6]. In their study, Assmann *et al.* provide crucial insight into NK cell metabolism that would help the manipulation of these cells for improved clinical response[4]. The key role of Srebp transcription factors is particularly relevant in the context of cancer and chronic inflammation, in which cholesterol derivatives known to inhibit Srebp activation are produced by tumor cells or macrophages[11,12]. Future work should determine whether Srebp-dependent metabolic reprogramming is defective in patients with cancer. Moreover, other metabolic pathways are probably disrupted in the tumor microenvironment. The immunosuppressive cytokine TGF-β is abundant in many tumors, and TGF-β signaling in NK cells has been shown to inhibit the activity of the serine-threonine kinase mTOR[13]. Consequently, deficiency in TGF-β signaling increases mTOR activity in IL-15-stimulated NK cells and improves NK cell–mediated control of experimental metastasis[13]. Interestingly, although mTOR might regulate several steps in the Srebp process, Assmann *et al.* observe that in cytokine-activated NK cells, inhibition of Srebp does not disrupt mTORC1 signaling. This suggests that targeting both mTOR pathways and Srebp pathways in NK cells might provide synergistic therapeutic effect.

Finally, the present study[3] provides additional evidence that manipulating metabolic pathways in immune cells can influence therapeutic efficacy[4]. The combination of IL-12, IL-15 and IL-18 endows NK cells with potent anti-tumor properties and is now preferentially used for the *in vitro* stimulation of NK cells before adoptive transfer[6]. Importantly, Assmann *et al.* show that inhibiting Srebp activation during stimulation with IL-12, IL-15 and IL-18 abolishes the therapeutic effect of adoptively transferred mouse NK cells against subcutaneous tumors[4]. Establishing an adequate NK cell metabolic program, either through manipulation of the microenvironment or through the *in vitro* manipulation of NK cells for subsequent adoptive transfer, should improve current anticancer therapies.

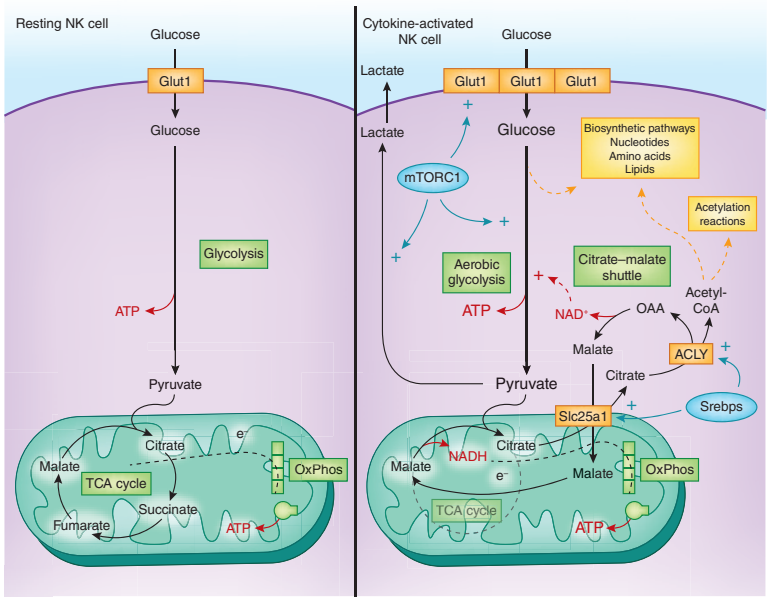

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Figure 1 Killer cell metabolism. Resting NK cells take up basal amounts of glucose that are metabolized to pyruvate through glycolysis. Pyruvate then enters the tricarboxylic acid (TCA) cycle in the mitochondria to generate electrons (e^-) that fuel OxPhos. Following activation, NK cells upregulate their intake of glucose that is metabolized through aerobic glycolysis. This process is dependent on mTORC1, which controls transcription of the gene encoding the glucose transporter Glut1, as well as that of genes encoding enzymes that control aerobic glycolysis. Aerobic glycolysis provides biosynthetic precursors for the synthesis of macromolecules, although so far there is no evidence of the importance of this pathway in NK cells. Srebps regulate transcription of the genes encoding the citrate–malate transporter Slc25a1 and ATP-citrate lyase (ACLY), two crucial components of this shuttle. The citrate–malate shuttle breaks the tricarboxylic acid cycle to export mitochondrial citrate into the cytosol, where it is then cleaved into acetyl-CoA and oxaloacetate (OAA). Oxaloacetate is then converted into malate and transported to the mitochondria. This process transfers electrons from the cytoplasm to the mitochondria; this leads to the generation of cytosolic NAD^+, which serves as a co-factor for glycolysis, and to mitochondrial NADH, which provides electrons for OxPhos. Cytosolic acetyl-CoA generated by the citrate–malate shuttle might feed lipid-synthesis pathways or regulate gene expression through the control of acetylation reactions. Overall, glycolysis and OxPhos rates are increased in cytokine-activated NK cells, which leads to enhanced generation of ATP.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

1. Pearce, E.L., Poffenberger, M.C., Chang, C.H. & Jones, R.G. *Science* **342**, 1242454 (2013).
2. Kidani, Y. *et al. Nat. Immunol.* **14**, 489–499 (2013).
3. Shimano, H. & Sato, R. *Nat. Rev. Endocrinol.* http://dx.doi.org/10.1038/nrendo.2017.91 (29 August 2017).
4. Assmann, N. *et al. Nat. Immunol.* **18**, 1197–1206 (2017).
5. Vivier, E. *et al. Science* **331**, 44–49 (2011).
6. Guillerey, C., Huntington, N.D. & Smyth, M.J. *Nat. Immunol.* **17**, 1025–1036 (2016).
7. Donnelly, R.P. *et al. J. Immunol.* **193**, 4477–4484 (2014).
8. Marçais, A. *et al. Nat. Immunol.* **15**, 749–757 (2014).
9. Chang, S. & Aune, T.M. *Proc. Natl. Acad. Sci. USA* **102**, 17095–17100 (2005).
10. Gardiner, C.M. & Finlay, D.K. *Front. Immunol.* **8**, 367 (2017).
11. York, A.G. & Bensinger, S.J. *J. Exp. Med.* **210**, 1653–1656 (2013).
12. Gold, E.S. *et al. Proc. Natl. Acad. Sci. USA* **111**, 10666–10671 (2014).
13. Viel, S. *et al. Sci. Signal.* **9**, ra19 (2016).