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Bcl-6 gets T cells off the sugar

Kevin Man & Axel Kallies

Kevin Man and Axel Kallies are with the Molecular Immunology Division at the Walter and Eliza Hall Institute of Medical Research, Parkville, Australia. Email: kallies@wehi.edu.au.

Transcriptional regulator Bcl-6 represses aerobic glycolysis in $CD8^+$ and $CD4^+$ T_H1 T cells.

During the course of an adaptive immune response T cell metabolism is dynamically regulated and subject to differentiation stage-specific regulation. In particular, antigen-triggered T cell activation is characterized by the transition from the catabolic state of naïve or quiescent cells, to the anabolic state of rapidly proliferating effector cells[1]. During this process the cells rewire their metabolic profile and switch from oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) to aerobic glycolysis. The transcription factors that mediate this metabolic transition in T cells remain poorly defined. In this issue of *Nature Immunology*, Weinmann and colleagues reveal that the BTB-zinc finger transcription factor Bcl-6 is a critical regulator of the metabolic changes that parallel the transition of effector to memory T cells[2]. Specifically, Bcl-6 acts as a repressor of genes involved in aerobic glycolysis that are upregulated by high environmental interleukin 2 (IL-2) during the effector phase of the immune response. Thus, this study offers important insight into how the formation of long-term memory cells is linked to transcriptional regulation of distinct metabolic pathways.

When naïve T cells encounter foreign antigen in conjunction with other immune modulatory signals, such as costimulation and IL-2, they undergo rapid clonal expansion and differentiate into effector or memory precursor T cells. During this process they reprogram their mitochondrial metabolism to aerobic glycolysis, also known as the 'Warburg effect'[3]. In aerobic glycolysis, glucose derived pyruvate in the cytosol is converted to lactate, and small amounts of pyruvate enter the mitochondria

to be oxidized to carbon dioxide in the tricarboxylic acid (TCA) cycle. In contrast to activated T cells, resting T cells utilize OXPHOS and FAO to fulfill their metabolic requirements generating chemical energy in the form of adenosine triphosphate (ATP) from the breakdown of pyruvate in the TCA cycle. Although aerobic glycolysis, employed by activated cells, is a relatively inefficient process, it produces intermediary metabolites that fuel anabolic biosynthetic pathways that support rapid proliferation and function of effector T cells. This includes synthesis of nucleotides, amino acids and lipids required to generate cellular progeny[3]. Upon clearance of the antigen, most effector T cells undergo apoptosis, whereas a proportion of memory precursor cells give rise to long-lived memory T cells. Although the metabolic profile of memory T cells is similar to that of naïve T cells, they are unique in that they have larger mitochondria and actively transcribe genes involved in mitochondrial biogenesis and fatty acid uptake, which is stimulated by the memory-promoting cytokine IL-15 (ref. 4). Therefore, during memory formation activated T cells revert back to catabolic metabolic pathways, reengaging in OXPHOS and FAO for energy production[5] (**Fig. 1**).

Gene regulatory networks that guide the transition from mitochondrial OXPHOS to aerobic glycolysis are only beginning to be elucidated, and even less is known about the transcription factors that regulate the reversion from aerobic glycolysis to OXPHOS and FAO during memory development. T cell activation induces early transcriptional regulators, including c-Myc[6] and estrogen-related receptor alpha (ERRα)[7], that are important for inducing the expression of genes involved in glycolysis and other intermediary metabolic pathways. Another T cell receptor (TCR)-dependent transcription factor, IRF4, is required for sustained glycolysis in effector $CD8^+$ T cells. Genetic ablation of IRF4 results in profound impairment in clonal expansion and loss of antigen-specific effector T cells during viral or bacterial infection[8]. Furthermore, IRF4 is required for the efficient expression of HIF-1α, an oxygen-sensitive key transcriptional regulator of metabolic processes that mediates the transition from OXPHOS to aerobic glycolysis and allows for metabolic adaptation to hypoxic microenvironments. HIF-1α can also be induced by Toll-like receptor signaling and mTOR complex 1 kinase activity even under conditions of normal oxygen tension[9]. Maintenance of HIF-1α expression in $CD8^+$ T cells requires

sustained IL-2 signaling, and it is partly through this mechanism that IL-2 promotes effector differentiation and glycolysis[10].

In their current study, Weinmann and colleagues uncover that HIF-1α and Bcl-6 reciprocally regulate genes controlling glucose metabolism in an IL-2 sensitive manner[2]. Using a variety of *in vitro* approaches conducted under low and high IL-2 conditions in $CD4^+$ T_H1 and $CD8^+$ T cells, the authors confirm earlier results [11], showing that genes involved in glucose metabolism are upregulated by high amounts of IL-2. This result correlated with increased promoter occupancy of c-Myc and HIF-1α at genes involved in glycolysis. Conversely, low IL-2 conditions favored Bcl-6 expression and DNA binding activity, allowing Bcl-6-dependent repression of the same glycolytic genes. The authors further demonstrate that the T-box transcription factor T-bet, critical for T_H1 and $CD8^+$ effector differentiation, is also required for the IL-2–dependent increase in the expression of glycolytic genes[2]. T-bet antagonized Bcl-6–dependent repression by interacting with Bcl-6 and masking its DNA-binding domain (**Fig. 1**). Whether T-bet can directly impact on glycolysis by affecting transcription of components of the glycolytic pathway remains to be determined. Expression of T-bet is augmented by IL-2 signals[12], providing an indirect link between cytokine availability and metabolic activity regulated by Bcl-6. In this context it is important to consider that T_H1 cells in comparison to follicular T helper cells express only low amounts of Bcl-6. Thus, further studies will be required to elucidate whether Bcl-6 is important for repressing the glycolytic gene program in T_H1 differentiation in the context of viral or bacterial infection. Furthermore, it remains to be tested whether genetic ablation of *Bcl6 in vivo* leads to increased glycolytic flux and whether this is truly relevant for $CD8^+$ T cell memory.

Availability of IL-2 and expression of the high affinity IL-2 receptor CD25 (*Il2rα*) were previously demonstrated to determine effector versus memory differentiation in both $CD4^+$ and $CD8^+$ T cells. In particular, IL-2 promoted effector differentiation at the expense of memory formation, acting through the reciprocal regulation of the transcription factors Bcl-6, Eomes and Blimp-1. In a similar manner, strong IL-2 signaling directed activated $CD4^+$ T cells into T_H1 differentiation but inhibited T follicular helper (T_{FH}) cell development[12]. Intriguingly, glycolysis was recently shown

to be required for full effector function of T cells, such as efficient cytokine secretion[13]. Thus, maintenance of aerobic glycolysis in an IL-2–dependent manner provides a rational for why effector cells expressing high amounts of CD25 are more likely to develop into short-lived effector T cells and have enhanced effector function[12].

Down-regulation of aerobic glycolysis, which characterizes the transition of effector to memory cells, does not simply lead to a 'default' reacquisition of OXPHOS and FAO. Recent work has shown that memory-specific pathways need to be activated, including intrinsic *de novo* fatty acid synthesis and lipolysis, which are important for memory development and survival[5,14]. It is intriguing to speculate that in addition to its repressive effects, Bcl-6 and other transcription factors involved in memory T cell development may also play active roles in initiating memory specific metabolic programs. Consistent with such a model, recent data suggest that Bcl-6 is required for the induction of genes involved in lipid metabolism, at least in adipose tissue[15]. Further work is necessary to fully unravel the complex interactions between Bcl-6 and other transcriptional regulators and to fully understand how these factors influence cellular fate and differentiation in the context of memory development.

Over the last few years, much progress was made in shedding light on the complex roles that transcription factors play during effector and memory T cell development. The results of Weinmann and colleagues add to an emerging model in which at least some of these factors not only impact on the transcriptional regulation of genes directly involved in effector and memory function but coordinate cellular metabolic function as well[2]. Given the strikingly different characteristics of naïve, effector and memory cells, and the high metabolic 'costs' that are associated with rapid proliferation and effector function, a tight link between the regulation of cellular differentiation and metabolism would be evolutionary beneficial. Uncovering how transcription factors contribute to the appropriate regulation of metabolic pathways required for the progression of immune responses remains a fascinating topic and will undoubtedly reveal new ways to influence and shape T cell responses.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Figure 1. Bcl-6 represses IL-2-induced glucose metabolism. During an adaptive immune response T cell activation and high amounts of IL-2 results in rapid upregulation of c-Myc and HIF-1α, both critical for the induction of components of the glycolytic pathway and the metabolic switch from switch from oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO) to aerobic glycolysis. TCR engagement also induces expression of IRF4, essential for the maintenance of aerobic glycolysis during clonal population expansion. Inflammatory cytokines and high amounts of IL-2 promote the development of effector T cells that express T-bet, which by repressing the activity of Bcl-6 prevents downregulation of the genes involved in glycolysis. During the development of memory T cells, clearance of antigen and less IL-2 available will lead to the downregulation of c-Myc, HIF-1α and IRF4. At the same time increased Bcl-6 expression in conjunction with lower amounts of T-bet will release the repressive activity of Bcl-6, leading to the repression of glycolysis and reengagement of OXPHOS and FAO.

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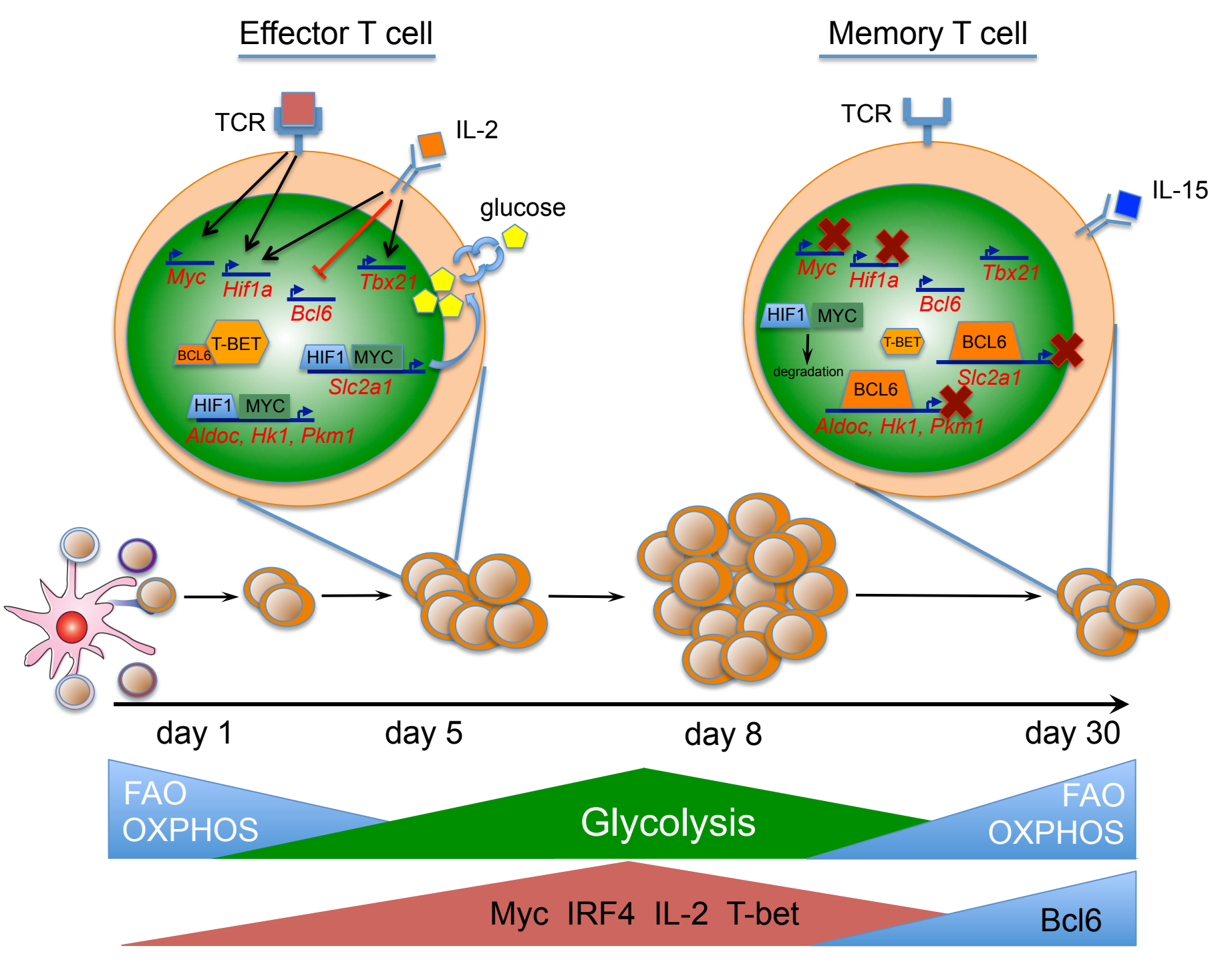
Effector T cell
Memory T cell
TCR
IL-2
glucose
Myc
Hif1a
Tbx21
Bcl6
T-BET
BCL6
HIF1
MYC
Slc2a1
Aldoc, Hk1, Pkm1
TCR
IL-15
Myc
Hif1a
Tbx21
Bcl6
HIF1
MYC
degradation
T-BET
BCL6
Slc2a1
BCL6
Aldoc, Hk1, Pkm1
day 1
day 5
day 8
day 30
FAO
OXPHOS
Glycolysis
FAO
OXPHOS
Myc IRF4 IL-2 T-bet
Bcl6