The understanding of how Tregs develop can facilitate our ability to use them as immune therapies against inflammation and autoimmune diseases. However, as a result of plasticity of $CD8^+$ T cells, naïve $CD8^+$ T cells can give a potential rise to effectors that are beneficial or detrimental, dependent on conditions. Zhao and colleagues [4] have established an experimental condition that specifically induces IL-10-producing $CD8^+$ Tregs without eliciting the Tc1 effector fate. The discovery that Cdkn2a and Blimp-1 are master regulators in promoting IL-10-producing $CD8^+$ T cell differentiation will lead to further exploration of those pathways and enhance our understanding of related cell-fate programs.

ACKNOWLEDGMENTS

This work was supported by grants from the U.S. National Institutes of Health (AI051626 and AI065566) to A.A. We thank members in the August laboratory and other colleagues in the Department of Microbiology and Immunology at Cornell University for discussion and feedback.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

1. Endharti, A., Okuno, Y., Shi, Z., Misawa, N., Toyokuni, S., Ito, M., Isobe, K., Suzuki, H. (2011) CD8+CD122+ regulatory T cells (Tregs) and CD4+ Tregs cooperatively prevent and cure CD4+ cell-induced colitis. *J. Immunol.* **186,** 41–52.
2. Sun, J., Dodd, H., Moser, E. K., Sharma, R., Braciale, T. J. (2011) CD4+ T cell help and innate-derived IL-27 induce Blimp-1-dependent IL-10 production by antiviral CTLs. *Nat. Immunol.* **12,** 327–334.
3. Kaech, S. M., Cui, W. (2012) Transcriptional control of effector and memory CD8+ T cell differentiation. *Nat. Rev. Immunol.* **12,** 749–761.
4. Zhao, Y., Zhao, H., Sun, Y., Hao, J., Qi, X., Zhou, X., Wu, Z., Wang, P., Kaech, S. M., Weaver, C. T., Flavell, R. A., Zhao, L., Yao, Z., Yin, Z. (2013) IL-4 induces a suppressive IL-10-producing $CD8^+$ T cell population via a Cdkn2a-dependent mechanism. *J. Leukoc. Biol.* **94**.
5. Noble, A., Giorgini, A., Leggat, J. A. (2006) Cytokine-induced IL-10-secreting CD8 T cells represent a phenotypically distinct suppressor T-cell lineage. *Blood* **107,** 4475–4483.
6. Roncarolo, M. G., Bacchetta, R., Bordignon, C., Narula, S., Levings, M. K. (2001) Type 1 T regulatory cells. *Immunol. Rev.* **182,** 68–79.
7. Oliver, J., Stolberg, V., Chensue, S., King, P. (2012) IL-4 acts as a potent stimulator of IFN-γ expression in CD8+ T cells through STAT6-dependent and independent induction of eomesodermin and T-bet. *Cytokine* **57,** 191–199.
8. Huang, W., Hu, J., August, A. (2013) Cutting edge: innate memory CD8+ T cells are distinct from homeostatic expanded CD8+ T cells and rapidly respond to primary antigenic stimuli. *J. Immunol.* **190,** 2490–2494.
9. Weinreich, M., Odumade, O., Jameson, S., Hogquist, K. (2010) T cells expressing the transcription factor PLZF regulate the development of memory-like CD8+ T cells. *Nat. Immunol.* **11,** 709–716.
10. Huang, L. R., Chen, F. L., Chen, Y. T., Lin, Y. M., Kung, J. T. (2000) Potent induction of long-term CD8+ T cell memory by short-term IL-4 exposure during T cell receptor stimulation. *Proc. Natl. Acad. Sci. USA* **97,** 3406–3411.
11. Cretney, E., Xin, A., Shi, W., Minnich, M., Masson, F., Miasari, M., Belz, G. T., Smyth, G. K., Busslinger, M., Nutt, S. L., Kallies, A. (2011) The transcription factors Blimp-1 and IRF4 jointly control the differentiation and function of effector regulatory T cells. *Nat. Immunol.* **12,** 304–311.
12. Calame, K. (2008) Activation-dependent induction of Blimp-1. *Curr. Opin. Immunol.* **20,** 259–264.
13. Zhu, Y., Ju, S., Chen, E., Dai, S., Li, C., Morel, P., Liu, L., Zhang, X., Lu, B. (2010) T-bet and eomesodermin are required for T cell-mediated antitumor immune responses. *J. Immunol.* **185,** 3174–3183.
14. Cimmino, L., Martins, G. A., Liao, J., Magnusdottir, E., Grunig, G., Perez, R. K., Calame, K. L. (2008) Blimp-1 attenuates Th1 differentiation by repression of ifng, tbx21, and bcl6 gene expression. *J. Immunol.* **181,** 2338–2347.
15. Dunn, D. E., Herold, K. C., Otten, G. R., Lancki, D. W., Gajewski, T., Vogel, S. N., Fitch, F. W. (1987) Interleukin 2 and concanavalin A stimulate interferon-γ production in a murine cytolytic T cell clone by different pathways. *J. Immunol.* **139,** 3942–3948.
16. Almontashiri, N. A., Fan, M., Cheng, B. L., Chen, H. H., Roberts, R., Stewart, A. F. (2013) Interferon-γ activates expression of p15 and p16 regardless of 9p21.3 coronary artery disease risk genotype. *J. Am. Coll. Cardiol.* **61,** 143–147.
17. Shin, H., Blackburn, S. D., Intlekofer, A. M., Kao, C., Angelosanto, J. M., Reiner, S. L., Wherry, E. J. (2009) A role for the transcriptional repressor Blimp-1 in CD8(+) T cell exhaustion during chronic viral infection. *Immunity* **31,** 309–320.

KEY WORDS:
IL-10 · Th2 · regulatory T cells · Cdkn2a

Editorial: "Presenting" an adaptive role for AMPK

By Morgan D. Fullerton and Gregory R. Steinberg[1]

Division of Endocrinology and Metabolism, Department of Medicine, McMaster University, Hamilton, Ontario, Canada

RECEIVED JUNE 18, 2013; REVISED JULY 10, 2013; ACCEPTED JULY 19, 2013. DOI: 10.1189/jlb.0613335

SEE CORRESPONDING ARTICLE ON PAGE 1113

The maintenance of cellular defense systems and removal of pathogens are energetically demanding processes that require integration of multiple metabolic checkpoints to maintain immune cell energy homeostasis [1]. The importance of this interplay between pathogen and host metabolic function involves alterations to multiple biological systems ranging from the control of glucose homeostasis to changes in electrolyte and amino acid metabolism [2, 3]. Given the importance of energy metabolism in immunity, it is not surprising that the AMP-activated protein kinase (AMPK) has emerged as an important regulator of inflammatory responses in immune cells (for review, see refs. [4, 5]). AMPK is a highly conserved serine/threonine kinase that plays a crucial role in the regulation of energy metabolism. AMPK, an $\alpha\beta\gamma$ heterotrimer (multiple isoforms

Abbreviations: HIF-1α=hypoxia-inducible factor 1α, mTOC1=mammalian target of rapamycin complex 1, SIRT1=sirtuin 1

1. Correspondence: Div. of Endocrinology and Metabolism, Dept. of Medicine, HSC 4N63, McMaster University, 1280 Main St. West, Hamilton, Ontario, L8N 3Z5, Canada. E-mail: gsteinberg@mcmaster.ca

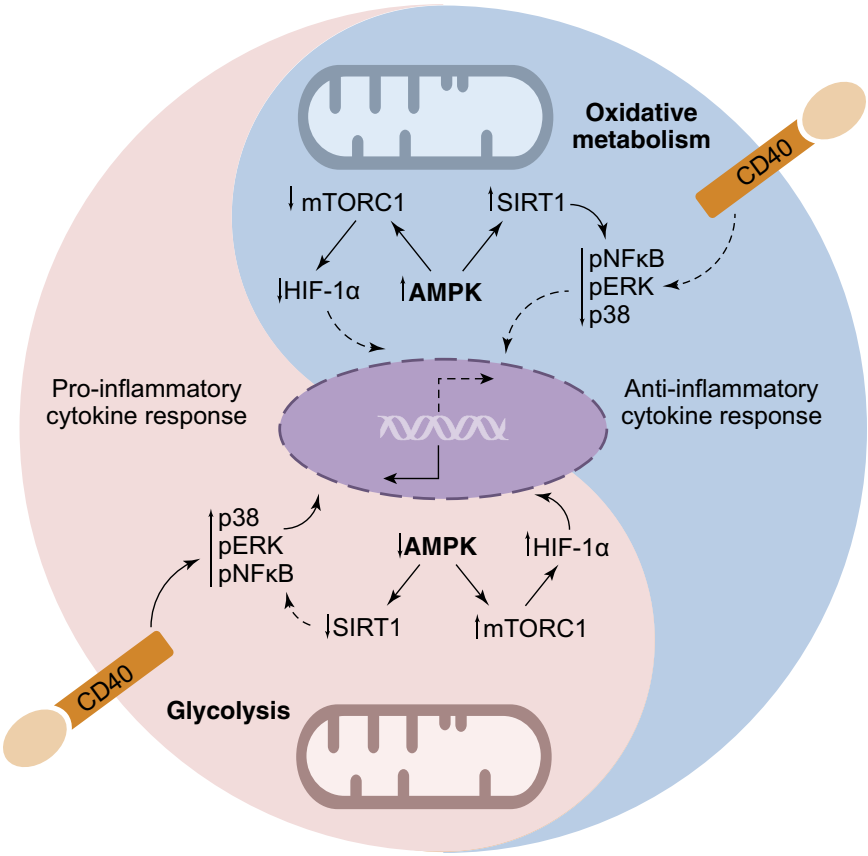

Figure 1. Schematic representation of AMPK signaling in APCs (macrophages or DCs) and possibly T cells. AMPK limits NF-κB and MAPK-mediated transcription of proinflammatory cytokines, potentially via SIRT1. In addition, AMPK inhibits mTORC1, which attenuates HIF-1α-mediated transcription of glycolytic genes, promoting oxidative metabolism. p, Phosphorylation.

of each subunit exist), phosphorylates targets that switch off ATP-depleting processes and turn on ATP-generating pathways [6]. In times of energy deprivation, the concentration of AMP and ADP rises, leading to increased, activating phosphorylation of Thr172 of the α-catalytic subunit. The tumor suppressor LKB1 is thought to be the main upstream kinase of AMPK under conditions of energy deprivation; however, calcium-dependent activation via CaMKKβ canalso phosphorylate and activate AMPK in certain cell types [6]. In addition to energy deficiency, pharmaceutical, small molecule, and endogenous/hormonal activators of AMPK have been identified. The antidiabetic drug metformin, the AMP analog aminoimidazole carboxamide ribonucleotide (AICAR), and many xenobiotics, such as berberine, are all compounds that alter or mimic alterations in the adenylate charge (AMP:ADP:ATP) and result in indirect activation of AMPK [6]. A small molecule, developed by Abbott Laboratories (A769662; Abbott Park, IL, USA), activates AMPK by inhibiting dephosphorylation—an effect requiring Ser108 of the β1-subunit [7]. Interestingly, the active component of aspirin, which mediates its anti-inflammatory effects—salicylic acid—also activates AMPK directly via this mechanism [8].

Whereas previous studies had recognized the intimate relationship between inflammation and AMPK in endothelial cells [9] and muscle [10], the first investigation into the role of AMPK in immune cells was by Sag et al. [11], who demonstrated that the expression of a dominant-negative or constitutively active AMPK exacerbated or blunted LPS-induced inflammation in mouse macrophages, respectively. Since this initial report, numerous studies have continued to define an important role for AMPK in macrophage-, T cell-, and DC-associated immune function in response to classical inflammatory agents or lipid-induced inflammation and obesity [12–17]. In addition, AMPK has been implicated in regulating the metabolic switch between glycolysis (the predominant energy-generating pathway in classically activated/proinflammatory immune cells) and oxidative phosphorylation (the main energy-generating pathway in alternatively/anti-inflammatory immune cells) [5]. In this issue of *JLB*, Carroll et al. [18] add to the growing body of evidence, indicating a critical role for AMPK in mediating immune function by modulating inflammatory responses in Antigen Presenting Cells (APCs) such as macrophages, DCs and T-cells.

With the use of bone marrow-derived macrophages from mice lacking AMPK α1, Carroll et al. [18] first confirm their previous findings using dominant-negative adenoviruses [11], in which in response to LPS, AMPK deficiency potentiates proinflammatory responses. The authors next looked to extend these findings and test the hypothesis that AMPK is critical for proper T cell response to antigen presentation. The surveillance and ingestion of foreign pathogens via phagocytosis are important functions of macrophages and DCs. Once internalized, APCs migrate into the lymphatic circulation, pathogens are enzymatically digested, and the antigens are presented to effector T cells. The presence of AMPK α1 in macrophages and DCs attenuated antigen-induced Th1 and Th17 (IFN-γ and IL-17, respectively) responses in isolated $CD4^+$ T cells. Moreover, in the absence of AMPK α1 in APCs and T cells, Th1 and Th17 responses were increased further. To probe the APC–T cell interaction, Carroll et al. sought to determine if AMPK was important in regulating CD40 signal transduction. Similar to findings with LPS stimulation, the CD40 ligand (soluble CD154) increased proinflammatory and inhibited anti-inflammatory responses in APCs. Interestingly, unlike LPS, which is known to decrease AMPK activity [11, 12], CD154 did not reduce AMPK Thr172 phosphorylation. Finally, the authors demonstrate that in response to CD40 signaling, NF-κB and MAPK phosphorylation is transiently elevated, where markers of the Akt signaling pathway are modestly inhibited downstream of CD154.

There have been several recent reports implicating a role for T cell AMPK in

modulating metabolic flux as well as cytokine responses [15–17]. However, that there may be a common, synergistic function for AMPK that span branches of adaptive and innate immunity is novel. In an in vitro setting, Carroll et al. [18] demonstrated that the presence of AMPK in APCs and T cells lessens the resultant inflammatory responses. As with all new concepts, many questions remain. First and foremost is how AMPK might be mediating its anti-inflammatory effects. Activation of AMPK has been shown to reduce NF-κB activation via SIRT1-mediated deacetylation of p65 at Lys310 in macrophages [14] (**Fig. 1**). The results shown here by Carroll et al. [18] in DCs are in keeping with this notion, although direct mechanistic evidence for inhibition of NF-κB is not presented. In addition, there remains the possibility that there are novel downstream (direct) targets of AMPK that dampen inflammation, although this has yet to be shown.

Naïve and memory T cells, as well as anti-inflammatory (alternatively activated) macrophages and DC populations, are metabolically tuned to efficiently derive ATP via oxidative phosphorylation, where there are low basal levels of glucose uptake and protein synthesis. Differentiation to effector T cells confers the opposite metabolic programming, with high rates of glycolysis and protein synthesis. This process, known as the Warburg effect, has been shown to be regulated via AMPK and its coordination of mTORC1 and subsequent inhibition of protein synthesis through HIF-1α [5, 15] (Fig. 1). The results of Carroll et al. [18] demonstrate that AMPK limits proinflammatory responses in macrophages, DCs, and T cells under various stimuli. However, these results to do not address whether AMPK is critical for the regulation of oxidative versus glycolytic metabolism and if so, whether this is the mechanism that influences inflammatory responses. As the authors speculate, it will be interesting to assess whether CD154 (in addition to other APC–T cell stimuli) induces a switch toward glycolysis, similar to that observed upon LPS and IFN-γ stimulation, and whether AMPK signaling limits this phenomenon. In keeping with AMPK dictating metabolic programming, macrophage AMPK directs increases in fatty acid oxidation, which is essential for suppressing lipid-induced inflammation and the development of obesity-induced insulin resistance [12]. Taken together, evidence in the macrophage, T cell, and possibly now DCs provides support for AMPK in regulating metabolic processes that direct immune function.

Whereas previous studies have established a critical role of AMPK in macrophages (12) and T cells (15, 16) in intact organisms, it will also be interesting to ascertain whether the pronounced inflammatory responses observed in AMPK α1-deficient APCs in vitro will translate in the in vivo systems. Given the sequential coordination of inflammatory responses to acute infection, perhaps reduced or limited AMPK signaling is necessary to initiate effector responses for pathogen disposal (by promoting the Warburg effect); although it should be noted that Carroll et al. [18] do not describe differences in the phagocytic capacity of APCs in vitro. As the resolution of inflammation is achieved, AMPK signaling would then be essential to transition back to a quiescent and more oxidative state. Importantly, CD40 is not exclusive to APCs but is found on other tissue types (endothelial and smooth muscle). Therefore, the results described here should direct new investigations into the interactions of AMPK and CD40 signaling in chronic inflammatory conditions, such as atherosclerosis, inflammatory bowel disease, and obesity-induced metabolic dysfunction.

ACKNOWLEDGMENTS

M.D.F. is a CIHR Banting Postdoctoral Fellow and G.R.S. is a Canada Research Chair in Metabolism and Obesity. The laboratory of G.R.S. is supported by operating grants from the Canadian Institutes of Health Research, the Canadian Diabetes Association and the Heart and Stroke Foundation of Ontario.

REFERENCES

1. Medzhitov, R. (2010) Inflammation 2010: new adventures of an old flame. *Cell* **140,** 771–776.
2. Beisel, W. R. (1972) Interrelated changes in host metabolism during generalized infectious illness. *Am.J. Clin. Nutr.* **25,** 1254–1260.
3. Beisel, W. R. (1975) Metabolic response to infection. *Ann.Rev. Med.* **26,** 9–20.
4. Fullerton, M. D., Steinberg, G. R., Schertzer, J. D. (2013) Immunometabolism of AMPK in insulin resistance and atherosclerosis. *Mol. Cell. Endocrin.* **366,** 224–234.
5. O'Neill, L. A., Hardie, D. G. (2013) Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature* **493,** 346–355.
6. Steinberg, G. R., Kemp, B. E. (2009) AMPK in health and disease. *Physiol. Rev.* **89,** 1025–1078.
7. Scott, J.W., van Denderen, B.J.W., Jørgensen, S.B., Honeyman, J., Steinberg, G.R., Oakhill, J.S., Iseli, T., Koay, A., Gooley, A.R., Stapleton, D., Kemp, B.E. (2008) Role of AMP-activated Protein Kinase β Subunit in Autoregulation and Thienopyridone Drug Sensitivity, *Chemistry and Biology*, **15,** 1220–30.
8. Hawley, S. A., Fullerton, M. D., Ross, F. A., Schertzer, J. D., Chevtzoff, C., Walker, K. J., Peggie, M. W., Zibrova, D., Green, K. A., Mustard, K. J., Kemp, B. E., Sakamoto, K., Steinberg, G. R., Hardie, D. G. (2012) The ancient drug salicylate directly activates AMP-activated protein kinase. *Science* **336,** 918–922.
9. Cacicedo, J. M., Yagihashi, N., Keaney J. F., Jr., Ruderman, N. B., Ido, Y. (2004) AMPK inhibits fatty acid-induced increases in NF-κB transactivation in cultured human umbilical vein endothelial cells. *Biochem. Biophys. Res. Commun.* **324,** 1204–1209.
10. Steinberg, G. R., Michell, B. J., van Denderen, B. J., Watt, M. J., Carey, A. L., Fam, B. C., Andrikopoulos, S., Proietto, J., Gorgun, C. Z., Carling, D., Hotamisligil, G. S., Febbraio, M. A., Kay, T. W., Kemp, B. E. (2006) Tumor necrosis factor α-induced skeletal muscle insulin resistance involves suppression of AMP-kinase signaling. *Cell. Metab.* **4,** 465–474.
11. Sag, D., Carling, D., Stout, R. D., Suttles, J. (2008) Adenosine 5′-monophosphate-activated protein kinase promotes macrophage polarization to an anti-inflammatory functional phenotype. *J. Immunol.* **181,** 8633–8641.
12. Galic, S., Fullerton, M. D., Schertzer, J. D., Sikkema, S., Marcinko, K., Walkley, C. R., Izon, D., Honeyman, J., Chen, Z. P., van Denderen, B. J., Kemp, B. E., Steinberg, G. R. (2011) Hematopoietic AMPK β1 reduces mouse adipose tissue macrophage inflammation and insulin resistance in obesity. *J. Clin. Invest.* **121,** 4903–4915.
13. Krawczyk, C. M., Holowka, T., Sun, J., Blagih, J., Amiel, E., DeBerardinis, R. J., Cross, J. R., Jung, E., Thompson, C. B., Jones, R. G., Pearce, E. J. (2010) Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* **115,** 4742–4749.
14. Yang, Z., Kahn, B. B., Shi, H., Xue, B. Z. (2010) Macrophage α1 AMP-activated protein kinase (α1AMPK) antagonizes fatty acid-induced inflammation through SIRT1. *J. Biol. Chem.* **285,** 19051–19059.
15. MacIver, N. J., Blagih, J., Saucillo, D. C., Tonelli, L., Griss, T., Rathmell, J. C., Jones, R. G. (2011) The liver kinase B1 is a central regulator of T cell development, activation, and metabolism. *J. Immunol.* **187,** 4187–4198.
16. Rolf, J., Zarrouk, M., Finlay, D. K., Foretz, M., Viollet, B., Cantrell, D. A. (2013) AMPKα1: a glucose sensor that controls CD8 T-cell memory. *Eur. J. Immunol.* **43,** 889–896.
17. Michalek, R. D., Gerriets, V. A., Jacobs, S. R., Macintyre, A. N., MacIver, N. J., Mason, E. F., Sullivan, S. A., Nichols, A. G., Rathmell, J. C. (2011) Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. *J. Immunol.* **186,** 3299–3303.
18. Carroll K.C., Viollet B., Suttles J. (2013) AMPKα1 deficiency amplifies proinflammatory myeloid APC activity and CD40 signaling. *J. Leukoc. Biol.* **94,** 1113–1121.

KEY WORDS:
immunometabolism · oxidative metabolism · inflammation · obesity · insulin resistance · AMP activated protein kinase · immunity