# Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from Myc-dependent to HIF1α-dependent

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**As a phenotypically plastic cellular population, macrophages change their physiology in response to environmental signals. Emerging evidence suggests that macrophages are capable of tightly coordinating their metabolic programs to adjust their immunological and bioenergetic functional properties, as needed. Upon mitogenic stimulation, quiescent macrophages enter the cell cycle, increasing their bioenergetic and biosynthetic activity to meet the demands of cell growth. Proinflammatory stimulation, however, suppresses cell proliferation, while maintaining a heightened metabolic activity imposed by the production of bactericidal factors. Here, we report that the mitogenic stimulus, colony-stimulating factor 1 (CSF-1), engages a myelocytomatosis viral oncogen (Myc)-dependent transcriptional program that is responsible for cell cycle entry and the up-regulation of glucose and glutamine catabolism in bone marrow-derived macrophages (BMDMs). However, the proinflammatory stimulus, lipopolysaccharide (LPS), suppresses Myc expression and cell proliferation and engages a hypoxia-inducible factor alpha (HIF1α)-dependent transcriptional program that is responsible for heightened glycolysis. The acute deletion of Myc or HIF1α selectively impaired the CSF-1– or LPS-driven metabolic activities in BMDM, respectively. Finally, inhibition of glycolysis by 2-deoxyglucose (2-DG) or genetic deletion of HIF1α suppressed LPS-induced inflammation in vivo. Our studies indicate that a switch from a Myc-dependent to a HIF1α-dependent transcriptional program may regulate the robust bioenergetic support for an inflammatory response, while sparing Myc-dependent proliferation.**

metabolism | macrophage | cell cycle | Myc | HIF1α

The cells of the immune system are constantly exposed to environmental challenges and are capable of tailoring their metabolic programs to meet distinct physiological needs. Macrophages, like other immune cells, rapidly change their physiology in response to various environmental cues. Macrophages undergo proliferation in response to mitogenic stimuli, such as colony-stimulating factor 1 (CSF-1) [also known as macrophage CSF (M-CSF)], and this cellular turnover is essential for macrophage homeostasis and may occur in mature macrophages, bypassing the need for self-renewing progenitors (1, 2). Proliferating macrophages consume considerable amounts of energy and require de novo synthesis of macromolecules to support their growth and proliferation (3–6). Therefore, macrophages must coordinately regulate metabolic programs to meet their bioenergetic and biosynthetic demand during proliferation. Despite the emerging view that extracellular signaling events dictate cell growth, proliferation, and death, in part by modulating metabolic activities in cancer cells and T lymphocytes, the precise mechanisms and crucial players of reprogramming metabolism during macrophage proliferation are incompletely understood.

Upon encountering an invading microorganism, the bioenergetic potential in macrophages quickly shifts away from fulfilling the needs of cell proliferation to mount a robust response to resolve the immunological insult. The combination of the bacterial component, lipopolysaccharide (LPS), and the proinflammatory cytokine, IFN-γ, triggers the differentiation of M1 macrophages, in a process often referred to as the classical activation program (7, 8). To mount a rapid and effective immune response against highly proliferative intracellular pathogens, M1 macrophages produce nitric oxide (NO), reactive oxygen species (ROS), and proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-12. This process is rapid and energy-intensive and therefore requires a reconfiguration of metabolic programs that may provide the host with a competitive bioenergetic advantage against pathogens. Earlier studies suggest that macrophages exit from the cell cycle during M1 differentiation, indicating a potential coordination between metabolic regulation and macrophage physiology (9–15).

M1 macrophage differentiation via proinflammatory stimulation induces the expression of inducible nitric oxide synthase (iNOS), resulting in an iNOS-mediated breakdown of arginine to produce NO and promote glucose catabolic routes, largely through aerobic glycolysis and the pentose phosphate pathway (PPP) (12, 16, 17). Heightened glycolysis is required for ATP generation in M1

## Significance

Macrophages maintain homeostatic proliferation in the presence of mitogens whereas encounters with invading microorganisms inhibit proliferation and engage a rapid proinflammatory response. Such cell fate change requires an extensive reprogramming of metabolism, and the regulatory mechanisms behind this change remain unknown. We found that myelocytomatosis viral oncogen (Myc) plays a major role in regulating proliferation-associated metabolic programs. However, proinflammatory stimuli suppress Myc and cell proliferation and engage a hypoxia-inducible factor alpha (HIF1α)-dependent transcriptional program that is responsible for heightened glycolysis. Our work indicates that a switch from a Myc-dependent to a HIF1α-dependent transcriptional program may regulate the robust bioenergetic support for inflammatory response, while sparing Myc-dependent proliferation.

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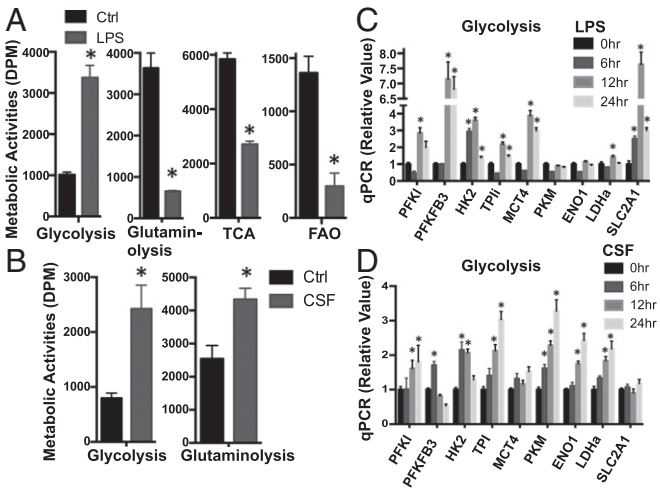


**Fig. 1.** Proinflammatory and mitogenic stimulation differentially drives macrophage metabolic reprogramming. (*A* and *B*) Untreated BMDMs (Ctrl) and LPS-stimulated (*A*) or CSF-stimulated BMDMs (*B*) were collected at 24 h after stimulation and were used for measuring the generation of $^3H_2O$ from [3-$^3$H]-glucose (glycolysis) or from [9,10-$^3$H]-palmitic acid (fatty acid beta-oxidation) and from [U-$^{14}$C]-glutamine (glutaminolysis) or from [2-$^{14}$C]-pyruvate (TCA). (*C* and *D*) RNAs were isolated from BMDMs collected at the indicated time after LPS (*C*) or CSF (*D*) stimulation and used for real-time PCR analyses of metabolic genes in the glycolytic pathway and in the glutaminolytic pathway. The relative gene expression was determined by the comparative $C_T$ method, also referred to as the $2^{-\Delta\Delta C}{}_T$ method. Error bars represent SD from the mean of triplicate qPCR reactions. Data are representative of three independent experiments. *P* values were calculated with Student's *t* test. *P* values of <0.05 were considered significant. An equal number of cells were used in the radioisotopic experiments. DPM, disintegrations per min.

macrophages and also provides precursors for lipid and amino acid biosynthesis, all of which may support intracellular membrane reorganization and the production and secretion of proinflammatory cytokines (17–20). Meanwhile, the increase of PPP-derived NADPH supports the production of reduced glutathione and therefore limits oxidative stress in M1 macrophages (12, 21, 22).

The transcriptional induction of glycolytic enzymes, such as phosphoglycerate kinase (PGK), glucose transporter-1 (GLUT-1), and ubiquitous 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (uPFK2), is involved in promoting glycolysis in M1 macrophages (23, 24). Emerging evidence also suggests that the fine-tuning of the activity of Pyruvate Kinase M2 (PKM2) is required for an optimized inflammatory response in various pathological contexts (25, 26). In addition to its role in promoting the transcription of proangiogenic factors and proinflammatory cytokines in macrophages, the transcriptional factor hypoxia-inducible factor alpha (HIF1α) may also regulate the transcription of the above glycolytic enzymes (16, 24, 27). Conversely, a decrease in carbohydrate kinase-like protein (CARKL) is implicated in the shuttling of glucose catabolism to the oxidative arm of the PPP in M1 macrophages (21). Beyond these metabolic regulations, previous studies have identified gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, in extracts of human peripheral blood monocyte-derived macrophages (28). The related "GABA (γ-aminobutyric acid) shunt" pathway provides a source of succinate, the intracellular level of which determines the stability of HIF1α and its proinflammatory activity in M1 macrophage (16).

These findings prompted us to ask how macrophages coordinate their metabolic programs to meet their distinct physiological needs in response to mitogenic signaling versus proinflammatory signaling. Our studies show that CSF-1–driven mitogenic signaling engages the myelocytomatosis viral oncogen (Myc)-dependent transcriptome, promoting cell proliferation and catabolism of glucose and glutamine whereas LPS-driven inflammatory signaling suppresses Myc-dependent proliferation and enhances the HIF1α-dependent transcription of glycolytic enzymes, leading to heightened aerobic glycolysis. This change may allow M1 macrophages to divert bioenergetics and biosynthetic resources away from supporting proliferation, thus optimizing metabolic capacity to fulfill the needs of an inflammatory response. Our results further emphasize the important role of HIF1α-dependent glycolysis in the modulation of M1 macrophage function in vivo by demonstrating that the inhibition of glycolysis by 2-deoxyglucose (2-DG) or genetic deletion of HIF1α significantly suppresses inflammation in a murine sepsis model.

## Results

**Macrophage Metabolic Reprogramming in Response to Proinflammatory and Mitogenic Stimulation.** Like many other immune cells, macrophages can rapidly adjust their metabolic activity in response to various environmental cues. CSF-1 (also known as M-CSF) is the main macrophage mitogen, driving the survival, proliferation, and maturation of macrophages. A combination of microbial components, such as LPS plus IFN-γ, however, can result in classically activated macrophages (also known as M1 macrophages) that exert rapid and effective proinflammatory and microbicidal responses. To understand how macrophages adapt their metabolic programs to meet the bioenergetic demand from mitogenic stimuli or to mediate the immune effector function required by immunological insult, we deprived bone marrow-derived macrophages (BMDMs) of CSF-1 for 24 h and then either restimulated BMDMs with CSF-1 or stimulated BMDMs with LPS and IFN-γ (M1 induction) for 24 h.

After the above treatments, we used radiochemical-based approaches to follow the metabolic activities in these cells. Consistent with early studies (12, 17–20, 23, 29), M1 macrophages significantly up-regulated glycolysis in a time-dependent manner, indicated by the

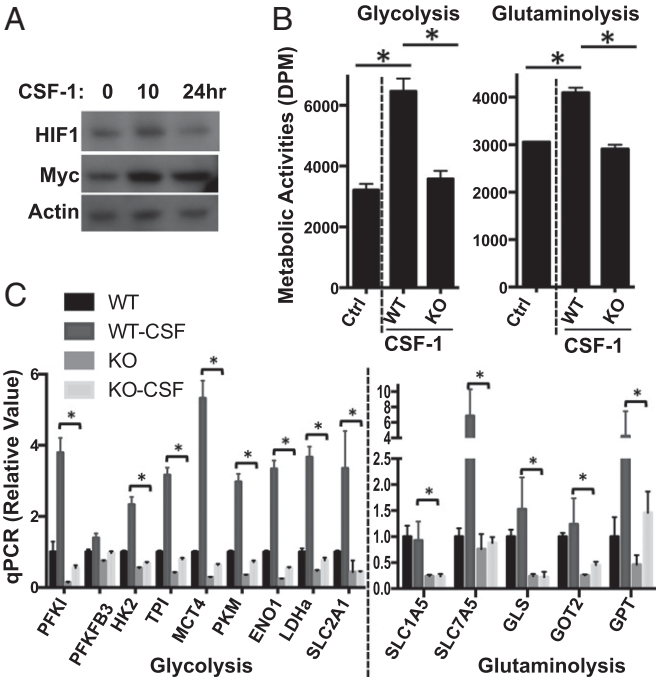


**Fig. 2.** Myc is required for CSF-driven proliferation and metabolic reprogramming in macrophage. (*A*) The protein levels of HIF1α and c-Myc in BMDMs collected at the indicated time after CSF stimulation were determined by Western blot. (*B* and *C*) BMDMs generated from either RosaCreERTam–, *Myc*$^{flox/flox}$ mice (WT) or RosaCreERTam+, *Myc*$^{flox/flox}$ mice (KO) were pretreated with 500 nM 4OHT (+4OHT). Untreated BMDMs (Ctrl) or CSF-stimulated BMDM (CSF) were used for measuring indicated metabolic activities (*B*) or the mRNA expression of indicated metabolic gene expression (*C*). The relative gene expression was determined as described in detail in Fig. 1. Error bars represent SD from the mean of triplicate qPCR reactions. Data are representative of two independent experiments. *P* values were calculated with Student's *t* test. *P* values of <0.05 were considered significant. An equal number of cells were used in the radioisotopic experiments.

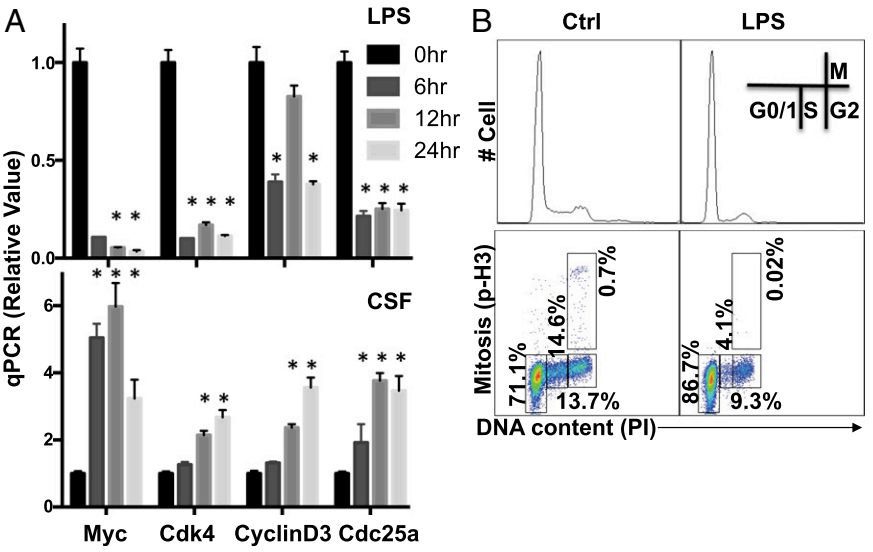


**Fig. 3.** Metabolic rewiring during macrophage M1 polarization is associated with cell cycle blockage. (*A*) BMDMs cultured in differentiation medium (with the presence of CSF-1) collected at the indicated time after LPS stimulation (*Upper*) and CSF stimulation (*Lower*) were used to determine the mRNA level of indicated genes by qPCR. The relative gene expression was determined as described in detail in Fig. 1. Error bars represent SD from the mean of triplicate qPCR reactions. Data are representative of two independent experiments. *P* values were calculated with Student's *t* test. *P* values of <0.05 were considered significant. (*B*) Untreated BMDMs (Ctrl) and LPS-stimulated (LPS) were collected at 24 h after stimulation. The cell cycle profile in indicated groups was determined as the combinational staining of propidium iodide (PI) and p-Histone H3 antibody (p-H3) by flow cytometry.

detritiation of [3-$^{3}$H]-glucose (Fig. 1*A* and Fig. S1*A*). In contrast, mitochondria-dependent pyruvate oxidation through the tricarboxylic acid (TCA) cycle, indicated by $^{14}CO_2$ release from [2-$^{14}$C]-pyruvate; mitochondria-dependent fatty acid β-oxidation (FAO), indicated by the detritiation of [9,10-$^{3}$H]-palmitic acid; and glutamine consumption through oxidative catabolism, indicated by $^{14}CO_2$ release from [U-$^{14}$C]-glutamine, were markedly down-regulated upon LPS and IFN-γ stimulation (Fig. 1*A*). Consistent with these results, oxygen consumption was also significantly dampened in M1 macrophages (Fig. S1*B*). In CSF-1–treated macrophages, however, we observed a significant up-regulation of both glycolysis and glutamine oxidative catabolism (Fig. 1*B*). Taken together, our metabolic and transcriptional profiling demonstrates that mitogenic stimulation with CSF-1 or proinflammatory stimulation with LPS plus IFN-γ differentially enhances or suppresses glutamine catabolism. However, both conditions promote glycolysis.

**Macrophage Metabolic Reprogramming Is Associated with Changes in the Metabolic Gene Transcriptome.** Mitogenic and proinflammatory stimulation drives distinct transcriptional programs, including cell cycle and inflammation, respectively. We therefore hypothesized that induction of the metabolic gene transcriptome in macrophages is associated with metabolic rewiring upon CSF-1 or LPS plus IFN-γ stimulation. We focused on the metabolic pathways that are up-regulated upon stimulation and measured the mRNA levels of metabolic genes involved. Consistent with the results from our metabolic assays (Fig. 1*A*), the expression of mRNAs encoding glycolytic enzymes and transporters was induced, but the expression

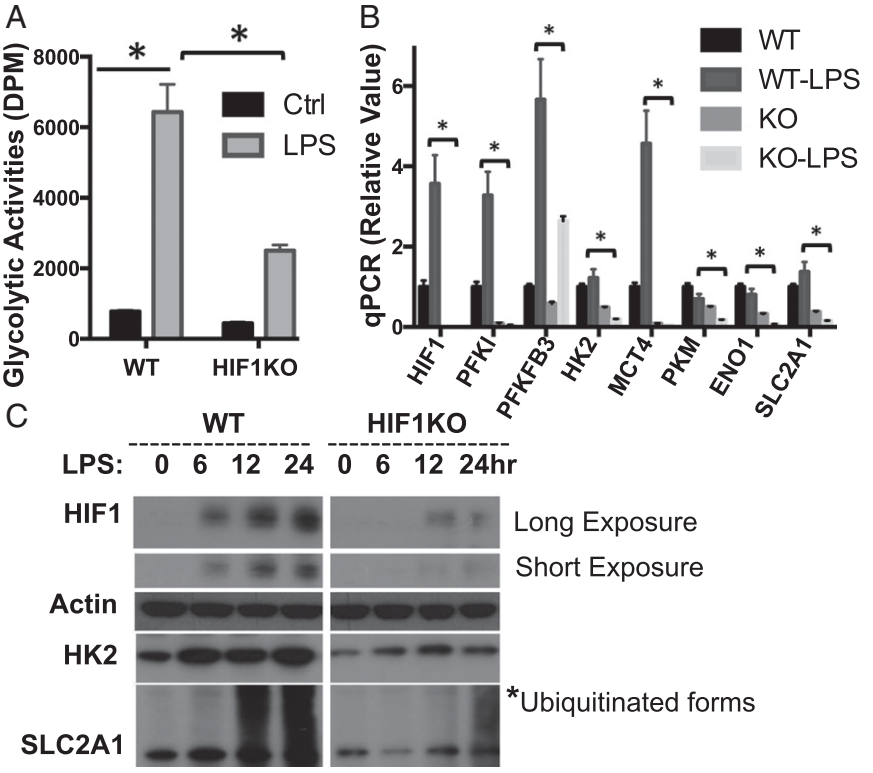


**Fig. 4.** HIF1α is required for LPS-driven glycolysis in macrophage. (*A* and *B*) BMDMs generated from either RosaCreERT2-, *HIF1α*$^{flox/flox}$ mice (WT) or RosaCreERT2+, *HIF1α*$^{flox/flox}$ mice (KO) were pretreated with 500 nM 4OHT (+4OHT). Untreated BMDMs (Ctrl) or LPS-stimulated BMDM (LPS) were collected 24 h after stimulation. The rate of glycolysis (*A*) and the expression of indicated mRNA (*B*) were determined by radioactive tracer-based assay and qPCR, respectively. The relative gene expression was determined as described in detail in Fig. 1. Error bars represent SD from the mean of triplicate qPCR reactions. Data are representative of two independent experiments. *P* values were calculated with Student's *t* test. *P* values of <0.05 were considered significant. (*C*) BMDMs in indicated groups were collected at the indicated time after LPS stimulation. The protein levels of indicated genes were determined by Western blot. Of note, these blots were from two transferred membranes but from the same batch of samples (lysates). The actin result was a representative result from reprobing of a stripped blot. An equal number of cells were used in the radioisotopic experiments.

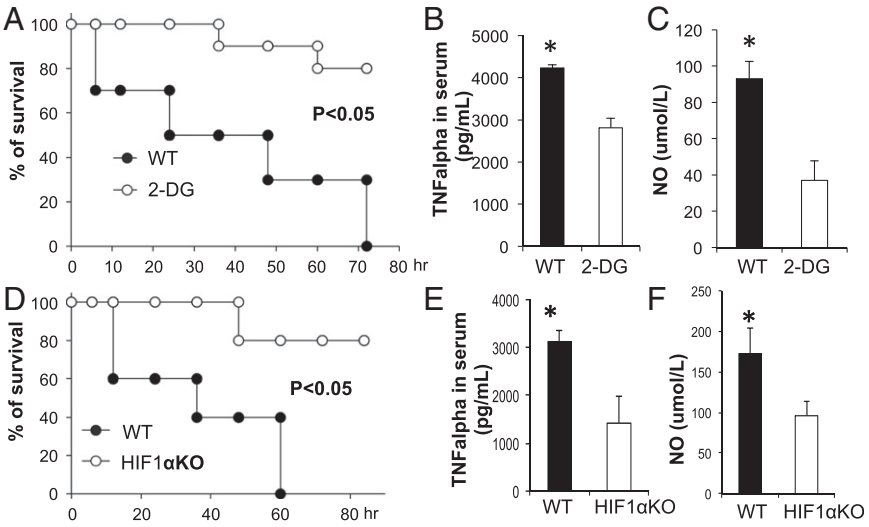


**Fig. 5.** Genetic deletion of HIF1α or pharmacologic blockage of glycolysis reduces severity of LPS-induced sepsis. (*A–C*) Age-matched BL6 mice were injected (i.p.) with PBS (solvent) or 2-DG (2 g/kg body weight) daily starting at 6 h before LPS (10 mg/kg) injection. The survival curve was plotted (*A*, $n = 10$). At 36 h, the serum was collected, and TNFα and NO levels were examined by ELISA and Greiss reagent, respectively (*B* and *C*). (*D–F*) Age-matched BL6 mice (WT) or LysM-Cre, HIF-1α$^{flox/flox}$ (KO) mice were injected (i.p.) with LPS (10 mg/kg). The survival curve was plotted (*D*, $n = 10$). At 36 h, the serum was collected, and TNFα and NO levels were examined by ELISA and Greiss reagent, respectively (*E* and *F*). Data are representative of two independent experiments. *P* values were calculated with Student's *t* test. *P* values of <0.05 were considered significant.

of mRNAs encoding essential metabolic enzymes in glutamine catabolism and the TCA cycle was suppressed after LPS plus IFN-γ stimulation (Fig. 1*C* and Fig. S1 *C* and *D*). Similarly, the expression of mRNAs encoding glycolytic enzymes and transporters and the expression of mRNAs encoding essential metabolic enzymes in glutamine catabolism were induced upon CSF-1 stimulation (Fig. 1*D* and Fig. S1*E*). Taken together, these results are consistent with our metabolic activity data and indicate that the regulation of metabolic gene transcription is associated with macrophage metabolic rewiring upon mitogenic or proinflammatory stimulation.

**CSF-1 Stimulation Drives a Myc-Dependent Metabolic Rewiring in Macrophages.** We next explored the molecular mechanisms behind the regulation of metabolic gene transcription and metabolic rewiring upon CSF-1 stimulation in macrophages. Previous studies have revealed that transcription factors HIF1α and myelocytomatosis oncogene (Myc) are involved in regulating glycolysis and glutaminolysis, respectively, in both cancer cells and immune cells (30–36). Quantitative polymerase chain reaction (qPCR) analysis revealed that the mRNA of Myc was significantly up-regulated in macrophages upon CSF-1 stimulation (Fig. S2*B*). Western blot analysis further confirmed the up-regulation of Myc at the protein level (Fig. 2*A*). The protein level of HIF1α remained unchanged in macrophages upon CSF-1 stimulation (Fig. 2*A*).

We next asked whether the up-regulation of Myc is required for CSF-1–driven metabolic rewiring in macrophages. Previously, we established a mouse model (Myc$^{flox/flox}$, CreERTam), where a tamoxifen-induced Cre recombinase deletes Myc floxed alleles in an acute manner. To obtain an efficient deletion ex vivo, we cultured freshly established BMDMs for 2 d in the absence (WT) or in the presence (KO) of 4-hydroxytamoxifen (4OHT). An efficient deletion of Myc was observed at the protein (Fig. S2*A*) and mRNA (Fig. S2*B*) levels. After CSF-1 stimulation, the level of Myc, the up-regulation of glycolysis, glutaminolysis, and the expression of metabolic genes involved were significantly dampened in Myc KO macrophages upon CSF-1 stimulation (Fig. 2 *B* and *C*). Together, these results suggest that the acute deletion of Myc impairs CSF-1–induced metabolic rewiring in macrophages.

**Myc Is Required for CSF-1–Driven Macrophage Proliferation.** Previous studies have suggested an essential role for Myc in the CSF-1–induced mitogenic response in macrophages (37). Thus, we tested the requirement of Myc in macrophage proliferation. We cultured BMDMs established from Myc$^{flox/flox}$, CreERTam mice for 2 d in the absence (WT) or in the presence (KO) of 4OHT, during which CSF-1 was also withdrawn from culture media. After CSF-1 re-addition, the cell cycle profile was determined by FACS analysis of the DNA content and mitotic index. We found that CSF-1 stimulation increased the percentage of macrophages in S-phase and in mitosis and that acute deletion of Myc abolished these changes (Fig. S2*C*). Previous studies have revealed that G1-S phase regulators, cyclin-dependent kinase 4 (CDK4), cyclin D3, and CDC25a, are downstream targets of Myc in transformed cells. To determine whether these cell cycle regulators are also under the control of Myc in macrophages, we examined the mRNA expression of these genes by qPCR and found that all three genes were up-regulated in a Myc-dependent manner upon CSF-1 stimulation (Fig. S2*B*). Together, these results suggest that the acute deletion of Myc impairs CSF-1–driven proliferation in macrophages.

**Proinflammatory Stimulation with LPS and IFN-γ Suppresses Myc Expression and Proliferation in Macrophages.** We next determined the impact of proinflammatory stimulation on Myc expression in macrophages. We first examined the expression of the Myc gene by qPCR upon CSF-1 stimulation or LPS plus IFN-γ stimulation. Although CSF-1 induced Myc mRNA expression, LPS plus IFN-γ dramatically suppressed Myc mRNA expression (Fig. 3*A*). Western blot analysis further confirmed the down-regulation of Myc at the protein level (Fig. S3*B*). Consistent with these changes in Myc expression, the mRNA expression of cell cycle regulators CDK4, cyclin D3, and CDC25a was up-regulated in a time-dependent manner upon CSF-1 stimulation, yet was down-regulated in macrophages upon LPS plus IFN-γ stimulation (Fig. 3*A*). Western blot analysis confirmed the down-regulation of CDK4, cyclin D3, and CDC25a at the protein level (Fig. S3*B*). To further determine the impact of proinflammatory stimulation on cell proliferation, we analyzed the cell cycle profile by FACS. Upon LPS plus IFN-γ stimulation, the percentage of macrophages in S-phage and mitosis was significantly reduced (Fig. 3*B*).

Stimulation with LPS plus IFN-γ up-regulates glycolysis and induces the expression of glycolytic genes in macrophages (Fig. 1 *A* and *C*). The down-regulation of Myc upon proinflammatory stimulation suggests that Myc is not required for the increase in glycolysis and the induction of glycolytic genes. Our metabolic activity analysis further validated our hypothesis by showing that acute deletion of Myc did not impair the up-regulation of glycolysis upon LPS plus IFN-γ stimulation (Fig. S3*A*). Together, these results demonstrate that proinflammatory stimulation suppresses Myc expression and proliferation in macrophages.

**HIF1α Is Required for Proinflammatory Stimulation-Driven Metabolic Rewiring in Macrophages.** Having excluded the requirement for Myc in regulating glycolysis in macrophages upon proinflammatory stimulation, we tested the requirement for HIF1α. Previous studies have implicated HIF1α as an essential transcriptional factor that regulates myeloid cell and lymphocyte development and

inflammatory function (27, 33–36). HIF1α is required for the transcription of proinflammatory cytokines and metabolic genes involved in glycolysis in M1 macrophages (16, 24). Consistent with these studies, qPCR analysis and Western blot analysis revealed an induction of HIF1α upon LPS plus IFN-γ stimulation at the mRNA and protein level, respectively (Fig. S4 *A* and *B*).

We have previously established a mouse model ($HIF1\alpha^{flox/flox}$, CreERT2), which allows us to delete HIF1α floxed alleles in an acute manner and therefore avoid any potential developmental defect caused by lineage-specific deletion. To determine the requirement of HIF1α in M1 macrophage glycolysis, we cultured BMDMs for 2 d in the absence (WT) or in the presence (KO) of 4OHT. Following stimulation with LPS plus IFN-γ, the level of HIF1α, glycolytic activity, and the expression of glycolytic genes were examined (Fig. 4). We found that the acute deletion of HIF1α significantly dampened LPS-induced glycolysis and the expression of metabolic genes (Fig. 4). The blunted, but not ablated, glycolysis and the expression of metabolic enzymes is likely due to the presence of residual HIF1α-expressing WT macrophages, as evidenced by HIF1α immunoblot (Fig. 4*C*). We have shown that LPS stimulation suppresses Myc expression (Fig. 3*A* and Fig. S3*B*) and induces glycolysis in the absence of Myc (Fig. S3*A*). We further examined whether HIF1α is required for suppressing Myc after LPS stimulation. LPS stimulation suppressed Myc expression in both HIF1α WT and KO macrophages (Fig. S4 *C* and *D*). Similarly, iNOS, a critical proinflammatory effector in LPS-stimulated macrophages, was not involved in suppressing Myc (Fig. S4 *E* and *F*). Together, these results suggest that the acute deletion of HIF1α impairs LPS plus IFN-γ –induced, but not Myc-dependent, metabolic rewiring in macrophages.

**Inhibition of Glycolysis or HIF1α Deletion Dampens Macrophage Proinflammatory Responses in a Murine Sepsis Model.** To further our investigation of heightened glycolysis during proinflammatory responses, we next evaluated the effects of blocking glycolysis with a pharmacological inhibitor, 2-DG, against lethal endotoxemia in an LPS-induced in vivo model of septic shock. Mice were treated i.p. with LPS at 10 mg/kg in the presence or absence of 2-DG, and mortality was monitored over an 80-h period. This high dose of LPS was chosen because it led to a mortality rate of >90% in WT B6 mice. The administration of 2-DG 6 h before the induction of septic shock conferred significant protection against lethal endotoxemia (Fig. 5*A*). Similarly, 2-DG treatment simultaneously reduced the serum levels of TNF-α and NO (Fig. 5 *B* and *C*). In agreement with the above findings, the deletion of HIF1α in cells of myeloid linage (LysM-Cre) significantly protected mice from death upon septic shock (Fig. 5*D*) and reduced the serum levels of TNF-α and NO (Fig. 5 *E* and *F*). One caveat of the above experiments is that both the systemic administration of 2-DG and the LysM-Cre–mediated deletion of HIF1α could affect the function of neutrophils. To address this concern, we first assessed the LysM-Cre activity using YFP reporter mice (R26-stop-EYFP) and found that a comparable percentage of macrophages and neutrophils were $YFP^+$, indicating that HIF1α is deleted in both macrophages and neutrophils in LysM-Cre, $HIF1\alpha^{fl/fl}$ mice (Fig. S5*A*). We further analyzed the distribution of immune cell populations before and after LPS treatment in WT and LysM-Cre, $HIF1\alpha^{fl/fl}$ mice and observed that there was no difference in the percentages of examined cell types, before or after LPS treatment (Fig. S5*B*). Next, we examined the expression of TNFα in macrophages and neutrophils that were isolated from LPS-induced sepsis mice. Although the LysM-Cre–mediated deletion of HIF1α in the myeloid cell lineage or systemic administration of 2-DG significantly reduced TNFα expression in macrophages, HIF1α deletion in neutrophils had a mild and statistically insignificant effect on TNFα expression (Fig. S6*A*). Finally, we applied the Gr1 antibody to deplete neutrophils in both WT and LysM-Cre, $HIF1\alpha^{fl/fl}$ mice and then challenged the mice with LPS. We found that neutrophil-depleted LysM-Cre, $Hif\text{-}1\alpha^{fl/fl}$ mice displayed a significantly longer survival time compared with neutrophil-depleted WT mice (Fig. S6*B*), indicating that the protection conferred by HIF1α deletion is due to the macrophage, not neutrophil population. (Fig. S6*B*). Collectively, these data suggest that targeting HIF1α protects against experimental lethal endotoxic shock and sepsis partly by inhibiting glycolysis in macrophages.

## Discussion

The proper development and function of all metazoan immune systems require the strict coordination of nutrient metabolism and bioenergetic capacity with immune cell proliferation and differentiation. Rapidly evolving pathogens exert a selective pressure on the integration of metabolism and immunity, which leads to the convergence of the signaling pathways that mediate nutrient processing (metabolism) and pathogen sensing (immunity). As such, our immune system is able to maintain homeostasis while remaining ready to elicit rapid and robust immune responses under diverse metabolic and immune conditions. As front-line effectors of innate immunity, macrophages can enter into the cell cycle upon mitogenic stimulation or can elicit a robust inflammatory response upon microbial challenge. Both the cell growth during proliferation and the cytokine production associated with the inflammatory response exhibit high bioenergetic and biosynthetic demands from macrophages. The inability to accommodate these demands would result in homeostatic imbalances in the immune system and possibly immunodeficiency and autoimmunity. We found that the Myc-dependent transcriptional program is responsible for cell cycle entry and the up-regulation of glucose and glutamine catabolism in BMDMs upon mitogenic stimulation. However, proinflammatory stimulation suppresses Myc-dependent cell proliferation while engaging a HIF1α-dependent transcriptional program to maintain heightened glycolysis in M1 macrophages. The switch between the Myc- and HIF1α-dependent transcriptional programs may ensure that inflammatory M1 macrophages have sufficient metabolic capacity to support their effector function, while limiting fuel use associated with cell proliferation. Whereas recent studies clearly demonstrate an essential role for metabolic reprogramming in inflammatory activation of macrophages (27, 38, 39), our studies implicate the switch in key transcriptional factors as an important mechanism of optimizing metabolic support during the inflammatory response.

The heightened glycolysis in proliferating or M1 macrophages is reminiscent of metabolic features in tumor cells, where aerobic glycolysis (the Warburg effect) is driven by aberrant oncogenic signals (31, 40, 41). Acting alone or in concert, dysregulation of Myc and HIF1α, two key transcription factors that regulate the expression of metabolic genes, plays an essential role in reprograming metabolism to support tumor growth (30, 32, 42, 43). Notably, heightened aerobic glycolysis has also been implicated as a key metabolic feature of many immune cells, such as T cells, B cells, and dendritic cells, upon activation (44–46). Interestingly, the ligation of the T-cell receptors (TCR) or B-cell receptors (BCR) induces the expression of both Myc and HIF1α in T cells and B cells, respectively (33, 34). This change is also accompanied by a cell growth and proliferation burst after T- or B-cell activation. However, only Myc, but not HIF1α, is required for driving activation-induced T-cell or B-cell metabolic reprogramming (33, 34). In contrast, increased glycolysis is also seen in differentiating $T_H17$ cells and during B-cell development in bone marrow, and this metabolic change is under the control of HIF1α (36, 47, 48). As such, the switch between the Myc- and HIF1α-dependent metabolic regulation in immunity may represent a general mechanism for fine-tuning metabolic homeostasis to support the divergent needs of immune function.

Emerging evidence has shown that a reconfiguration of glucose catabolism toward aerobic glycolysis and the pentose phosphate shunt (PPP) in M1 macrophages is integral to their host-defense properties (12, 17, 21, 25, 26). Glutaminolysis is a glutamine catabolic process during which the carbons of glutamine are oxidized and converted into $CO_2$ and pyruvate largely through the TCA cycle in mitochondria (49, 50). One recent study revealed that glutamine is required for M2 polarization in macrophages. During LPS-stimulated M1 polarization, the integrated transcriptional-metabolic profiling revealed two metabolic break points in the metabolic flow of the TCA cycle, which suggests a defective TCA cycle and likely a suppressed glutaminolysis (51, 52). This finding is consistent with

our finding of a reduction of glutamine oxidation in M1 macrophages. However, other specialized amino acid catabolic routes may be selectively induced in M1 macrophages. As such, the arginine catabolic pathway and recycling pathway have been implicated in dictating polarization and immune function of macrophages (53). Beyond this metabolic feature, the catabolism of GABA via the GABA shunt may play a critical role in channeling glutamate to the TCA cycle to provide succinate in M1 macrophages. Succinate, an anaplerotic substrate of the TCA cycle, may stabilize HIF1α and thus enhance its proinflammatory activity in M1 macrophages (16). In addition, genetic modulation of metabolic enzymes involved in glucose catabolism, such as PKM2, uPFK2, hexokinase (HK), and CARLK, significantly impacts on LPS-induced inflammatory immune responses in macrophages (21, 23–26). Collectively, these metabolic alterations enable the inflammatory functions of M1 macrophages. The manipulation of metabolic programs or their upstream regulatory signaling molecules can have a profound impact on the immune outcome.

## Experimental Procedures

Animal protocols were approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital, the Research Institute at Nationwide Children's Hospital, and Fudan University. The detailed procedures of the endotoxin-induced model of sepsis, bone marrow-derived macrophage (BMDM) generation, qPCR analysis, Western blot analysis, metabolic activity analysis, and statistical analysis are described in *SI Experimental Procedures*.

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