**Glycerol phosphate shuttle enzyme GPD2 regulates macrophage inflammatory responses**

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Macrophages are key regulators of innate immunity and inflammation during microbial infection. On detection of the gram-negative bacterial component LPS, macrophages are activated (henceforth LPS activation) to coordinate the induction of innate and adaptive immune responses by producing chemoattractants and inflammatory cytokines. However, in response to overwhelming infection or protracted exposure to LPS, macrophages are initially activated but then shift to a tolerant state (henceforth LPS tolerance). Tolerant macrophages mount a blunted response to further LPS challenge and produce markedly lower amounts of inflammatory cytokines that contribute to the induction of whole-body immunosuppression. Such immunosuppression limits the detrimental effects of sustained inflammation, which otherwise damages multiple organ systems and leads to mortality. Therefore, acute microbial infection elicits an inflammatory response that confers host defense, but persistent and overwhelming microbial infection triggers inflammation followed by a protracted period of tissue-protective immunosuppression.

Cellular metabolism is increasingly appreciated for its role in regulating macrophage activation and functions, as well as innate immunity and inflammation. Cellular metabolism interfaces with signal transduction and other regulatory processes, and genetic and pharmacological modulation of metabolism can influence macrophage activation and functions. In macrophages activated by interleukin-4 (IL-4), which orchestrate type 2 inflammatory responses like tissue repair and fibrosis, oxidative metabolism is increased to boost macrophage activation. For example, increased oxidative metabolism upregulates the production of citrate, a tricarboxylic acid (TCA) cycle intermediate, which is used by ATP-citrate lyase (ACLY) to generate a nucleocytosolic pool of acetyl coenzyme A (acetyl-CoA). Because acetyl-CoA is the metabolic substrate for histone acetylation, ACLY-dependent acetyl-CoA production links increased oxidative metabolism to enhanced expression of IL-4-inducible genes. In contrast, a metabolic hallmark of LPS-stimulated macrophages is a robust shutdown of oxidative metabolism; this limits the availability of acetyl coenzyme A for histone acetylation at genes encoding inflammatory mediators and thus contributes to the suppression of inflammatory responses. Therefore, GPD2 and the glycerol phosphate shuttle integrate the extent of microbial stimulation with glucose oxidation to balance the beneficial and detrimental effects of the inflammatory response.

Glycerol phosphate shuttle enzyme GPD2 regulates glucose oxidation to drive inflammatory responses. GPD2, a component of the glycerol phosphate shuttle, boosts glucose oxidation to fuel the production of acetyl coenzyme A, acetylation of histones and induction of genes encoding inflammatory mediators. While acute exposure to LPS drives macrophage activation, prolonged exposure to LPS triggers tolerance to LPS, where macrophages induce immunosuppression to limit the detrimental effects of sustained inflammation. The shift in the inflammatory response is modulated by GPD2, which coordinates a shutdown of oxidative metabolism; this limits the availability of acetyl coenzyme A for histone acetylation at genes encoding inflammatory mediators and thus contributes to the suppression of inflammatory responses. Therefore, GPD2 and the glycerol phosphate shuttle integrate the extent of microbial stimulation with glucose oxidation to balance the beneficial and detrimental effects of the inflammatory response.
of acetyl-CoA, thus contributing to the inability to induce *Il6* and *Il1b*. Such changes in glucose oxidation are regulated by GPD2, a rate-limiting enzyme in the glycerol phosphate shuttle (GPS). We propose that GPD2-mediated glucose oxidation has a unique role in integrating the duration and extent of microbial stimulation to the balance of inflammatory response induction and suppression.

**Results**  
**ACLY supports inflammatory gene induction during LPS activation.** In IL-4-stimulated macrophages, increased oxidative metabolism bolsters macrophage activation in part by supporting the activity of ACLY, which produces a nucleocytosolic pool of acetyl-CoA that can be used for histone acetylation. We investigated the role of ACLY in macrophages acutely exposed to LPS (0–3 h of LPS stimulation, throughout the study), during the period of LPS activation. LPS stimulation of bone marrow-derived macrophages (BMDMs) for 0.5–2 h increased phosphorylation of the Ser455 residue in ACLY (Fig. 1a); this is an activating phosphorylation, indicating that LPS signaling enhanced ACLY activation. Furthermore, treatment with the ACLY inhibitor SB 204990 attenuated LPS-inducible histone acetylation at the *Il6* and *Il1b* gene promoters (Fig. 1b), as well as LPS- and ACLY-inducible expression of *Il6* and *Il1b* messenger RNA, compared to no inhibitor (Fig. 1c). Two structurally distinct ACLY inhibitors, BMS-303141 and Medica16, similarly reduced LPS-induced histone acetylation and the expression of *Il6* and *Il1b* mRNA (Supplementary Fig. 1a,b), suggesting specificity in the effects of the ACLY inhibitors. Inhibition of p300 with the selective inhibitor C646 reduced LPS-inducible *Il6* and *Il1b* promoter histone acetylation and *Il6* and *Il1b* mRNA expression (Supplementary Fig. 1c,d), consistent with the idea that histone acetylation and histone acetyltransferases, including the p300-CBP coactivator family in particular, support LPS-inducible gene expression (11–13). These findings indicated that ACLY activity fueled histone acetylation and inflammatory gene induction in LPS-activated macrophages.

**Glucose oxidation fuels inflammatory gene induction in LPS activation.** Because ACLY-dependent production of acetyl-CoA depends on the availability of the TCA cycle intermediate citrate and oxidative metabolism (14, 15), that ACLY activity supported LPS-inducible inflammatory gene induction was in apparent conflict with the idea that LPS-stimulated macrophages shut down oxidative metabolism. Measurement of oxygen consumption indicated that a 1 h LPS stimulation of BMDMs triggered a small, but significant and highly reproducible increase in mitochondrial oxidative metabolism compared to no stimulation (Fig. 2a). Oxygen consumption began to decline after 2 h, followed by a sustained decrease in oxygen consumption below that of unstimulated BMDMs between 4 and 24 h (Supplementary Fig. 2a and data not shown), probably explaining previous conclusions that LPS-stimulated macrophages shut down oxidative metabolism. Because LPS stimulation enhances glucose use (Supplementary Fig. 2b and ref. 16), we tested if the increase in oxidative metabolism after 1 h of LPS stimulation could be fueled by glucose oxidation. Indeed, treatment with the glucose use inhibitor 2-deoxyglucose (2DG) abrogated such increase in oxidative metabolism, compared to no treatment (Fig. 2a).

To further test whether glucose oxidation supports acetyl-CoA production and inflammatory gene induction in LPS-activated BMDMs, we performed glucose isotope tracing using uniformly 13C-labeled glucose. LPS stimulation for 1.5–3 h enhanced the labeling of glucose-derived carbons into m + 2 citrate-isocitrate and m + 2 acetyl-CoA compared to no stimulation (Fig. 2b,c), indicating that glucose oxidation fueled acetyl-CoA production during LPS activation. Tracing glucose carbons all the way into histones indicated increased labeling of histones in LPS-stimulated BMDMs compared to no stimulation (Fig. 2d). 2DG treatment attenuated LPS-inducible histone acetylation at the promoters of *Il6* and *Il1b* (Fig. 2c) and expression of *Il6* and *Il1b* mRNA (Fig. 2f) compared to no 2DG treatment, indicating that glucose metabolism supported histone acetylation. 2DG reduced the production of IL-6 in LPS-activated BMDMs (Fig. 2g), while production of mature IL-1β protein, which requires inflammasome activation in BMDMs (17), was examined no further. Incubation of BMDMs in glucose-free media also led to diminished induction of *Il6* and *Il1b* histone acetylation and mRNA expression (Supplementary Fig. 2c,d). These analyses suggested that availability of acetyl-CoA can be limiting for histone acetylation and inflammatory gene induction during LPS activation, and that LPS stimulation boosts glucose oxidation, leading to increased acetyl-CoA production, histone acetylation and inflammatory gene induction.

**GPD2 regulates glucose use and oxidation in LPS activation.** During the cytosolic phase of glucose oxidation, glucose-derived electrons are transferred to nicotinamide adenine dinucleotide (NAD⁺) at the step catalyzed by glyceraldehyde-3-phosphate...
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which were viable and fertile and appeared relatively normal (data not shown). Gpd2−/− BMDMs had normal expression of CD11b and F4/80 (Supplementary Fig. 3d), but lacked GPD2 protein and activity as determined by immunoblotting (Fig. 3d) and Seahorse extracellular flux analysis of G3P-driven oxygen consumption (Fig. 3e).

In glucose tracing experiments, 1.5–3 h LPS stimulation of wild-type (WT; Gpd2+/+) BMDMs led to a marked increase in the abundance of total and m+3 G3P (Fig. 3f), indicative of enhanced flux through the GPS, while in Gpd2−/− BMDMs, the abundance of total and m+3 G3P was further increased (Fig. 3f), which is consistent with the accumulation of the GPD2 substrate in the absence of GPD2 activity. The 2DG-sensitive, LPS-inducible increase in oxidative metabolism apparent in WT BMDMs (Fig. 2a and Fig. 3g) was diminished in Gpd2−/− BMDMs (Fig. 3g), indicating a role for GPD2 in mediating glucose oxidation in LPS-activated macrophages. Importantly, the ability of LPS to augment glucose uptake was impaired in Gpd2−/− BMDMs compared to WT BMDMs (Fig. 3h), indicating that GPD2 activity is rate-limiting for glucose use in LPS-activated macrophages. Compared to WT BMDMs, Gpd2−/− BMDMs had less LPS-inducible glucose labeling into many glycolytic and TCA intermediates (Supplementary Fig. 4) and aerobic glycolysis (Fig. 3i), had less LPS-inducible glucose labeling into many glycolytic and TCA intermediates (Supplementary Fig. 4) and aerobic glycolysis (Fig. 3i), indicating a role for GPD2 in mediating glucose oxidation in LPS-activated macrophages. Importantly, the ability of LPS to augment glucose uptake was impaired in Gpd2−/− BMDMs compared to WT BMDMs (Fig. 3h), indicating that GPD2 activity is rate-limiting for glucose use in LPS-activated macrophages. Compared to WT BMDMs, Gpd2−/− BMDMs had less LPS-inducible glucose labeling into many glycolytic and TCA intermediates (Supplementary Fig. 4) and aerobic glycolysis (Fig. 3i), in line with reduced LPS-inducible glucose use and oxidation. These observations indicated that the GPS was a critical regulator of glucose use and oxidation in LPS-activated macrophages.

**Impaired inflammatory gene induction in LPS-activated Gpd2−/− BMDMs.** We next investigated how reduced glucose use and oxidation in Gpd2−/− BMDMs affected LPS activation. LPS-inducible dehydrogenase (GAPDH). The malate-aspartate shuttle (MAS) is thought to be ubiquitously deployed during glucose oxidation to transfer reduced nicotinamide adenine dinucleotide (NADH) to complex I of the ETC while regenerating cytosolic NADH2. However, the ability of glucose carbons to label histones within 1.5 h of labeling (Fig. 2d) relative to steady-state histone labeling in other contexts (approximately 24 h)23,24, suggested an alternative mechanism for rapidly mobilizing glucose oxidation in LPS-activated macrophages.

By applying global metabolomics to unstimulated and LPS-stimulated BMDMs, we identified the GPS, a glycolytic shunt that delivers glucose-derived electrons to the ETC, as a top metabolic pathway regulated during LPS activation (Fig. 3a). In the GPS, the flux of glucose carbons to histones is diminished in Gpd2−/− BMDMs compared to WT BMDMs (Fig. 3g), indicating a role for GPD2 in mediating glucose oxidation in LPS-activated macrophages. Importantly, the ability of LPS to augment glucose uptake was impaired in Gpd2−/− BMDMs compared to WT BMDMs (Fig. 3h), indicating that GPD2 activity is rate-limiting for glucose use in LPS-activated macrophages. Compared to WT BMDMs, Gpd2−/− BMDMs had less LPS-inducible glucose labeling into many glycolytic and TCA intermediates (Supplementary Fig. 4) and aerobic glycolysis (Fig. 3i), in line with reduced LPS-inducible glucose use and oxidation. These observations indicated that the GPS was a critical regulator of glucose use and oxidation in LPS-activated macrophages.

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Fig. 3 | The GPD2 enzyme GPD2 regulates glucose oxidation in LPS-activated macrophages. a, Global steady-state metabolite profiling of BMDMs, unstimulated or LPS-stimulated for 3 h (n = 4). The enriched pathways are shown and are ranked according to P value. b, Schematic depicting the spatial and biochemical position of the GPD2 at the nexus between glycolysis and electron transport. c, qPCR analysis of GPD2 gene expression in BMDMs stimulated with LPS for 1.5 h. d, Immunoblot analysis of GPD2 in unstimulated WT and Gpd2−/− BMDMs. e, Seahorse extracellular flux analysis of OCR in permeabilized WT and Gpd2−/− BMDMs (n = 6). f, Bar graph shows the fold change in OCR after G/R/A injection. g, 13C6-glucose tracing into glycerol 3-phosphate (presented as peak areas for each isotopologue) in WT and Gpd2−/− BMDMs (n = 3). h, Seahorse analysis of basal and maximal mitochondrial OCR in WT and Gpd2−/− BMDMs, unstimulated or stimulated with LPS for 1 h (n = 6). i, Uptake of 2-13C deoxy-d-glucose in WT and Gpd2−/− BMDMs stimulated with LPS for the indicated times (n = 3). j, Seahorse analysis of ECAR in WT and Gpd2−/− BMDMs stimulated with LPS for 0–2 h (n = 6). Data are from one experiment representative of two (a), three (c,d,f,i) or four (e) independent experiments. The mean (c,e-i) ± s.e.m. (e-i) are shown. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (two-tailed Student’s t-test).

labeling of glucose carbons into citrate and acetyl-CoA was reduced in Gpd2−/− BMDMs compared to WT BMDMs (Fig. 4a,b). LPS-inducible histone acetylation at the Il6 and Il1b promoters was also attenuated in Gpd2−/− BMDMs (Fig. 4c), leading to diminished Il6 and Il1b mRNA expression (Fig. 4d) and IL-6 production (Fig. 4e), compared to WT BMDMs. Together, these findings indicated that in LPS-activated macrophages, GPD2 activity boosted glucose oxidation, leading to enhanced carbon substrate availability for histone acetylation and increased inflammatory gene induction (Supplementary Fig. 5a).

Glucose use suppresses inflammatory gene induction in LPS tolerance. During the course of LPS exposure, there is a transition from LPS activation to tolerance10. Acute LPS stimulation (0–3 h) of BMDMs led to the induction of Il6 and Il1b mRNA during the period of LPS activation, while prolonged LPS stimulation (12–24 h) led to an inability to induce Il6 and Il1b mRNA after a second LPS challenge during the period of LPS tolerance (Supplementary Fig. 6a,b; see refs. 34). Our data indicated that such shift from inflammatory response induction to suppression was accompanied by a shift from increased to decreased oxidative metabolism (Supplementary Fig. 2a). Because increased oxidative metabolism supports inflammatory gene induction during LPS activation, we tested whether the decrease in oxidative metabolism in LPS-tolerant BMDMs limited the availability of acetyl-CoA for histone acetylation and contributed suppression of inflammatory gene induction. In contrast to naïve BMDMs, where 3 h LPS stimulation induced glucose conversion into citrate and acetyl-CoA, LPS did not drive glucose conversion into citrate and acetyl-CoA in BMDMs tolerized by 24 h LPS stimulation (Fig. 5a,b), suggesting reduced LPS-induced glucose oxidation and acetyl-CoA production in tolerant macrophages.

Serendipitously, we found that treating BMDMs with 2DG during a 12 h LPS tolerance induction led to a rescue of oxygen consumption compared to no 2DG treatment (Fig. 5c). 2DG treatment during 24 h LPS tolerance induction was also associated with recovery of Il6 and Il1b promoter histone acetylation, Il6 and Il1b mRNA expression and IL-6 production compared to no 2DG
GPD2 inhibitor iGP-1 attenuated the ability of acute LPS stimulation, tolerance-associated inhibition of oxygen consumption was attenuated compared to their WT counterparts (Fig. 5e–f).

We next investigated the mechanisms by which GPD2-GPS activity mediated glucose oxidation during LPS stimulation (Supplementary Fig. 7a–c), but rescued the tolerance-associated shutdown of oxidative metabolism and suppression of Il6 and Il1b mRNA expression triggered by prolonged LPS stimulation (12–24 h) (Supplementary Fig. 7d–f), compared to no inhibitor treatment. Of note, LPS-inducible degradation and resynthesis of IκBα and activating phosphorylation of IRF3 in naïve ortolerized Gpd2−/−BMDMs was similar to that of their WT counterparts (Fig. 6e,f). Therefore, GPD2 did not exert broad effects on LPS signaling to influence histone acetylation and inflammatory gene induction during LPS stimulation.

GPD2 regulates RET in tolerized macrophages. We next investigated a potential role for GPD2-mediated glucose oxidation in regulating RET during LPS tolerance. Multiple factors are thought to modulate such shutdown, including induction of inducible nitric oxide synthase, IRG1 and RET12–15, as well as repression of the TCA cycle enzyme isocitrate dehydrogenase-1, which allows carbon flux to be shunted from the TCA cycle toward isocitrate production. Over a time course of LPS stimulation, WT and Gpd2−/−BMDMs had similar expression of Nos2, lir1 (Acod1) and Idh1 mRNA (Supplementary Fig. 8a) and levels of itaconate (Supplementary Fig. 8b).

We next investigated a potential role for GPD2-mediated glucose oxidation in regulating RET during LPS tolerance. In Gpd2−/−BMDMs, stimulated with LPS for 0–3 h, treatment with the GPD2 inhibitor JIG-1 attenuated the ability of acute LPS stimulation (0–3 h) to induce oxidative metabolism, Il6 and Il1b promoter histone acetylation and Il6 and Il1b mRNA expression during LPS activation (Supplementary Fig. 7a–c), but rescued the tolerance-associated shutdown of oxidative metabolism and suppression of Il6 and Il1b promoter histone acetylation and Il6 and Il1b mRNA expression triggered by prolonged LPS stimulation (12–24 h) (Supplementary Fig. 7d–f), compared to no inhibitor treatment. Of note, LPS-inducible degradation and resynthesis of IκBα and activating phosphorylation of IRF3 in naïve or tolerized Gpd2−/−BMDMs was similar to that of their WT counterparts (Fig. 6e,f). Therefore, GPD2 did not exert broad effects on LPS signaling to influence histone acetylation and inflammatory gene induction during LPS stimulation.

**Fig. 4** GPD2 activity influences inflammatory gene induction in LPS-activated macrophages by regulating acetyl-CoA production and histone acetylation. a,b, 13C6-glucose tracing into citrate-isocitrate (n = 3) (a) and acetyl-CoA (n = 4) (b) in WT and Gpd2−/− BMDMs, stimulated with LPS for 0–1 h, presented as LPS-induced fold change in percentage m + 2 enrichment. c, ChIP-qPCR analysis of histone acetylation in Il6 and Il1b promoter regions in WT and Gpd2−/−BMDMs stimulated with LPS for 0–1 h (n = 3). d, qPCR analysis of Il6 and Il1b gene expression in WT and Gpd2−/− BMDMs stimulated with LPS for 0–3 h (n = 2). e, ELISA for IL-6 production in culture supernatants of WT and Gpd2−/− BMDMs stimulated as in d (n = 4). Data are from one experiment representative of three (a,b) or four (c–e) independent experiments. The mean (a–e) = s.e.m. (a,b,e) are shown. ***P ≤ 0.001, ****P ≤ 0.0001 (two-tailed Student’s t-test).
While rotenone treatment increased the levels of superoxide in WT unstimulated BMDMs, it decreased the levels of superoxide in WT BMDMs tolerized by 12 h LPS stimulation (Fig. 7a,b), indicating that RET was induced after prolonged LPS exposure. The ability of rotenone to reduce superoxide was diminished in that RET was induced after prolonged LPS exposure. The ability of rotenone treatment increased the levels of superoxide in WT unstimulated BMDMs, it decreased the levels of superoxide in WT unstimulated BMDMs, which is indicative of robust FET, and modestly enhanced NADH in WT BMDMs tolerized by 12 h LPS stimulation (Fig. 7c,d), which is indicative of reduced FET and/or increased RET because the net NADH amount is determined by the relative magnitudes of FET and RET. However, rotenone did not differentially modulate NADH levels between unstimulated and tolerized Gpd2−/− BMDMs or between 2DG-treated and untreated WT tolerized BMDMs (Fig. 7c,d), indicating that GPD2 deficiency could lead to a recovery of oxidative metabolism, acetyl-CoA production, histone acetylation and inflammatory gene induction in tolerized macrophages. Therefore, GPS activity supports inflammatory response induction during acute LPS exposure and inflammatory response suppression during prolonged LPS exposure, acting as a rheostat to link the duration of LPS exposure to the balance of inflammatory response induction and suppression (Supplementary Fig. 5b).
GPD2 activity suppresses inflammatory responses during septic shock. To ask whether GPD2 regulated inflammatory responses in vivo, we used a model of in vivo LPS tolerance, where a sublethal dose of LPS protects against septic shock triggered by a subsequent lethal dose of LPS. In contrast to counterparts that did not receive the sublethal LPS pretreatment, WT mice receiving the pretreatment markedly suppressed circulating amounts of IL-6, as detected 6h after lethal LPS challenge (Fig. 8a). Compared to their WT counterparts, Gpd2−/− mice receiving the pretreatment had increased circulating amounts of IL-6, indicating attenuated suppression of inflammatory responses (Fig. 8a). Furthermore, the drop in body temperature induced 6h after lethal LPS challenge was exaggerated in Gpd2−/− mice compared to WT mice (Fig. 8b), indicating an augmented response to lethal LPS challenge. Finally, WT mice that received a sublethal LPS pretreatment survived the subsequent lethal LPS challenge, while Gpd2−/− mice succumbed (Fig. 8c), suggesting a profound defect in LPS tolerance and immunosuppression in the absence of GPD2. Collectively, the data indicate that GPD2 suppressed inflammatory responses to protect against a drop in body temperature and mortality during septic shock.

Discussion

In this study, we made several findings regarding the role of glucose use in controlling the fate of macrophages exposed to LPS. We showed that oxidative metabolism was increased in LPS-activated BMDMs, fueling the production of acetyl-CoA to provide carbon substrate for histone acetylation at the Il6 and Il1b promoters. Our findings define a previously unknown role for glucose use in LPS-stimulated macrophages—namely glucose oxidation—as well as an underpinning basis by which such glucose use drove LPS activation. We also showed that the acetyl-CoA-producing enzyme ACLY was
We identified GPD2 and the GPS as key regulators of glucose oxidation in LPS-stimulated macrophages. Like the MAS, the GPS generates cytosolic NAD⁺ to maintain redox balance during glucose oxidation; unlike the MAS, GPS activity is restricted to a limited number of tissues because GPD2 is expressed in a tissue-specific manner. The role of GPD2 in macrophages is unknown, except that its biochemical activity is increased after microbial infection. Here we showed that LPS stimulation increased flux through the GPS by augmenting glucose use (which stimulates substrate delivery to the GPS), GPD2 expression and the intrinsic activity of GPD2. Such increase of GPS flux is noteworthy given that macrophages can deploy the MAS to maintain glucose oxidation, and suggests a nonredundant role for the GPS in regulating macrophage responses to LPS.

Although shutdown of oxidative metabolism is a metabolic hallmark of LPS-stimulated macrophages, its role in modulating macrophage responses to LPS has been unclear. Here we showed that such shutdown occurred at 12–24 h after LPS stimulation, after the initial increase in oxidative metabolism, and contributed to reduced acetyl-CoA production, histone acetylation and inflammatory gene induction in tolerant macrophages. We propose that shutdown of oxidative metabolism is central to metabolic reprogramming during LPS tolerance, while induction of aerobic glycolysis is presumably a compensatory mechanism to support cellular bioenergetics (that is, ATP production) in the face of oxidative metabolism shutdown.

We found that GPD2 and the GPS were important regulators of tolerance-associated shutdown of oxidative metabolism. Inhibiting glucose use or GPD2 deficiency was sufficient for partial restoration of oxidative metabolism, leading to a partial recovery of histone acetylation and inflammatory gene induction in tolerant macrophages.
as well as increased IL-6 production and mortality during septic shock. We propose a model where GPS activity influences oxidative metabolism to modulate a shift from inflammatory response induction to suppression. During acute LPS exposure, GPS activity drives FET and acetyl-CoA production to enhance histone acetylation and inflammatory gene induction; however, during prolonged LPS exposure, sustained GPS activity eventually saturates electron transport capacity to trigger RET and limit acetyl-CoA production for histone acetylation and inflammatory gene induction. Therefore, GPS activity triggered RET as a consequence of its initial boost of FET, allowing the GPS to integrate the duration of LPS exposure with the activity of the ETC. In this manner, GPS activity supported inflammatory responses after acute microbial exposure, but contributed to inflammatory response suppression after prolonged microbial exposure. Inflammation confers host defense at the expense of tissue damage and must be carefully regulated to balance its beneficial and detrimental effects; GPS activity, by integrating the extent of microbial exposure to inflammatory response induction versus suppression, may provide one such balancing mechanism.

Our detailed metabolic analyses implicated histone acetylation at inflammatory genes as a key target by which GDP2 regulates inflammatory gene expression. In contrast, we found no broad effects of GDP2 deficiency on LPS-inducible nuclear factor kappa B and IR3 activation, or LPS-inducible gene expression as assessed by RNA sequencing (data not shown). However, we do not exclude the possibility that GDP2 activity impinges on specific aspects of LPS signaling to contribute to its regulation of inflammatory gene expression. For example, GDP2-mediated glucose oxidation could influence the expression of specific signaling proteins, via effects on promoter histone acetylation and protein acetylation, an effect nonexclusive with its modulation of histone acetylation at inflammatory genes. Additionally, how GDP2-mediated glucose oxidation and acetyl-CoA production can modulate histone acetylation and the expression of a subset of LPS-inducible genes is unclear. One possibility is that ACLY is recruited to chromatin, producing a local pool of acetyl-CoA that regulates histone acetylation in a gene-specific manner. Precedence for such gene-specific association and regulation has been established by the metabolic machinery that produces S-adenosyl-L-methionine, the substrate for histone and DNA methylation.

Our findings indicate that increased oxidative metabolism is a common feature of LPS- and IL-4-activated macrophages, and that one consequence of this process is enhanced provision of acetyl-CoA, which is otherwise limiting for histone acetylation and inducible gene expression. Therefore, polarizing signals like LPS and IL-4 mobilize oxidative metabolism to drive optimal gene induction and macrophage activation. In the case of LPS-stimulated macrophages, this process is further exploited to eventually influence oxidative metabolism shutdown and inflammatory gene suppression. In this regard, our conclusions appear in conflict with those of a recent study showing that RET-associated reactive oxygen species production drives Il1b induction. However, that study did not perform a kinetic analysis of LPS stimulation. In our study, we examined glucose oxidation, oxidative metabolism, FET/RET and inflammatory gene induction or suppression over a time course, allowing us to address how dynamic changes to oxidative metabolism and FET/RET could affect the dynamic changes to inflammatory gene induction or suppression that occur over the course of LPS exposure.

In conclusion, we propose that GDP2-GPS-mediated glucose oxidation has a unique, nonredundant role in optional control of inflammatory gene induction and suppression. Compared with the MAS, GPS activity may allow for rapid and robust glucose oxidation that drives ETC flux and inflammatory gene expression during LPS activation, but in the event of sustained LPS exposure, contributes to RET induction. Therefore, deployment of the GPS in the high metabolic state of LPS-stimulated macrophages enables GDP2 activity to act as a rheostat that couples the duration of LPS exposure to ETC directionality to enable appropriate modulation of inflammatory response induction and suppression.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at [https://doi.org/10.1038/s41590-019-0453-7](https://doi.org/10.1038/s41590-019-0453-7).

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Author contributions

P.K.L. designed and performed the experiments and analyzed the data. J.J., A.N., M.S., H.I.A. and J.L. performed the experiments and analyzed the data. P.K.L. prepared the figures. P.K.L. and T.H. wrote the manuscript. T.H. supervised the project, including the experimental design and data analysis. R.X., M.T.D., H.J., N.W.S., X.G. and J.W.L. performed the metabolite profiling and provided related technical expertise. M.R.M. and Y.K. helped with the data analysis. E.T.C. provided technical expertise.

Competing interests

The authors declare no competing interests.

Additional information

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**Methods**

**BMDM culture.** BMDMs from male and female mice were differentiated and cultured as described previously. For LPS activation (0–3 h post-LPS), BMDMs were stimulated with 100 ng/ml LPS (LPS Ultra Pure; Sigma-Aldrich). All of the metabolic parameters associated with LPS activation, including glucose uptake and oxidation, glucose tracing into TCA intermediates and acetyl-CoA, ACLY phosphorylation, glucose labeling into histones and promoter histone acetylation, were assessed at early time points after LPS stimulation (0–3 h), while the downstream consequences of such metabolic events, that is, inflammatory gene induction and cytokine production, were assessed 3 h after LPS stimulation. For downstream consequences of such metabolic events, that is, inflammatory gene expression, were assessed at early time points after LPS stimulation (0–3 h), while the downstream consequences of such metabolic events, that is, inflammatory gene induction and cytokine production, were assessed 3 h after LPS stimulation.

For LPS tolerance (12–28 h post-LPS), BMDMs were challenged with 100 ng/ml LPS for 24 h to induce tolerance, washed and then rechallenged with 10 ng/ml LPS for the desired amount of time. In such experiments, unstimulated ( naïve) BMDMs were incubated in media for 24 h, washed and challenged with 10 ng/ml LPS to provide responsive cell controls. Pharmacological inhibition of ACLY (80–160 µM; SB 203906; Tox or Bio), GPD2 (300 µM; Mitochondrial GPDH Inhibitor, IGP; Calbiochem) were achieved by pretreating BMDMs with inhibitors for 1 h before LPS activation. For rescue of tolerance, BMDMs were pretreated for 1 h before activation, but drugs were not added back after washing before restimulation. Additional drugs include the ACLY inhibitors BMS-303141 and Medicinal and the p38 inhibitor Cs64.

**Seahorse assays.** Basal and LPS-induced changes in oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured with a Seahorse XF96 Extracellular Flux Analyzer (Agilent Technologies). For experiments in intact cells, assays were performed in Seahorse XF Assay Medium (Agilent Technologies) supplemented with 2 µCi/ml [3H]-labeled glucose (pH 7.4 with NaOH) and 800 µM ATP, and wait and measure durations were set to 2, 3 and 3 min, respectively. For the mitochondrial stress test, oligomycin, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) and rotenone/antimycin A were sequentially injected to achieve final concentrations of 1, 1.5 and 2 µM. Glycolysis tests were performed in unstimulated XF Assay Medium; 2 g/L glucose from Glucose-free USM and 2-DG were sequentially injected to achieve final concentrations of 11 mM, 1 µM and 500 mM, respectively. Non-mitochondrial OCR was subtracted from the mean pre-oligomycin OCR and the mean post-FCCP OCR to calculate basal and maximal mitochondrial OCR, respectively. For experiments in permeabilized cells, assays were performed in mannose sucrose buffer supplemented with 0.1% BSA and adjusted to pH 7.2 with KOH (MAS-BSA buffer). Cells were permeabilized by injection of perfringolysin O (Plasma Membrane Permeabilizer reagent; Agilent Technologies) followed by 4 µl ice-cold 10% trichloroacetic acid and extracted.

**Steady-state metabolism and relative metabolic flux.** For steady-state analysis of metabolic networks, BMDMs were stimulated for the desired period of time and then lysed and polar metabolites extracted using 80:20 methanol:water (HPLC Grade Solvent; Sigma-Aldrich). Extracts were dried under nitrogen gas followed by liquid chromatography–mass spectrometry (Beth Israel Deaconess Medical Center Mass Spectrometry Facility) and analyzed using a library of 300 species, including those involved in glycolysis, the GPS and the TCA cycle, with 13C-glucose followed by acid extraction of acetylated histones. Radioisotope incorporation was assessed in technical duplicates by scintillation counting and normalized to total protein concentration.

**Glucose uptake.** Basal and LPS-inducible glucose uptake in BMDMs was measured using [2-3H]deoxy-o-glucose (PerkinElmer) in Krebs–Ringer bicarbonate-HEPES buffer. Counts per minute (cpm) were measured in triplicate cell cultures and then normalized to protein concentration in each sample.

**NADH analysis.** For NADH autofluorescence, BMDMs were excited at 340/26 nm on an Eclipse Ti-S microscope (Nikon). The emitted signal was collected using a 460/80 nm band-pass filter. Autofluorescence was analyzed for at least 100 cells per condition, per time point using the Fiji image processing software v.2.0. Duplicate cell cultures were analyzed for each condition tested.

**Chromatin immunoprecipitation (ChiP).** Acetylation at inflammatory gene promoters was measured by ChiP assay. Promoters as defined as the region between 500 base pairs and the transcription start site. The antibodies used were as follows: anti-acetyl-histone H3 (catalog no. 06-599; Merck Millipore); anti-histone H3 (acetyl K27) (catalog no. ab4729; Abcam); anti-acetyl-histone H4 (catalog no. 06-866; Merck Millipore); and IgG (catalog no. sc-2027; Santa Cruz Biotechnology). Fold enrichment was calculated as ChiP signals normalized to input. Technical triplicates were analyzed for each condition tested.

**Gene expression.** RNA was isolated from BMDMs using RNA-Beew (Tel-Test) according to the manufacturer’s protocol. Complementary DNA synthesis was performed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR (qPCR) was run on a C1000 Thermal Cycler (Bio-Rad Laboratories) and expression of target genes was calculated by the 2−ΔΔCT method using the CFX Manager software v.2.1 (Bio-Rad Laboratories). The relative expression values for target genes were calculated by normalization to the expression of hypoxanthine phosphoribosyltransferase. Duplicate cell cultures were analyzed for each condition tested.

**Generation of Gpd2−/− mice.** Gpd2−/− mice were generated on the C57BL/6J background using a guide RNA targeting exon 6 of mGpd2 (oligo 1: CACCGGC TACCGTCAATGAGACA; oligo 2: CGGATGCAGTATCTGTTCAAAA); gRNA and Cas9 RNA were injected into eggs followed by implantation into recipient female mice at the BWH Transgenic Mouse Core. Mice were backcrossed twice to C57BL/6J mice. BMDMs from two independently generated lines were used with similar results.

**Mice.** WT C57BL/6J mice (The Jackson Laboratory) and whole-body Gpd2−/− mice were maintained under specific pathogen-free conditions at the Harvard TH. Chan School of Public Health in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines. Male and female mice between 6 and 12 weeks of age were used for in vivo LPS tolerance experiments and as a source of bone marrow for BMDM cultures. All protocols were approved by the LAI, Harvard Medical School and the BWH IACUC. Mice were injected with vehicle (saline) or 3 mg kg−1 LPS via intraperitoneal injection followed by 30 mg kg−1 LPS intraperitoneally 24 h later. Serum IL-6 and internal body temperature (TH-5 Thermalert Clinical Monitoring Thermometer; Physitemp) were measured for 6 h after lethal LPS challenge. Survival of WT and Gpd2−/− mice that received both challenges was recorded as an indication of differential endotoxin tolerance in vivo.

**Immuno blot and ELISA.** For immunoblotting, BMDMs were lysed in 1% NP-40 or radioimmunoprecipitation assay buffer; protein concentration was determined using the Bradford method or Micro BCA Protein Assay Kit (Pierce). Primary antibodies were used anti-phospho–ACLY-S455 (1:1,000, Cell Signaling Technology, cat# 4331) and anti-ACLY (1:1,000, Cell Signaling Technology, cat# 4332), anti-GPD2 (1:800; Proteintech, cat# 17219-1-AP), anti-ICB (1:1,000; Santa Cruz Biotechnology, SC-371), anti-phospho-IRF3 (1:1,000, Cell Signaling Technology, cat# 4947) and anti-a-ubulin (1:5,000; Sigma-Aldrich, cat# T6074). For ELISA, cell culture supernatants and mouse whole blood were spun down (500 at 400 g and 20 min at 13,000 g), diluted appropriately in 5% BSA and assayed for IL-6 using standard ELISA kits (BioLegend) according to the manufacturer’s protocols. For the in vitro LPS tolerance experiments, cell culture supernatants in the unstimulated (tolerized) and LPS-stimulated (tolerized) conditions were collected after LPS washout and rest or restimulation for 4 h (refer to Fig. 5c, time of collection indicated by ‘asay’), allowing for the measurement of IL-6 produced during tolerance rather than activation. Supernatants were analyzed from duplicate or quadruplicate cell cultures, indicated by n in the figure legends.

**Flow cytometry.** Efficiency of macrophage differentiation from bone marrow–derived progenitors was determined by staining day 7 BMDMs with anti-F4/80 (clone BM8) and anti-CD11b (clone M1/70) antibodies (BD Biosciences) for 30 min at 4°C in fluorescence-activated cell sorting buffer (2% FCS), followed by washes and fixation in 2% Ultra Pure paraformaldehyde. Mitochondrial superoxide
was measured by MitoSOX Red Mitochondrial Superoxide Indicator (Thermo Fisher Scientific) according to the manufacturer’s protocol. Briefly, BMDMs were left unstimulated or stimulated with LPS for the indicated times and then labeled with 2.5 μM MitoSOX Red in the final 15 min of treatment, followed by washout and no fixation. The mean fluorescence intensity of MitoSOX in the phycoerythrin channel was measured to determine levels of mitochondrial superoxide at the time of labeling. Duplicate cell culture samples were run for each condition in every experiment. All samples were acquired on a FACS Fortessa flow cytometer (BD Biosciences); the FlowJo software v.10.4 (FlowJo LLC) was used to analyze the data.

**Statistical analysis.** Data are shown as the mean ± s.e.m. or s.d., as indicated in the figure legends. Statistical significance was determined using a two-tailed Student’s t-test, two-way analysis of variance (ANOVA) with Sidak’s multiple comparisons test or Mantel–Cox test using Prism v.7.0a (GraphPad Software), as indicated in the figure legends. All statistical tests were performed using Prism, except for the pathway enrichment analysis of steady-state metabolite profiling, for which MetaboAnalyst was used to generate the statistics. * P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001 and **** P ≤ 0.0001, respectively.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**
The data supporting the findings of this study are available from the corresponding author upon request.

**References**
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
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  Give P values as exact values whenever possible.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Software and code

Policy information about availability of computer code

- BDFACS Diva and Bio-Rad CFX Manager software were used for collecting FACS and qPCR data, respectively.
- Seahorse XF96 Extracellular Flux Analyzer software was used for determining rates of oxygen consumption and extracellular acidification.
- NAD[P]H autofluorescence traces were collected using NIS-Elements AR Analysis software.

- FlowJo v10.4 and Bio-Rad CFX Manager were used for analyzing FACS and qPCR data, respectively.
- Seahorse data was analyzed using Wave.
- NAD[P]H autofluorescence data was analyzed using Fiji image processing software.
- Metaboanalyst 2.0 and FluxFix were used for analyzing steady-state and substrate tracing metabolomics data.
- Final graphs were made in Graphpad Prism v7, and figures were arranged and edited in Adobe Illustrator 12.0.0.
- Schematics were made using a combination of Microsoft PowerPoint and Adobe Illustrator.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
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- A list of figures that have associated raw data
- A description of any restrictions on data availability

There are no restrictions on data availability.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-list.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For Seahorse analysis in BMDMs, we used n=6-8 because we have seen that this sample size is needed in most cases to account for inability to use replicates because of machine error or plate edge effects and observe a significant difference. For most other studies in BMDMs, statistical methods were not used to predetermine sample size; rather, we used n=2 or n=3 because we have seen that these sample sizes make in vitro experiments sufficiently powered to observe reproducible differences. For mice experiments, sample size calculation was made using G*Power based on differences in cytokine levels seen in the in vivo BMDM experiments; t-tests of our data confirmed a significant difference.

Data exclusions

No data were excluded.

Replication

Data reproducibility was confirmed by three independent experiments. All data produced from successfully executed experiments were reproducible across time and between lab personnel.

Randomization

Animals were randomly assigned to different experimental groups.

Blinding

Blinding was not specifically introduced into our experimental design because in most cases the same investigator was responsible for the entirety of the experiment. However, most of our experiments have a quantitative end-point so subconscious bias is not an issue.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a Involved in the study
☐ ☒ Antibodies
☒ ☒ Eukaryotic cell lines
☐ ☒ Palaeontology
☒ ☒ Animals and other organisms
☒ ☒ Human research participants
☑ Clinical data

Methods

n/a Involved in the study
☒ ChiP-seq
☒ Flow cytometry
☒ MRI-based neuroimaging

Antibodies

Antibodies used

For Immunoblot analysis:

Total ACLY (Cell Signaling, cat #: 4332) 1:1000
p-ACLY S455 (Cell signaling, cat# 4331) 1:1000
GPD2 (Proteintech, cat # 17219-1-AP) 1:800
alpha-Tubulin (Sigma-Aldrich, cat # T6074) 1:5000
IkB (Santa Cruz, SC-371) 1:1000
pRF3 (Cell Signaling, cat#4947) 1:1000

For ChIP:

acetylated H3 (Millipore 06-599)
acetylated H3K27 (Abcam ab4729)
acetylated H4 (Millipore 06-866)
IgG (Santa Cruz, SC-2077)

For FACS:

F4/80 (Biolegend, cat #: 123108, clone # BM8, lot #, B222019) 1:100
CD11b (BD Biosciences, cat # 557397, clone # M1/70, lot # 66680) 1:100
Validation

All antibodies have been validated for the species and application by the vendor and have been published on previously. All antibodies had validation statement provided on the website of the manufacturer.

Animals and other organisms

Policy information about studies involving animals, ARRIVE guidelines recommended for reporting animal research

<table>
<thead>
<tr>
<th>Laboratory animals</th>
<th>Male and female C57BL/6J mice and Gpd2-/- mice between 6 weeks to 4 months of age were used.</th>
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<tbody>
<tr>
<td>Wild animals</td>
<td>No wild animals were used.</td>
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<tr>
<td>Field-collected samples</td>
<td>Samples were not collected from the field.</td>
</tr>
<tr>
<td>Ethics oversight</td>
<td>Wild-type C57BL/6J mice (The Jackson Laboratory) and whole-body Gpd2-/- (KO) mice were maintained under specific pathogen-free conditions at the Harvard T.H. Chan School of Public Health in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. Male and female mice were used for in vivo LPS tolerance experiments and as a source of bone marrow for BMDM culture between 8 and 12 weeks of age. All protocols were approved by the IACUC of Harvard Medical School.</td>
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Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
☐ All plots are contour plots with outliers or pseudocolor plots.
☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

BMDMS were counted and plated at 1x10^6 cells per stain for the F4/80 and CD11b staining, and plated at 0.5x10^6 cells per stain for the MitoSox staining. For F4/80 and CD11b staining, cells were stained for 30 minutes in FACS buffer on ice protected from light with both antibodies at 1:1000 dilution. After incubation cells were washed with FACS buffer 2ce and fixed with 2% PFA. For MitoSox staining, 2.5 μM MitoSox was added to cells in culture for 15 minutes followed by one wash in PBS and collection in PBS. Cells were acquired without fixation.

Instrument

BD FACS Fortessa, model no. 649225

Software

FACS Diva for acquisition and FlowJo for analysis

Cell population abundance

No sorting was done.

Gating strategy

For F4/80 and CD11b staining, F4/80+CD11b+ cells were gated of FSC/SSC. For MitoSox staining, histograms were generated based on FSC/SSC and plotted as PE MFI.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.