Autocrine IFN-I inhibits isocitrate dehydrogenase in the TCA cycle of LPS-stimulated macrophages

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**Graphical abstract**

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Macrophage activation in response to LPS is coupled to profound metabolic changes, typified by accumulation of the TCA cycle intermediates citrate, itaconate, and succinate. We have identified that endogenous type I IFN controls the cellular citrate/α-ketoglutarate ratio and inhibits expression and activity of isocitrate dehydrogenase (IDH); and, via 13C-labeling studies, demonstrated that autocrine type I IFN controls carbon flow through IDH in LPS-activated macrophages. We also found that type I IFN–driven IL-10 contributes to inhibition of IDH activity and itaconate synthesis in LPS-stimulated macrophages. Our findings have identified the autocrine type I IFN pathway as being responsible for the inhibition of IDH in LPS-stimulated macrophages.

Introduction

Macrophages activated with LPS (so-called M1 macrophages) have a TCA cycle that is altered at 2 distinct steps, leading to the accumulation of citrate, succinate, and itaconate (1). The mechanisms leading to the increased levels of these metabolites following LPS activation are not fully understood. It has been found that LPS inhibits gene expression of isocitrate dehydrogenase (IDH) (2, 3), the enzyme that converts isocitrate to α-ketoglutarate and a key checkpoint in the TCA cycle. Repression of Idh mRNA leads to reduced α-ketoglutarate levels and accumulation of citrate in activated macrophages (2, 3). Inhibition of Idh represents the first “break” in the TCA cycle of LPS-treated macrophages (2–4). Citrate can promote inflammatory responses in macrophages and is also a precursor for the generation of itaconate (4). The second break in the TCA cycle is associated with accumulation of succinate, which is derived from enhanced glutaminolysis (3). Oxidation of succinate by succinate dehydrogenase (SDH) in the electron transport chain drives macrophage ROS and IL-1β production (5). Apart from being a powerful antibacterial agent (6), itaconate inhibits the enzymatic activity of SDH in LPS-stimulated macrophages (7).

Type I IFNs are constitutively produced in a range of tissues and cells, and are critical regulators of innate and adaptive immune response (8). LPS-stimulated macrophages are a substantial source of IFN-β, which in turn signals via its receptors (IFNAR1 and IFNAR2) to induce production of mediators such as IL-12p70, NO, and IL-10 (9). Given the recent interest in the autocrine action of cytokines (e.g., IL-10) in regulating macrophage metabolism (10), we wanted to address the role of endogenous type I IFN in regulating the profound metabolic changes observed in LPS-activated macrophages. Here we report that the type I IFN pathway is responsible for the truncated TCA cycle at IDH and accumulation of citrate in LPS-stimulated macrophages.

Results and Discussion

Autocrine type I IFN promotes TCA cycle fragmentation and citrate accumulation in LPS-treated macrophages. To investigate whether endogenous type I IFN is involved in regulating macrophage metabolism, we stimulated bone marrow–derived macrophages (BMMs) from WT or ifnar1−/− mice with LPS (100 ng/mL) or vehicle (PBS) for 24 hours and profiled changes in metabolite levels by gas chromatography–mass spectrometry (GC-MS). As expected, LPS treatment of WT BMMs led to accumulation of itaconate, succinate, and citrate (refs. 2, 3, and Figure 1A; see Supplemental Tables 1–4 for the full list of metabolites; supplemental material available online with this article; https://doi.org/10.1172/JCI127597DS1). LPS treatment also led to reduced levels of isocitrate and α-ketoglutarate in WT BMMs, in agreement with an LPS-induced break point in the TCA cycle at IDH (2).

Similar to others (11), we observed that IFN-β treatment enhanced expression of Irg1 (the gene encoding the enzyme that synthesizes itaconate; ref. 6) (Supplemental Figure 1A) and that autocrine type I IFN facilitated LPS-mediated induction of Irg1 in BMMs (Supplemental Figure 1B). However, LPS treatment of ifnar1−/− BMMs did increase Irg1 gene transcription (Figure 1A and Supplemental Figure 1B), and they expressed Irg1 protein levels similar to those in WT BMMs (Figure 1A). Blockade of endogenous IFN-β with a neutralizing Ab significantly reduced, but did not completely abrogate, Irg1 expression in LPS-stimulated BMMs (Supplemental Figure 1C) and had no observable impact on Irg1 protein levels (Supplemental Figure 1D). Irg1 gene (Supplemental...
Autocrine type I IFN promotes TCA cycle fragmentation and citrate accumulation in LPS-treated macrophages. Metabolic profiling of WT and Ifnar1–/– BMMs stimulated or not with LPS (100 ng/mL) for 24 hours was carried out by GC-MS. (A) Schematic diagram of the TCA cycle, with the abundance of individual metabolites quantified. Data (fold change) are presented as histograms (n = 6). Gene and protein levels of Irg1 were determined by qPCR (n = 4) and Western blot analysis. (B) Citrate, aconitate, itaconate, and α-ketoglutarate levels determined by GC-MS in WT BMMs stimulated with LPS (100 ng/mL, 24 hours) in the presence of 50 U/mL of an IFN-β-neutralizing Ab or isotype control IgG (250 ng/mL, corresponding to 50 U/mL IFN-β Ab). P values were determined by 1-way ANOVA (A) or Student’s t test (B). α-KG, α-ketoglutarate; Ct, control.
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Blockade of endogenous type I IFN with a neutralizing anti–IFN-β Ab increased carbon flow through IDH in LPS-stimulated BMMs. 13C-glucose incorporation in succinate increased from approximately 2% ± 1% in LPS plus control Ab–treated BMMs to 12% ± 1% in LPS plus anti–IFN-β Ab–treated BMMs (Figure 2B). The restored carbon flow through IDH in LPS + anti–IFN-β Ab-treated BMMs is consistent with the reduced citrate and aconitate as well as the elevated α-ketoglutarate pools in these cells (Figure 1B). In line with our findings in Ifnar1–/– BMMs (Supplemental Figure II), we found that LPS plus anti–IFN-β Ab-treated WT BMMs had enhanced flux toward itaconate (Supplemental Figure II). These findings suggest that the elevated itaconate pools observed in the absence of endogenous type I IFN in LPS-activated BMMs (Figure 1, A and B) are supported by increased flux of glucose-derived carbon toward itaconate. Our data indicate that autocrine type I IFN controls the carbon flow through IDH in LPS-stimulated macrophages.

**Figure 2. Autocrine type I IFN controls carbon flow through IDH in LPS-treated macrophages.** 13C-glucose labeling of citrate and succinate in (A) BMMs (WT and Ifnar1–/–) stimulated or not with LPS (100 ng/mL, 24 hours) or (B) WT BMMs stimulated with LPS (100 ng/mL, 24 hours) in the presence of IFN-β Ab (50 U/mL) or Ct Ab (250 ng/mL, corresponding to 50 U/mL anti–IFN-β Ab). Cells were treated with 13C-glucose (10 mM) for 24 hours (i.e., labeling was initiated at the time of LPS treatment). 13C incorporation was analyzed by GC-MS. Mean percentage labeling ± SEM (n = 3) is shown. See Supplemental Figure 1, I and J, for 13C-glucose labeling of itaconate. Circle sizes are scaled with respect to pool sizes relative to untreated WT BMMs in A, or to BMMs + LPS + Ct Ab in B, for each metabolite.
We next treated WT and Ifnar1–/– BMMs with LPS or poly(I:C) over 24 hours and determined total IDH activity. IDH activity was impaired by approximately 40% after 24 hours of LPS treatment in WT BMMs (Figure 3E), whereas IDH activity in Ifnar1–/– BMMs was unchanged following LPS activation. This distinct response meant that IDH activity in LPS-treated Ifnar1–/– BMMs was approximately 2-fold higher than in their WT counterparts. Poly(I:C), a potent inducer of type I IFN (13), blocked IDH activity in WT BMMs at 24 hours (Figure 3F), with no impact observed in Ifnar1–/– BMMs. There was a tendency for both LPS and poly(I:C) to reduce IDH activity at 6 hours, but this was not statistically significant. In agreement with the restored carbon flow through IDH in LPS plus anti–IFN-β Ab–treated WT BMMs (Figure 2B), we found that IDH enzymatic activity in these cells was restored (Figure 3G). Exogenous IFN-β treatment of WT BMMs led to a significant reduction in IDH activity (Figure 3H), and LPS-treated human monocyte–derived macrophages (MDMs) also had significantly impaired IDH activity, which could be replicated by exogenous IFN-β (Figure 3I).
relative to that in untreated BMMs. Consistent with this finding, accumulation of citrate (Figure 4B) and aconitate (Figure 4C) was reduced in BMMs treated with LPS plus anti–IL-10 mAb relative to LPS plus control mAb–treated BMMs. Blockade of endogenous IL-10 increased Irg1 expression in LPS-treated BMMs (Figure 4, D and E) but, interestingly, led to a reduction in the itaconate pool size in these cells (Figure 4F). Our data agree with an earlier study finding that the first break in the TCA cycle was intact in LPS-treated Il10–/– BMMs (16). A small flux in metabolite concentration can induce significant changes in enzyme activity (17). The data presented here suggest that blockade of endogenous IL-10 in LPS-stimulated macrophages reduces the accumulation of citrate and aconitate, the substrate for Irg1 (6), which in turn reduces the generation of itaconate, despite the increased Irg1 expression.

We measured the activity of aconitase, which catalyzes the conversion of citrate to isocitrate (1). In agreement with others (14), we found that LPS reduced aconitase activity (Supplemental Figure 1K); however, we found no significant difference in aconitase activity between LPS-treated WT and Ifnar1–/– BMMs. These data provide evidence that autocrine type I IFN is responsible for inhibition of IDH activity in LPS-stimulated macrophages.

**Type I IFN induction of IL-10 contributes to inhibition of IDH.** Type I IFN drives IL-10 release in macrophages (Supplemental Figure 1L) (9, 15), and IL-10 has a profound impact on macrophage metabolism (10). Given this, we first measured IDH activity in LPS-treated WT BMMs following IL-10 (100 ng/mL) or IFN-b (1000 U/mL, 24 hours, n = 6) treatment (Figure 4A). Total IDH activity in WT and Ifnar1–/– BMMs following LPS with or without IL-10 (100 ng/mL, 24 hours, n = 5). Citrate and aconitate in WT and Ifnar1–/– BMMs treated as in H (n = 6). Irg1 gene (n = 6) and protein in WT and Ifnar1–/– BMMs treated as in H. (M) Itaconate in WT and Ifnar1–/– BMMs treated as in H. Mean ± SEM. P values by 1-way ANOVA (A, D, G–K, and M) or Student’s t test (B, C, and F).
We next determined the impact of exogenous IL-10 on WT and Ifnar1−/− BMMs. Unlike IFN-β, IL-10 alone did not inhibit IDH activity in WT BMMs (Figure 4G). However, when combined with IFN-β, it tended to reduce IDH activity in these cells (Figure 4G). In combination with LPS, IL-10 led to further inhibition of IDH activity in WT BMMs but had no impact on IDH activity in Ifnar1−/− BMMs (Figure 4H). These data suggest that the ability of IL-10 to block IDH activity is dependent on the presence of type I IFN signaling in BMMs. We found by metabolic profiling that, consistent with its blockade of IDH activity in LPS-treated WT BMMs, exogenous IL-10 significantly elevated levels of citrate (Figure 4I) and aconitate (Figure 4I) in these cells. In comparison, IL-10 did not alter the pool sizes of either citrate or aconitate in LPS-treated Ifnar1−/− BMMs (Figure 4, I and J), in agreement with its inability to block IDH activity in these cells (Figure 4H). Irg1 expression (Figure 4, K and L) was partially blocked by IL-10 in LPS-treated WT BMMs. Blockade of Irg1 protein was more pronounced in LPS-treated Ifnar1−/− BMMs (Figure 4L). IL-10 also blocked IFN-β-induced Irg1 expression in WT BMMs (Supplemental Figure 1M). IL-10 inhibition of Irg1 expression in macrophages has been shown previously (18). Regarding itaconate levels, we found that IL-10 boosted itaconate synthesis in LPS-treated WT BMMs, whereas IL-10 decreased itaconate in LPS-treated Ifnar1−/− BMMs (Figure 4M). In summary, in LPS-treated WT BMMs, IL-10 blocked IDH activity, resulting in further accumulation of citrate and aconitate to drive itaconate synthesis. In LPS-treated Ifnar1−/− BMMs, IL-10 had no impact on IDH activity or substrate accumulation, enabling the IL-10–mediated suppression of Irg1, which is more evident in these cells, to impair itaconate synthesis.

We showed that LPS-treated Ifnar1−/− BMMs have increased carbon flux, via IDH, toward succinate (Figure 2A) and increased carbon flux, via Irg1, toward itaconate (Supplemental Figure 1I). This distinct carbon flow led to reduced citrate levels in LPS-treated Ifnar1−/− BMMs (Figure 1A), as citrate is utilized to fuel the accumulation of both succinate and itaconate. The elevated flux toward itaconate in LPS-treated Ifnar1−/− BMMs was consistent with their minimal IL-10 production (Supplemental Figure 1I), which uniquely blocked itaconate generation in these cells (Figure 4M). Our results are supported by a detailed analysis of LPS-treated MDMs, where transient induction of Irg1 and repression of Idh1 mRNA was correlated with the level of induction of IFN-β (19). Thus, in the absence of IL-10–mediated suppression of Irg1, carbon flux through Irg1 is increased, leading to the elevated itaconate levels observed in LPS-treated Ifnar1−/− BMMs. Overall, this study demonstrates that inhibition of IDH in the TCA cycle of LPS-stimulated macrophages is due to the autocrine type I IFN pathway.

Methods

See Supplemental Methods for a detailed description of all experimental procedures.

Study approval. All experiments were performed in accordance with the ethics approval obtained from the University of Melbourne Animal Ethics Committee.

Author contributions

AJF conceptualized the work. AJF, DPD, and DLT were responsible for methodology. AJF, MKSL, AA, MCL, KJB, and SD validated results. SD, AJM, MJM, KJB, DPD, and DLT were responsible for resources. AJF, MJM, ADC, AJM, and JAH participated in the writing of this article.

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