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Reshaping of Human Macrophage Polarization through Modulation of Glucose Catabolic Pathways

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Macrophages integrate information from the tissue microenvironment and adjust their effector functions according to the prevalent extracellular stimuli. Therefore, macrophages can acquire a variety of activation (polarization) states, and this functional plasticity allows the adequate initiation, regulation, and resolution of inflammatory responses. Modulation of the glucose metabolism contributes to the macrophage adaptation to the surrounding cytokine milieu, as exemplified by the distinct glucose catabolism of macrophages exposed to LPS/IFN-γ or IL-4. To dissect the acquisition of macrophage effector functions in the absence of activating cytokines, we assessed the bioenergetic profile of macrophages generated in the presence of GM-CSF (GM-MØ) or M-CSF (M-MØ), which do not release pro- or anti-inflammatory cytokines unless subjected to additional activating stimuli. Compared to M-MØ, GM-MØ displayed higher oxygen consumption rate and aerobic glycolysis (extracellular acidification rate [ECAR]), as well as higher expression of genes encoding glycolytic enzymes. However, M-MØ exhibited a significantly higher oxygen consumption rate/ECAR ratio. Surprisingly, whereas aerobic glycolysis positively regulated IL1B, TNF, and INHBA mRNA expression in both macrophage subtypes, mitochondrial respiration negatively affected IL6, IL1B, TNF, and CXCL10 mRNA expression in M-MØ. The physiological significance of these results became evident under low oxygen tensions, as hypoxia enhanced ECAR in M-MØ via HIF-1α and HIF-2α, increased expression of glycolytic enzymes and GM-MØ–specific genes, and diminished expression of M-MØ–associated genes. Therefore, our data indicate that GM-MØ and M-MØ display distinct bioenergetic profiles, and that hypoxia triggers a transcriptomic switch in macrophages by promoting a HIF-1α/HIF-2α-dependent increase in ECAR. The Journal of Immunology, 2015, 195: 000–000.
GLUCOSE CATABOLISM MODIFIES HUMAN MACROPHAGE POLARIZATION

Materials and Methods

Generation of human monocyte-derived macrophages

Human PBMCs were isolated from buffy coats from normal donors over Lymphoprep (Nycomed Pharma, Oslo, Norway) gradient according to standard procedures. Monocytes were purified from PBMCs by magnetic cell sorting using CD14+ microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes (0.5 × 10^6 cells/ml, ≥95% CD14+ cells) were cultured in RPMI 1640 supplemented with 10% FBS for 7 d in the presence of 5% CO2 and 94% N2.

Sample preparation for metabolomics analysis

Bicarbonate-free DMEM (Sigma-Aldrich) supplemented with 11.11 mM bicarbonate was used to freshen media for 2 h, and then cells were washed twice with DMEM to remove the bicarbonate from the media. Then, samples were centrifuged at 14,000 rpm for 10 min at 4°C.

Measurement of cellular respiration and extracellular acidification (bioenergetic profile)

The XF24 extracellular flux analyzer (Seahorse Biosciences, North Billerica, MA) was used to determine the bioenergetic profile of intact cells. Briefly, cells were seeded (200,000 cells/well) in XF24 plates (Seahorse Biosciences) and allowed to recover for 24 h. Cells were then incubated in bicarbonate-free DMEM (Sigma-Aldrich, St. Louis, MO), or 300 mM CoCl₂ for the indicated periods of time. Cells were routinely cultured in 21% O₂ and 5% CO₂ (normoxic conditions). Hypoxic conditions (1% O₂) were generated in a cell incubator under controlled anaerobic atmosphere with 5% CO₂ and 94% N₂.

Statistical analysis

Statistical analysis was performed using a Student t test, and a p value < 0.05 was considered significant.

Results

Human GM-MØ and M-MØ display a different bioenergetic profile

GM-MØ and M-MØ exhibit distinct phenotypes (3) and gene signatures (5), and they exert opposite effector functions on tumor cells and T lymphocytes (3–5, 20). To determine whether glucose metabolism lies at the basis of these phenotypic and functional differences, the mitochondrial respiration and glycolytic metabolism...
Moreover, the expression of specific gene markers, such as INHBA, SERPINE1, and CD163, in GM-MØ and M-MØ was also significantly different from each other. INHBA mRNA expression was upregulated in GM-MØ compared to M-MØ under both basal conditions and after stimulation with proinflammatory cytokines (Figs. 2A and 3C). Conversely, oligomycin treatment significantly decreased the expression of these markers in both macrophage subtypes. These results indicate that expression of proinflammatory cytokine mRNA (Fig. 3B) or the oligomycin-triggered expression of proinflammatory cytokine mRNA under basal conditions in both GM-MØ and M-MØ. Moreover, the expression of INHBA mRNA was positively correlated with the expression of proinflammatory cytokines (IL1B, TNF, and IL10) and significantly reduced the mRNA levels of the proinflammatory cytokine IL1B and SERPINE1 mRNA in M-MØ (Supplemental Fig. 1). Therefore, the expression of IL6, TNF, and IL1B mRNA is negatively regulated by oligomycin-sensitive mechanisms in M-MØ (Fig. 3D), as their enhanced expression is concomitant with the oligomycin-mediated OCR decrease. Because blockade of mitochondrial respiration also results in a subsequent downregulation of GM-MØ–associated markers (CD163 mRNA) and significantly reduced the mRNA levels of other GM-MØ–associated markers (EGLN3, MMP12, SERPINE1), IL23, or CXCL10 (Fig. 2B and not shown). Conversely, oligomycin (1 μM, 4 h) reduced OCR and led to a compensatory increase in ECAR (Fig. 2C), but it did not affect proinflammatory gene expression (Fig. 2D). Therefore, aerobic glycolysis contributes to the expression of IL6, TNF, and IL1B mRNA under basal conditions in GM-MØ. In the case of M-MØ, oxamate-mediated reduction of ECAR (Fig. 3A) enhanced IL6 gene expression (3-fold, Fig. 3B) and downregulated GM-MØ–associated marker INHBA and SERPINE1 mRNA (Fig. 3B), without influencing TNF and IL1B (Fig. 3B) or the M-MØ–associated marker CD163 mRNA (Supplemental Fig. 1). Conversely, unlike in GM-MØ, blockade of mitochondrial respiration by oligomycin (Fig. 3C) dramatically enhanced the mRNA expression level of the proinflammatory genes IL6, TNF, and IL1B in M-MØ (between 18- and 80-fold, Fig. 3D), increased CXCL10 mRNA to a lower extent, and left unaffected the levels of INHBA and SERPINE1 (Fig. 3D). Therefore, the expression of IL6, TNF, and IL1B mRNA is negatively regulated by oligomycin-sensitive mechanisms in M-MØ (Fig. 3D), as their enhanced expression is concomitant with the oligomycin-mediated OCR decrease. Because blockade of mitochondrial respiration also results in a subsequent decrease in ECAR enhancement (Fig. 3C), we next assessed whether oligomycin-mediated changes are solely caused by ATPase synthase inhibition or due to the secondary enhancement in ECAR. To this end, M-MØ were exposed to the ATP synthase inhibitor in the presence or absence of oxamate. As shown in Fig. 3E, inhibition of lactate dehydrogenase prevented the oligomycin-induced increase in TNF, IL1B, and CXCL10 mRNA, whereas it had no effect on IL6 mRNA levels. Therefore, it can be concluded that enhanced ECAR contributes to the oligomycin-triggered expression of proinflammatory cytokine mRNA.

As a whole, the above results indicate that expression of proinflammatory cytokine IL6, TNF, and IL1B mRNA is positively regulated by aerobic glycolysis in GM-CSF–primed macrophages and negatively regulated by mitochondrial respiration in macrophages primed by M-CSF. These results support the link between the metabolic profile and the production of IL6, TNF, and IL1B under non–activating conditions, and they demonstrate that the relationship of OCR/ECAR inversely correlates with the expression of TNF and IL1B in both GM-MØ and M-MØ. Moreover, note that although to a much lower extent, the expression of the anti-inflammatory cytokine gene IL10 is also sensitive to modulation of the bioenergetics profile, because oligomycin treatment weakly (<2-fold) upregulated IL10 expression, whereas oxamate diminished by 50% the levels of IL10 mRNA in M-MØ (Supplemental Fig. 1). Therefore, IL10 mRNA expression increases upon OCR inhibition (similar to proinflammatory cytokine mRNA) but is reduced upon ECAR inhibition by oxamate, thus suggesting that glucose catabolism differentially affects pro- and anti-inflammatory cytokines.

Along the same line, the link between glucose catabolism and the expression of polarization-associated markers in GM-MØ and M-MØ is not so straightforward. In the case of GM-MØ–associated markers, INHBA expression was reduced by oxamate in both macrophage subtypes, SERPINE1 was only dependent on aerobic glycolysis in M-MØ (Figs. 2B, 3B), and the expression of EGLN3 and MMP12 was not altered by oxamate (data not shown). Regarding M-MØ–associated markers, no significant effect of either oligomycin or oxamate on the expression of CD163, FOLR2, or HTR2B was observed (Supplemental Fig. 1 and data not shown).

Hypoxia triggers a polarization shift in human macrophages via modulation of the glycolytic metabolism

To clarify all of the above results in a more “physiological” context, we next sought to assess the influence of hypoxia (which
FIGURE 1. Characterization of the energy metabolism of GM-MØ and M-MØ macrophages. (A) OCR profile of human GM-MØ and M-MØ monitored using the Seahorse Biosciences extracellular flux analyzer. Cells were treated sequentially, as indicated, with 1 µM oligomycin (Oligo), 0.6 plus 0.4 µM FCCP, and 1 µM rotenone plus 1 µM antimycin A (Rot/AA). (B) Metabolic parameters obtained from the OCR profiling after subtraction of the rotenone/antimycin-insensitive respiration. Basal OCR is the oxygen consumption rate in the absence of effectors, ATP turnover is considered as the oligomycin-sensitive respiration, and maximal respiration is the OCR value in the presence of the uncoupler FCCP. Results are normalized according to protein concentrations and presented as mean ± SD of eight independent samples. (C) ECAR, a proxy for the rate of lactate production, measured in human GM-MØ and M-MØ under basal conditions and after the stimulation with 1 µM oligomycin. (D) Metabolic parameters obtained from ECAR profiling. Basal indicates the rate of glycolysis in the absence of effectors, and glycolytic capacity is the ECAR value after the inhibition with oligomycin of the mitochondrial ATP synthesis. Results are normalized according to protein concentrations and presented as mean ± SD of eight independent samples. (E) The bioenergetics profile of the cells is defined as the ratio between the basal OCR and basal ECAR. Results are presented as mean ± SD of eight independent samples. (F) Abundance of metabolites in GM-MØ and M-MØ, relative to GM-MØ. Data are presented as mean ± SD of three independent samples. (G) Gene expression levels of SLC2A1, HK3, H6PD, FBP1, PFKP, LDHA, PDK1, PKM2, PFKFB2, and PFKFB3 in GM-MØ and M-MØ determined by microarray analysis of three independent samples (filled bars) or by qRT-PCR (open bars). Data are presented as mean ± SD of three independent samples. (H–J) Bioenergetic profiling of GM-MØ and M-MØ under basal conditions (−) and after stimulation for 4 h with GM-CSF or M-CSF, respectively: (H) basal OCR, (I) basal ECAR, (J) OCR/ECAR ratio. Data are presented as mean ± SD of four independent samples. *p < 0.05, **p < 0.01, ***p < 0.001.
increases glycolysis and limits oxidative phosphorylation) on the expression of proinflammatory cytokines and polarization-associated genes. The positive correlation between an active glycolytic pathway and the expression of proinflammatory cytokine genes raised the possibility that the oligomycin-enhanced gene expression in M-MØ was caused by an augmented glycolysis (ECAR) secondary to the inhibition of mitochondrial respiration (see Fig. 3C). If so, and because glycolysis is greatly potentiated in response to low oxygen tension (21), GM-MØ and M-MØ should differ in their sensitivity to hypoxia: M-MØ would exhibit a higher hypoxia sensitivity, whereas GM-MØ would show a weaker hypoxia responsiveness, as expected from their higher content of glycolytic enzymes. In line with our prediction, exposure to the chemical hypoxia-mimicking agent CoCl₂ (4 h) caused a significant increase in ECAR in M-MØ but did not modify the bioenergetic profile of GM-MØ (Fig. 4A). Moreover, CoCl₂-induced hypoxia led to a significant enhancement in the expression of glycolytic enzyme-coding genes (SLC2A1, LDHA, PDK1, PKM2, PFKFB2, and PFKFB3) exclusively in M-MØ (Fig. 4B). Therefore, CoCl₂-induced hypoxia induces the acquisition of a GM-MØ-like glycolytic profile in M-MØ.

The relevance of the above findings was substantiated by assessing whether actual hypoxia (1% O₂, 24 h) also modified the basal phenotype of GM-MØ and M-MØ. In M-MØ, hypoxia (1% O₂, 24 h) induced the acquisition of GM-MØ-associated polarization genes SERPINE1, CCR2A, and INHBA (Fig. 5A), drastically reduced the expression of the M-MØ–associated markers SLC40A1, FOLR2, and MAFB (Fig. 5B), and led to a strong reduction in MAFB protein levels (Fig. 5C). Additionally, low oxygen tension induced M-MØ to acquire tumor cell growth inhibitory activity (Fig. 5D), a property exclusively displayed by GM-MØ (5). Therefore, unlike GM-MØ, M-MØ are extremely
sensitive to hypoxia (1% O₂) and respond to low oxygen levels by increasing GM-MØ-specific genes, losing M-MØ-specific markers and acquiring functions that are characteristic of proinflammatory GM-MØ.

Because all of the above parameters were evaluated in the absence of macrophage-activating cytokines or TLR ligands, we next assessed whether hypoxia differentially affected the response of GM-MØ and M-MØ to an activating stimulus. To this end, both macrophage subtypes were exposed to LPS under hypoxic conditions. At 1% O₂ (24 h), M-MØ responded to LPS by producing significantly higher levels of GM-MØ-related cytokines IL-12p40 and TNFα, but lower levels of anti-inflammatory CCL2 and IL-10 (9) (Fig. 5E). In contrast, the LPS-stimulated cytokine secretion from GM-MØ was not significantly modified by hypoxia (Fig. 5E). Essentially similar effects were observed on macrophages subjected to CoCl₂-simulated hypoxia (Fig. 5F). Therefore, hypoxia modifies the bioenergetic profile of M-MØ and induces the acquisition of GM-MØ-associated transcriptomic and functional properties in M-MØ.

To evaluate whether a causal relationship exists between the transcriptomic switch and the changes in glucose catabolism initiated by hypoxia in M-MØ, these macrophages were exposed to hypoxia in the presence of oxamate. As shown in Fig. 6A, CoCl₂-induced hypoxia triggered a significant increase in the expression of proinflammatory cytokine genes, whereas oxamate inhibited the hypoxia-enhanced mRNA expression of TNF, IL1B, and the GM-MØ-specific genes INHBA and SERPINE1. Additionally, oxamate reduced the hypoxia-augmented production of LPS-induced TNFα in M-MØ (Fig. 6B). These results further illustrate the link between the bioenergetic profile and the macrophage polarization state, and they demonstrate that the hypoxia-triggered shift in the transcriptomic and functional polarization of M-MØ is dependent, at least partly, on hypoxia-triggered changes in the macrophage bioenergetic profile.

HIF-1α and HIF-2α contribute to the polarization shift induced by hypoxia in human macrophages

Given the link between hypoxia-driven glycolytic changes and the macrophage polarization shift, and taking into account that HIF-1α and HIF-2α mediate most cellular responses to hypoxia, we sought to evaluate the role of both factors in the change of the bioenergetics profile that underlies the hypoxia-driven polarization switch in M-MØ (Fig. 6). No HIF-1α, and a low level of HIF-2α, was detected in M-MØ under normoxia, whereas CoCl₂-induced hypoxia led to a great accumulation of HIF-1α and a weak increase in HIF-2α levels (Supplemental Fig. 2). Because similar results were observed in GM-MØ, it can be hypothesized that the distinct bioenergetics profile of GM-MØ and M-MØ is not secondary to a differential content of HIF factors.

To definitively assess whether HIF factors mediate the change in the bioenergetics profile that leads to a proinflammatory polarization of M-MØ during hypoxia, the expression of HIF-1α and/or HIF-2α was knocked down in M-MØ before exposure to CoCl₂. siRNA-mediated knockdown resulted in a considerable decrease of the expression of each factor in CoCl₂-exposed M-MØ (Fig. 7A). The simultaneous siRNA-mediated knockdown of HIF-1α and HIF-2α significantly reduced the CoCl₂-augmented ECAR in M-MØ (Fig. 7B), a reduction that was not accomplished when each factor was silenced alone (Fig. 7B). Along the same line, knockdown of both factors diminished the expression of the glycolytic enzyme-encoding genes SLC2A1, LDHA, and PDK1, and/or LDHB, and a weak induction of SERPINE1 (Fig. 7D). Therefore, HIF-1α and HIF-2α contribute to the augmented expression of glycolytic enzyme-encoding genes, to the increase in ECAR, and to the polarization switch observed in M-MØ exposed to hypoxia.

Discussion

Glucose metabolism has been already shown to differ between macrophages exposed to Th1- or Th2-derived cytokines or TLR ligands (11). However, the glucose metabolism status in human macrophages exposed to either GM-CSF or M-CSF, which prime macrophages for proinflammatory (GM-MØ) or anti-inflammatory (M-MØ) responses, respectively (18), has not been previously addressed. GM-MØ and M-MØ greatly differ in their phenotypic, transcriptomic, and functional status (2–6) and, therefore, constitute a valid cellular system to address the contribution of glucose metabolism to the acquisition and maintenance of macrophage functions in the absence of TLR stimulation. In the present study we provide evidence that GM-MØ and M-MØ exhibit distinct bioenergetic profiles, that their distinct glucose metabolism leads to the acquisition and maintenance of a polarized macrophage functional profile.
catabolism determines their different transcriptional and functional programs, and that hypoxia triggers an HIF-1α/HIF-2α-dependent transcriptomic switch in macrophages through an increase in anaerobic glycolysis. As a whole, our results illustrate that the acquisition of polarization-specific features of human macrophages rely, at least partly, on glucose catabolism.
and that modulation of the glucose metabolism alters transcriptomic and functional features in macrophages.

Our findings indicate that, in terms of glucose catabolism, GM-MØ and M-MØ significantly differ in their preferential pathway, as GM-MØ exhibit a significantly higher level of aerobic glycolysis (OCR) and mitochondrial respiration (ECAR), as well as a lower OCR/ECAR ratio, than M-MØ. These results are in an apparent contradiction with those reported on murine macrophages, which were shown to be essentially glycolytic cells (11). This discrepancy might derive from the high levels of NO found in M1 murine macrophages, because NO inhibits mitochondrial respiration (22, 23). In contrast, NOS2 mRNA expression could not be detected in human GM-MØ (data not shown), in agreement with findings in human alveolar macrophages stimulated with IFN-γ plus LPS, where epigenetic methylation-dependent gene silencing explains the lack of NOS2 mRNA and protein expression (24). Thus, the differences between previous results and ours might be explained by the lack of NO production by GM-MØ (25, 26). Our findings have another important implication because GM-CSF or M-CSF just prime macrophages toward a pro- or anti-inflammatory state, but a secondary stimulus is required for triggering pro- or anti-inflammatory cytokine production. Therefore, and given the distinct bioenergetics profiles of GM-MØ and M-MØ, our results predict that both macrophage subtypes would respond differently to cytokines promoting overt polarization (IFN-γ, IL-4), further contributing to the plethora of macrophage polarization states. In support of this hypothesis, we have already observed that the signaling pathways and gene expression initiated by LPS on these macrophage subtypes differ both qualitatively and quantitatively (V.D. Cuevas and A.L. Corbi, unpublished results).

GM-MØ display a high expression of glycolytic enzymes and glycolysis-related metabolites, thus resembling the metabolic

FIGURE 6. Transcriptional and functional effects of CoCl2-induced hypoxia in M-MØ. M-MØ were exposed to hypoxic conditions (300 μM CoCl2, 4 h) in the presence or absence of 30 mM oxamate. (A) Relative mRNA expression of IL6, TNF, IL1B, IL23, CXCL10, INHBA, and SERPINE1 was determined by qRT-PCR using TBP mRNA as a reference. Results are expressed as the expression of each gene under the indicated conditions and relative to its expression under normoxic conditions. Data are shown as mean ± SD of three independent experiments. *p < 0.05, CoCl2 plus oxamate versus CoCl2; # p < 0.05, CoCl2 versus untreated. (B) TNF-α levels produced by M-MØ exposed to LPS (10 ng/ml) for 4 h, as determined by ELISA. Data are shown as mean ± SD of three independent experiments. *p < 0.05.

FIGURE 7. Bioenergetic and transcriptomic effects of targeting (siRNA) HIF-1α and HIF-2α in M-MØ. M-MØ were transfected with scramble (siScr), HIF-1α (siHIF-1α), HIF-2α (siHIF-2α), or a mix of both siRNAs (siHIF-1α plus HIF-2α) and then exposed to normoxic or hypoxic (300 μM CoCl2, 4 h) conditions. (A) Representative Western blot of HIF-1α and HIF-2α of transfected M-MØ. α-Tubulin protein levels were determined in parallel for loading control purposes. (B) ECAR of transfected M-MØ in hypoxic (300 μM CoCl2, 4 h). Data are shown as mean ± SD of three independent experiments. *p < 0.05. (C and D) Relative mRNA expression of SLC2A1, FBP1, PFKP, LDHA, PKD1, PKM2, PFKFB2, and PFKFB3 (C) and IL1B, INHBA, and SERPINE1 (D) was determined in M-MØ by qRT-PCR after hypoxia (300 μM CoCl2, 4 h) treatment. Results are expressed as relative expression (relative to TBP mRNA levels) and referred to each gene expression in scramble transfected M-MØ. Data are shown as mean ± SD of three independent experiments. *p < 0.05.
profile of glycolytic tumor cells and activated inflammatory immune cells (10). This shift toward aerobic glycolysis, known as the Warburg effect, rapidly provides immune cells with ATP and metabolic intermediates for the synthesis of immune and inflammatory proteins (27, 28). In the case of human macrophages, note that our results indicate that GM-MØ exhibit higher levels of PKM2 mRNA than M-MØ, which might contribute to the enhanced glycolysis observed in GM-MØ. Besides, higher levels of succinate are also seen in GM-MØ, further contributing to the similarity of the glucose metabolic profile between GM-CSF–primed human macrophages and tumor cells.

The glycolytic metabolism is known to regulate cytokine expression in activated immune cells (29–32), as IL-6 expression in murine monocyte cell lines is lowered upon downregulation of the glycolytic pathway by 2-deoxyglucose (33), TNF-α is downregulated by lactate (34), and IL-1β expression is enhanced by a TLR-dependent stabilization of HIF-1α (35), a process further enhanced by succinate accumulation (36). Our data show that IL6, IL1B, and TNF mRNA are positively regulated by the glycolytic pathway in proinflammatory GM-MØ; whereas a drop of GM-MØ ECAR levels correlates with reduced IL6, IL1B, and TNF mRNA levels, inhibition of mitochondrial respiration leaves the expression of these cytokine genes unaffected. In the case of M-MØ, IL1B and TNF and IL6 mRNA are regulated by an increase in ECAR levels secondary to the inhibition of oxidative phosphorylation by oligomycin treatment. Therefore, the expression of proinflammatory cytokine mRNA is also under the control of the glucose metabolism in human macrophages primed by either GM-CSF or M-CSF. Interestingly, the expression of IL10 mRNA also increases upon inhibition of oxidative phosphorylation (similar to proinflammatory cytokine mRNA) but is reduced by ECAR inhibition, suggesting that glucose catabolism differentially affects pro- and anti-inflammatory cytokines. The positive regulatory effect of the glycolytic pathway on the proinflammatory cytokine expression in M-MØ is also observed under hypoxic conditions, which promote the acquisition of GM-MØ–like transcriptional and functional features in M-CSF–dependent macrophages. Therefore, it can be hypothesized that GM-CSF, similar to hypoxia, primes macrophages for a more rapid and robust response to pathogens by enhancing glycolytic metabolism and, thus, proinflammatory cytokine expression. This “priming” action might therefore resemble the HIF-1α–mediated “training” that has been recently reported in monocytes exposed to β-glucan (37, 38).

Although low oxygen concentrations are found in certain tissues under physiological conditions, exacerbated tissue hypoxia is common to many inflammatory pathologies (16, 39, 40). One of the main features of the cellular response to hypoxia is a significant increase in glucose catabolism (41, 42). In the case of murine macrophages, the expression of hypoxia-inducible factors HIF-1α and HIF-2α appears to be dependent on the stimulatory agents: whereas M1-promoting factors induce the expression of HIF-1α, IL-4 primarily induces HIF-2α that regulates the expression of M2 marker genes (17). In human monocyte-derived macrophages, we have observed that both GM-MØ and M-MØ express HIF-1α and HIF-2α in response to hypoxia. M-MØ respond to hypoxia by changing their metabolic profile and acquiring a GM-MØ–like transcriptional and functional profile, with both changes dependent on both HIF-1α and HIF-2α. This hypoxia-driven response could explain the elevated number of proinflammatory macrophage founds in the synovium of rheumatoid arthritis patients (43), where the severe hypoxic milieu might promote or contribute to the activin A–dependent M-MØ to GM MØ switch (5, 6, 43). In contrast, hypoxia does not modify GM-MØ at the transcriptional (expression of polarization-associated genes) or functional (cytokine production) level (Fig. 5). We hypothesize that the lack of response to hypoxia of GM-MØ might be explained by their high glycolytic state, which resembles that of cells under hypoxic conditions. If so, the elevated ECAR level and the high expression of key enzymes of the glycolytic pathway seen in GM-MØ would impair a further increase in their glycolytic mechanisms, thus preventing the hypoxia-driven transcriptomic and functional effects seen in M-MØ.

In summary, our study provides new insights into the regulation of glucose catabolism and functional features by the glucose metabolism, thus suggesting that modulation of glucose metabolism represents a potential strategy to modify macrophage polarization under pathological conditions such as cancer or inflammatory diseases. In this regard, because high PDK1 expression correlates with increased aerobic glycolytic rate (44–46) and PKM2, the major isoform expressed in cancer cells (47, 48), promotes rapid glycolytic energy production through upregulation of HIF-1α target genes (GLUT1, EGLN3, PDK1, LDHA) (49, 50), it seems plausible that both enzymes (PDK1 and PKM2) might be appropriate targets to tackle the link between the glucose catabolism and the inflammatory response in macrophages.

Disclosures
The authors have no financial interests of conflict.

References
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Supplementary Figure 1. Effect of oligomycin and oxamate on the expression of CD163 and IL10 mRNA in M-MØ. M-MØ were left untreated or exposed for 4h to either 30 mM oxamate or 1 µM oligomycin, and relative mRNA expression of CD163 and IL10 was determined by qRT-PCR. Results are indicated as relative expression (relative to TBP mRNA levels) and referred to the expression of each gene in untreated M-MØ. Data are shown as mean ± SEM of five (CD163) or eight (IL10) independent experiments (**, p<0.01; ***, p<0.001).
Supplementary Figure 2. Expression of HIF in GM-MØ and M-MØ under normoxia or CoCl₂-induced hypoxia. GM-MØ and M-MØ were either untreated (−) or exposed to 300 μM CoCl₂ for 4h to induce hypoxia-like conditions. After 4 hours cells were lysed, and macrophage lysates subjected to Western blot using antibodies specific for either HIF-1α and HIF-2α. α-tubulin protein levels were determined in parallel for loading control purposes.