

A Metabolic Roadblock in Inflammatory Macrophages

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<http://dx.doi.org/10.1016/j.celrep.2016.09.085>

In this issue of *Cell Reports*, [Van den Bossche et al. \(2016\)](#) reveal that, once the M1 macrophage forms, the accompanying metabolic alterations in the mitochondria are irreversible, preventing differentiation into the more homeostatic M2 macrophage.

Macrophages are a frontline cell in host defense and inflammation, participating in the handling of infectious agents but also restoring homeostasis once the trauma has passed. As such, they are highly plastic and respond to different environmental cues by adopting different effector states, as required by the prevailing conditions. The best understood scenario consists of macrophages occurring in two quite distinct “flavors.” When the stimulus is lipopolysaccharide (LPS) from gram-negative bacteria in combination with interferon γ (IFN γ ; as might prevail during bacterial infection), they adopt an inflammatory phenotype (usually termed M1 macrophages, or as recently proposed, M[LPS + IFN γ]) ([Murray et al., 2014](#)). However, when activated by cytokines such as IL-4 or IL-13, they adopt a more reparative phenotype (M2 macrophages or M[IL-4]). These macrophages are also more prominent in tumors or during helminth infections and in allergy, where they contribute to pathology. Therefore, a lot of attention has focused on the molecular basis for these differing responses, one aim being to reprogram macrophages from one state to another for therapeutic gain. Attention has recently focused on metabolic differences between these states. M1 macrophages have impaired oxidative phosphorylation and rely on glycolysis for ATP production. This impairment is partly due to the Krebs cycle being broken in M1 macrophages, with the intermediates citrate and succinate taking on the roles of membrane biogenesis and HIF1 α activation, respectively ([Tannahill et al., 2013](#); [Jha et al., 2015](#); [Galván-Peña and O'Neill, 2014](#)). On the other hand, M2 macro-

phages have an intact Krebs cycle and use oxidative phosphorylation as the main means of ATP generation. In this issue of *Cell Reports*, [Van den Bossche et al. \(2016\)](#) provide new insights into this aspect of macrophage regulation by demonstrating that, once the macrophage has committed to being M1, it cannot be reprogrammed to an M2 by IL-4. However, the opposite is not apparent—the M2 can be converted to an M1 by the addition of LPS. The mechanism involves an impairment in the mitochondrial electron transport chain in the M1 macrophage that is caused by nitric oxide (NO) in the mouse and cannot be overcome. This study confirms the importance of metabolic reprogramming for macrophage function and might have relevance for therapeutic manipulation of macrophages in disease.

The study began with attempts to repolarize either human or mouse macrophages from M1 to M2 using IL-4. With the exception of arginase, a host of M2 markers were not inducible by IL-4. Intriguingly, IL-4 signaling (as indicated by STAT6 phosphorylation) was intact in this protocol. In contrast, LPS + IFN γ treatment was able to induce M1 markers in M2 macrophages, indicating that the M2 phenotype was more plastic. Importantly these results were confirmed in vivo. Metabolic analysis confirmed enhanced glycolysis and impaired oxidative phosphorylation in the M1 macrophage, with oxidative phosphorylation being more evident in the M2 macrophage. Further analysis revealed that M1 macrophages had a dysfunctional electron transport chain, being unable to respire under conditions where substrates were provided to

complexes I, II or III. IL-4 was unable to restore respiration in the M1 macrophage. Also of note was the observation that oligomycin (which inhibits the ATP synthase) or 2-deoxyglucose (which inhibits glycolysis) both suppressed the induction of M2 macrophages by IL-4. This indicates that glucose feeds the Krebs cycle for respiration in the M2 macrophage and that inhibition of this will prevent polarization to M2.

[Van den Bossche et al. \(2016\)](#) then turned to a possible mechanism of impaired respiration in the M1 macrophage. Previous studies in other cell types had pointed to NO as being able to block respiration by nitrosylating components in the electron transport chain ([Clementi et al., 1998](#)). Inducible NO synthase (iNOS) inhibition prior to LPS + IFN γ markedly improved respiration and also made the M1 macrophage amenable to reprogramming to an M2 phenotype by IL-4. It did not block induction of the M1 phenotype but instead made the M1 macrophage more responsive to IL-4 in terms of induction of M2 markers. Therefore, these results indicate that NO is the damaging agent for the electron transport chain, with this damage preventing IL-4 from repolarizing the macrophage to M2 ([Figure 1](#)).

[Van den Bossche et al. \(2016\)](#) further emphasize metabolic reprogramming as being a critical event in macrophage polarization. NO appears to play a critical role here, and inhibitors of iNOS might have potential as agents for promoting M2 polarization. An important point to note is that the mechanism may be different in humans. The authors demonstrate a similar lack of plasticity in

human M1 macrophages but the mechanism is unlikely to involve NO. They speculate that the metabolite taconite might be involved (Lampropoulou et al., 2016). The actual mechanism of irreversibility in the M1 phenotype requires further analysis, and as proposed by the authors, may entail epigenetic changes in key genes that might be responsible for the stability of the M1 phenotype. The work also highlights the importance of alterations in the electron transport chain in M1 macrophages as indicated in two other recent studies (Garaude et al., 2016; Mills et al., 2016). Succinate dehydrogenase (complex II) has been shown to be a key control point, driving reverse electron transport in complex I in the LPS-activated macrophage to promote reactive oxygen species generation, a hallmark of the M1 macrophage (Mills et al., 2016).

The study also provides new information on how M2 macrophages arise during the resolution of inflammation. The lack of plasticity in the formed M1 macrophage means that the M2 macrophage must come from precursor macrophages rather than the conversion of the M1 macrophage into M2. However, it might be possible to provide inhibitors that will promote this process in the context of an inflammatory environment. For example,

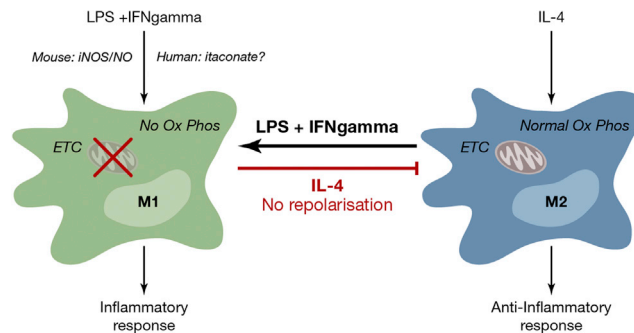


Figure 1. Metabolic Polarization of Macrophages

Treatment of macrophages with LPS + IFN γ leads to NO generation, which impedes the electron transport chain and respiration, driving the pro-inflammatory phenotype of these cells. IL-4 promotes oxidative phosphorylation, which promotes an anti-inflammatory phenotype. The inflammatory M1 macrophage cannot be repolarized to an M2 macrophage by IL-4 unless NO generation is blocked. However, the anti-inflammatory M2 macrophage can be repolarized to an M1 macrophage by LPS + IFN γ .

inhibition of SDH or of the enzyme PKM2 (which governs the switch to glycolysis) will promote an M2 phenotype in response to LPS (Palsson-McDermott et al., 2015). Further studies will help to unravel the complex metabolic events occurring in this most important of cell types and possibly point to therapeutic approaches for manipulating them in inflammatory and allergic diseases and cancer.

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