Interestingly, the effect of antibiotic treatment was limited to two nonadjuvanted vaccines, TIV and another viral subunit vaccine to polio. In contrast, immunization with alum-absorbed toxoids, the HibVenv protein as well as a live-attenuated yellow fever vaccine, were unaffected. Thus, the microbiota has no detectable effect on the induced humoral response to adjuvantated vaccines or attenuated pathogens. Flagellin has long been known to represent a potent immunostimulatory molecule and useful adjuvant. The presented data now reveal an unexpected role of naturally produced flagellin derived from the microbiota to provide costimulation for immune activation. Thus, the intestinal microbiota might effectively take the function of a natural adjuvant. This view is consistent with lessons learned from gut immunology: the microbiota is an essential driver of homeostasis and a key part of an effective immune system.

REFERENCES

For Macrophages, Ndufs Is Enough

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Proinflammatory macrophage activation is coupled to a metabolic switch toward glycolysis. In Cell Metabolism, Jin et al. (2014) show that this process is negatively regulated by mitochondrial electron transport chain complex I through both cell intrinsic and extrinsic pathways.

There is a link between anti-inflammatory properties and mitochondrial oxidative phosphorylation on the one hand, and classical activation and glycolytic metabolism on the other (O’Neill and Hardie, 2013). In their recent paper in Cell Metabolism, Jin and colleagues highlight the importance of complex I (CI) in regulating macrophage activation by showing that deletion of the CI component Ndufs4 causes a switch in the metabolic balance toward glycolysis and associated changes in the monocyte, macrophage, and osteoclast lineage that result in the development of inflammatory disease symptoms and abnormal bone density (Jin et al., 2014).

The electron transport chain (ETC), which links nutrient oxidation to ATP production by oxidative phosphorylation (OXPHOS), consists of complexes I–V in the mitochondrial inner membrane (Figure 1). CI is composed of >45 subunits and is highly regulated by pathways initiated by various extracellular signals (Papa et al., 2012). Unsurprisingly, mutations in genes encoding components of CI are associated with severe clinical disease (Papa et al., 2012). From an immunological perspective, CI is interesting not only because of its core function in metabolism but because along with complex III, it is a site of reactive oxygen species (ROS) production (Sena and Chandel, 2012). ROS plays a role in a range of innate cell functions, and indeed Toll-like receptor (TLR) signaling has been linked to increased production of mitochondrial ROS for bacterial killing (Sena and Chandel, 2012). However, ROS can be detrimental when produced in excess, and this can occur when CI function is compromised (Chen et al., 2007).

*Ndufs4-/- mice have been used to model CI deficiency and have been found to die from encephalomyopathy approximately 7 weeks after birth (Kruse et al., 2012). Using these mice, Jin and colleagues recovered three-weeks after birth, the absence of Ndufs4 led to systemic inflammation, apparent as increased levels of proinflammatory cytokines, systemically increased numbers of Ly6C+ monocytes and the development
of alopecia with macrophage skin infiltration. A component of this response was macrophage intrinsic, because macrophages grown from Ndufs4<sup>−/−</sup> bone marrow expressed inflammatory genes more strongly and anti-inflammatory genes less strongly in the steady state than did wild-type (WT) macrophages. Moreover, macrophages from Ly22-cre-Ndufs4<sup>fl/fl</sup> mice and Tek-cre-Ndufs4<sup>fl/fl</sup> mice, in which Ndufs4 deletion was restricted to monocytes and macrophages or cells of hematopoietic origin, respectively, also exhibited increased baseline expression of inflammatory genes. Nevertheless, these mice did not exhibit the skin disorder or monocyte and macrophage imbalances or early lethality of Ndufs4<sup>−/−</sup> mice. This finding led the authors to look for macrophage extrinsic factors that could contribute to the complex pathologic changes in the Ndufs4<sup>−/−</sup> animals.

Human cases of CI deficiency are marked by lactic acidosis, which reflects the fact that cells lacking functional CI have to utilize aerobic glycolysis to make ATP, and the major end product of this pathway is lactic acid (Figure 1). Jin and colleagues found that Ndufs4<sup>−/−</sup> mice also had elevated serum lactate levels, along with elevated serum triglycerides and free fatty acids (FAs) (Figure 1). The changes in lipids are consistent with the reported inhibitory effects of loss of CI function on mitochondrial FA oxidation (Leong et al., 2012) (Figure 1). Elevated serum lipids were not apparent in Alb-cre-Ndufs4<sup>fl/fl</sup> mice but were apparent in Alb-cre-Ndufs4<sup>fl/fl</sup> mice, in which Ndufs4 was deleted in hepatocytes. The authors reasoned that the inability of the liver (and other organs) to oxidize FAs in the absence of Ndufs4 and resultant increases in plasma triacylglycerols and free FAs could be a contributing extrinsic factor to the more aggressive pathologic features in whole body compared to conditional knockouts, because in type 2 diabetes and atherosclerosis, for example, elevated plasma triacylglycerols and free FAs are associated with inflammation. In these settings, inflammation and disease can be ameliorated by deletion of TLR2 and/or TLR4 (discussed in Erridge and Samani, 2009). In this vein, the authors found that the gross inflammatory conditions in Ndufs4<sup>−/−</sup>/Tek-2,Trl<sup>−/−</sup> mice were significantly diminished in Ndufs4,Tlr2,Trl<sup>−/−</sup> mice even though serum FAs, triglycerides, and lactic acid remained elevated. Furthermore, the targeting of TLR4 with a chemical inhibitor was able to prevent at least one of the consequences of Ndufs4 deficiency—alopecia.

In addition to documenting the effects of loss of CI function on macrophage biology, Jin et al. examined CI biology in osteoclasts. These cells, which like macrophages can also differentiate from monocytes, are responsible for bone resorption. Differentiation to osteoclasts is associated with mitochondrial biogenesis and increased oxidative metabolism. Consistent with this, bone density was greater in Ndufs4<sup>−/−</sup> mice than in WT mice. This was partially due to macrophage-intrinsic effects of the loss of Ndufs4 on osteoclast differentiation but was exacerbated by central metabolic processes in the liver, because changes in bone density were apparent in Alb-cre-Ndufs4<sup>fl/fl</sup>, as well as in Tek-cre-Ndufs4<sup>fl/fl</sup> mice, and were exacerbated when Ndufs4 was deleted in both osteoclasts and hepatocytes (Figure 1). Deletion of Ndufs4 in osteoclasts or hepatocytes had the powerful effect of preventing the development of LPS-induced experimental arthritis and bone loss despite the fact that inflammatory gene expression was exacerbated, indicating that, depending on context, loss of CI activity can have pronounced beneficial effects.

This work establishes the importance of CI in maintaining metabolic homeostasis in monocyte-derived cells. It emphasizes the balance between OXPHOS and aerobic glycolysis in the biology of these cells and indicates that facets of disease due to CI deficiency in people might reflect...
altered in macrophage biology that are amenable to therapeutic targeting of TLR signaling. Further, it addresses the issue of whether changes in metabolism are merely reflective of changes in cellular activation or rather play an initiating role in these processes; the fact that inhibiting "OXPHOS was able to prime macrophage activation in a cell-autonomous fashion provides strong support for the latter view. In this regard, this study makes intriguing and valuable contributions to our understanding of macrophage and osteoclast biology. However, as with many complex and wide-ranging studies, some interesting findings remained less deeply explored than others. Of particular interest here is the identity of the macrophage and osteoclast extrinsic signals that alter the biology of these cells in Ndufs4−/− mice and which appear to require TLR2 and/or TLR4. What are these exactly? The authors found that lactic acid, as well as palmitic and linoleic FAs, were able to activate macrophages and inhibit osteoclast differentiation in vivo and/or in vitro. However, while there has been considerable historic interest in the possibility that endogenous lipid species act directly as agonists for TLRs, this scenario is now questioned (Erridge and Samani, 2009). Other possible endogenous TLR agonists have emerged, such as the damage-associated molecular pattern molecule high-mobility group box 1 (HMGB1), and in other settings where plasma fats are elevated LPS from commensal bacteria has been implicated in promoting TLR4-dependent inflammation (discussed in Erridge and Samani, 2009). It is conceivable that Ndufs4 deletion could result in changes in HMGB1 release and/or in barrier epithelial integrity leading to low-grade endotoxemia, either of which might affect macrophage or osteoclast biology. With regard to lactic acid, Jin et al. are not the first to show a proinflammatory effect of this metabolite on macrophages, but these findings contrast with recently published data showing that lactic acid induces the expression of alternative activation markers that are linked to the regulation of inflammation (Colegio et al., 2014). It will be important to deconvolute this area because concentrations of lactic acid vary not only due to metabolic disease but also as a result of cancers and infections, and understanding the effects of this metabolite on macrophages and related cells might provide clues for the development of relevant therapeutics.

Consistent with the known importance of CI in ROS production, Ndufs4−/− mice exhibited evidence of increased ROS production in all tissues examined. Moreover, Ndufs4−/− macrophages made significantly more ROS at baseline than did WT macrophages and were induced to make additional ROS by palmitic acid. Consistent with the damaging effects of excessive ROS, Ndufs4−/− macrophages expressed the stress sensors Gadd45α/β and were more apoptotic. In patients with tumor necrosis factor receptor (TNFR)-associated periodic syndrome, excessive ROS sensitizes TLR4 signaling (Sena and Chandel, 2012), and it is tempting to conclude that a similar situation exists in Ndufs4−/− macrophages. However, a thought at least should be given to the possibility that the NLRP3 inflammasome is involved in the inflammatory consequences of loss of CI function. This innate sensor is localized to mitochondria and is activated by ROS (Sena and Chandel, 2012) and is able to initiate interleukin-1β (IL-1β) activation, which is then able to further activate macrophages to make additional inflammatory cytokines. The downstream inflammatory consequences of this pathway would be expected to be similar to those reported in the paper. This is particularly intriguing in light of the report that palmitic acid is able to activate the NLRP3 inflammasome through a ROS-dependent pathway (Wen et al., 2011).

In summary, the paper by Jin et al. provides insights into the role of CI at the interface between metabolism and immunity and provides further support for anti-inflammatory effects of OXPHOS. Indeed, in certain cases it seems that Ndufs4 is enough to prevent the emergence of life-threatening inflammation.

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