

## Improving Mitochondrial Function in Viral Infection: Targeting Cellular Metabolism

Host-mediated immunopathology is a common feature of respiratory viral pathogens, including influenza and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). For this reason, targeting of immune dysregulation that mediates lung injury is an attractive proposition that could be leveraged to generate therapeutics for broad classes of lung pathogens. In recent years, an increasing focus on structural and immune cell metabolism has revealed interesting findings regarding the balance between glycolysis and oxidative phosphorylation (OXPHOS) as the primary mechanism for ATP generation. Several studies, initially in macrophages, have implicated the transcription factor hypoxia-inducible factor (HIF)-1 $\alpha$  in mediating the shift toward glycolysis during infection (1). This phenomenon, originally described in cancer cells and often referred to as the “Warburg effect,” describes the shift to glycolysis as the source of ATP in cells under stress.

Recent studies have shown that infection of macrophages or lung epithelial cells with influenza, SARS-CoV-2, or respiratory syncytial virus results in increased glycolysis and stabilization of HIF-1 $\alpha$ , which forms a reinforcing feedback loop (2–6). Recruited and resident lung macrophages differ with respect to the extent of HIF-1 $\alpha$  effects, with infiltrating macrophages exhibiting greater glycolysis and inflammatory cytokine production after influenza infection (7). Interestingly, in lung epithelial cells, inhibiting HIF-1 $\alpha$  was shown *in vitro* to limit viral replication (4, 5, 8). Conversely, lung epithelium-specific deletion of HIF-1 $\alpha$  resulted in an increase viral burden, inflammation, and mortality in the influenza model (9). These studies are complemented by work done by Nolan and colleagues (10). They demonstrated that inhibiting glycolysis with 2-deoxy-D-glucose in mice infected with influenza worsened hypoxia and inflammation (10). These changes were seen in the absence of an effect on viral load, suggesting that alterations in cellular metabolism significantly impact disease outcome independently of antiviral immunity.

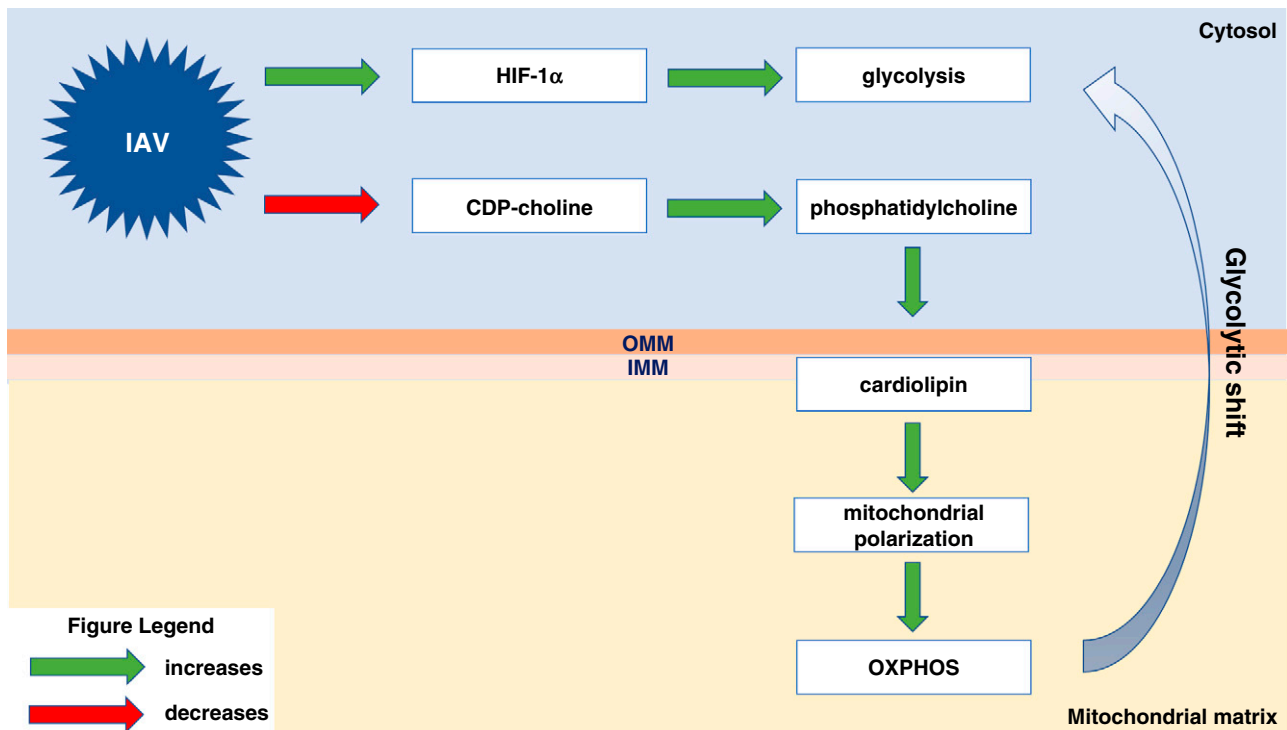
Alveolar type II cells are critical to the production of surfactant in the lung and are highly metabolically active. Woods and colleagues showed that influenza infection decreases the production of the major components of lung surfactant, including phosphatidylcholine and its precursor cytidine 5'-diphosphocholine (CDP-choline) (11). This finding implicated changes in lipid biosynthesis within alveolar type II cells in the pathogenesis of influenza infection. They later demonstrated that treatment of influenza-infected mice with CDP-choline resulted in decreased airway resistance and inflammation and increased oxygenation (12). A single dose 5 days after influenza infection was shown to have efficacy in mitigating reductions in lung function. These findings revealed the exciting potential for targeting alveolar type II cell metabolism and raised further questions regarding lipid biosynthesis and cell metabolism in the context of influenza infection.

In this issue of the *Journal*, Doolittle and colleagues (pp. 682–693) report on their exploration of the impact of CDP-choline treatment on mitochondrial function within alveolar type II cells using the influenza mouse model (13). Functional mitochondria are required for normal OXPHOS. CDP-choline serves as a precursor for phosphatidylcholine synthesis, a major component of the mitochondrial phospholipid bilayer and an acyl donor for cardiolipin, another mitochondrial phospholipid that is essential for cristae formation and maintenance of normal OXPHOS (14). Doolittle and colleagues show that glucose uptake was significantly increased in the lungs of influenza-infected mice. However, glucose uptake was not affected by CDP-choline treatment, suggesting that CDP-choline does not alter lung-specific increased rates of glycolysis. Next, they determined if OXPHOS is impaired in mitochondria isolated from infected alveolar type II cells. They found that influenza infection decreased basal oxygen consumption rate, which coincided with reduced ATP synthesis. Daily CDP-choline treatment restored basal oxygen consumption rate and ATP synthesis in alveolar type II cells from influenza-infected mice, indicating that CDP-choline treatment corrected impaired OXPHOS. Lactate is an acidic by-product of glycolysis, and its accumulation can be used as a proxy for glycolytic rate. Influenza infection caused an increase in the extracellular acidification rate, which coincided with increased lactate concentrations in the BAL fluid, regardless of CDP-choline treatment.

Doolittle and colleagues then looked more closely at the source of ATP production in alveolar type II cells. They found that influenza infection decreased ATP production by OXPHOS and increased ATP production by glycolysis. CDP-choline treatment restored ATP production by OXPHOS to mock-infected control levels, but it also exhibited high degrees of ATP production due to glycolysis, leading to higher total ATP concentrations. Together, these data indicate that CDP-choline treatment was able to restore OXPHOS to preinfection levels but did not block the glycolytic shift in alveolar type II cells. Mitochondria must maintain membrane potential to preserve OXPHOS capacity. Mitochondrial membrane potential was shown to be lower in alveolar type II cells from influenza-infected mice. Daily CDP-choline treatment prevented mitochondrial depolarization, likely contributing to CDP-choline's positive impact on OXPHOS. Electron leakage from complexes 1 and 2 of the electron transport chain can generate mitochondrial reactive oxygen species (mtROS), and Doolittle and colleagues found higher amounts of mtROS in alveolar type II cells from influenza-infected mice. CDP-choline treatment did not affect mtROS generation, indicating that increases in ROS are likely not caused by dysfunctional mitochondria. Alveolar type II cells from influenza-infected mice had a high number of structurally abnormal mitochondria with low surface area, which is suggestive of fragmentation. CDP-choline treatment restored

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**Figure 1.** The effect of influenza infection on alveolar type II cell metabolism. Influenza infection causes a reduction in cytidine 5'-diphosphocholine (CDP-choline) synthesis, which is a precursor for phosphatidylcholine, a mitochondrial phosphoprotein present in both the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM), where it acts as an acyl donor for cardiolipin. Cardiolipin is a mitochondrial phosphoprotein essential for proper cristae formation and stabilization of the electron transport chain super complexes, leading to maintenance of mitochondrial polarization and oxidative phosphorylation (OXPHOS). Without sufficient CDP-choline synthesis, alveolar type II cells lose the capacity to produce essential mitochondrial phosphoproteins, leading to mitochondrial depolarization and reduced OXPHOS. Paired with influenza-induced activation of hypoxia-inducible factor (HIF)-1 $\alpha$ , which promotes glycolysis, alveolar type II cells experience a glycolytic shift 6 days after infection. IAV = influenza A virus.

mitochondrial mass and partially restored mitochondrial morphology. Together, these data suggest that CDP-choline treatment is acting directly on alveolar type II mitochondria to restore their structure and function back to homeostatic levels.

Finally, Doolittle and colleagues examined the effect of CDP-choline on alveolar type II cell cardiolipin concentrations during influenza infection. They found that cardiolipin was decreased in alveolar type II cells from influenza-infected mice. The restoration of OXPHOS and mitochondrial membrane potential in alveolar type II cells in CDP-choline-treated mice was attributed to their ability to preserve cardiolipin concentrations, which help stabilize electron transport chain super complex assembly and cristae morphology. This mechanism potentially explains why CDP-choline treatment restored mitochondrial metabolism but did not affect glycolytic shift (Figure 1).

These findings bridge the gap between alveolar type II cell metabolism and lung function during influenza infection in mice. CDP-choline may be an attractive therapeutic that can preserve mitochondrial function and OXPHOS without altering the glycolytic shift during infection. This is an important aspect because inhibiting glycolysis *in vivo* was shown to be detrimental in influenza infection (9, 10). CDP-choline is a common dietary supplement available in many countries worldwide. Neurologic effects of repeated use of CDP-choline have been studied with unclear benefit or detriment; however, the use of CDP-choline acutely in infectious disease therapy

is largely unstudied. Recently, CDP-choline was discussed as a potential adjunctive therapy in coronavirus disease (COVID-19) (15). Promising findings in the mouse model underscore the need for additional work to define the potential for CDP-choline therapy in advanced preclinical models of influenza and SARS-CoV-2 infection. ■

**Author disclosures** are available with the text of this article at [www.atsjournals.org](http://www.atsjournals.org).

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## References

1. Knight M, Stanley S. HIF-1 $\alpha$  as a central mediator of cellular resistance to intracellular pathogens. *Curr Opin Immunol* 2019;60:111–116.
2. Codo AC, Davanzo GG, Monteiro LB, de Souza GF, Muraro SP, Virgilio-da-Silva JV, et al. Elevated glucose levels favor SARS-CoV-2 infection and monocyte response through a HIF-1 $\alpha$ /glycolysis-dependent axis. *Cell Metab* 2020;32:498–499.

3. Li S, Zhao F, Ye J, Li K, Wang Q, Du Z, *et al.* Cellular metabolic basis of altered immunity in the lungs of patients with COVID-19. *Med Microbiol Immunol (Berl)* 2022;211:49–69.
4. Morris DR, Qu Y, Agrawal A, Garofalo RP, Casola A. HIF-1 $\alpha$  modulates core metabolism and virus replication in primary airway epithelial cells infected with respiratory syncytial virus. *Viruses* 2020;12:1088.
5. Ren L, Zhang W, Zhang J, Zhang J, Zhang H, Zhu Y, *et al.* Influenza A virus (H1N1) infection induces glycolysis to facilitate viral replication. *Viral Sin* 2021;36:1532–1542.
6. Zhu B, Wu Y, Huang S, Zhang R, Son YM, Li C, *et al.* Uncoupling of macrophage inflammation from self-renewal modulates host recovery from respiratory viral infection. *Immunity* 2021;54:1200–1218.e9.
7. Woods PS, Kimmig LM, Meliton AY, Sun KA, Tian Y, O'Leary EM, *et al.* Tissue-resident alveolar macrophages do not rely on glycolysis for LPS-induced inflammation. *Am J Respir Cell Mol Biol* 2020;62:243–255.
8. Duan X, Tang X, Nair MS, Zhang T, Qiu Y, Zhang W, *et al.* An airway organoid-based screen identifies a role for the HIF1 $\alpha$ -glycolysis axis in SARS-CoV-2 infection. *Cell Rep* 2021;37:109920.
9. Zhao C, Chen J, Cheng L, Xu K, Yang Y, Su X. Deficiency of HIF-1 $\alpha$  enhances influenza A virus replication by promoting autophagy in alveolar type II epithelial cells. *Emerg Microbes Infect* 2020;9:691–706.
10. Nolan KE, Baer LA, Karekar P, Nelson AM, Stanford KI, Doolittle LM, *et al.* Metabolic shifts modulate lung injury caused by infection with H1N1 influenza A virus. *Virology* 2021;559:111–119.
11. Woods PS, Doolittle LM, Rosas LE, Joseph LM, Calomeni EP, Davis IC. Lethal H1N1 influenza A virus infection alters the murine alveolar type II cell surfactant lipidome. *Am J Physiol Lung Cell Mol Physiol* 2016;311:L1160–L1169.
12. Rosas LE, Doolittle LM, Joseph LM, El-Musa H, Novotny MV, Hickman-Davis JM, *et al.* Postexposure liponucleotide prophylaxis and treatment attenuates acute respiratory distress syndrome in influenza-infected mice. *Am J Respir Cell Mol Biol* 2021;64:677–686.
13. Doolittle LM, Binzel K, Nolan KE, Craig K, Rosas LE, Bernier MC, *et al.* Cytidine 5'-diphosphocholine corrects alveolar type II cell mitochondrial dysfunction in influenza-infected mice. *Am J Respir Cell Mol Biol* 2022;66:682–693.
14. Li XX, Tsoi B, Li YF, Kurihara H, He RR. Cardiolipin and its different properties in mitophagy and apoptosis. *J Histochem Cytochem* 2015; 63:301–311.
15. Turana Y, Nathaniel M, Shen R, Ali S, Aparasu RR. Citicoline and COVID-19-related cognitive and other neurologic complications. *Brain Sci* 2021;12:59.