Is the microbiome-induced glycolytic pathway a harbinger of acute exacerbation of idiopathic pulmonary fibrosis?

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Idiopathic pulmonary fibrosis (IPF) is a chronic age-related disease with increasing prevalence that is characterised by progressive loss of lung function, which contributes to accelerated morbidity and mortality. While the exact aetiology is unknown, studies in patients with IPF and murine models of lung fibrosis have established genetic mutations[1 2] and telomere dysfunction in alveolar epithelial cells[3] as molecular drivers that lead to a series of events involving aberrant repair, fibroblast activation, senescence reprogramming, epithelial to mesenchymal transition and inflammatory cell recruitment to the fibrotic niche. After onset, disease progression is variable, with some patients suffering acute exacerbation. Although the cause of acute exacerbation remains unknown, they may be encountered in patients exposed to secondary insults, such as environmental pollutants like chitin polysaccharides,[4 5] smoking and altered immune response.[6]

To better understand the aetiology of acute exacerbation, several groups investigated the role of microbiome in IPF.[7–10] Altered and increased bacterial burden is a common finding across all these studies. Molyneaux *et al* employed 16S bacterial ribosomal RNA sequencing to show there is increased bacterial burden in IPF lungs compared with control subjects and that patient survival was inversely correlated with bacterial burden.[7] In the Correlating Outcomes with biochemical Markers to Estimate Time-progression in IPF (COMET-IPF) study, Han and colleagues found a significant association between relative abundance of *Streptococcus* and *Staphylococcus* with IPF disease progression.[8] More recently, in a bleomycin model of lung fibrosis, O'Dwyer *et al* report that lung dysbiosis precedes peak lung injury and persists during fibrogenesis. Moreover, germ-free mice were protected from mortality resulting from pulmonary fibrosis.[9]

These studies demonstrate that microbial subtypes and cellular pathways are dysregulated during acute exacerbation in patients with IPF. Ingenuity pathway analysis (IPA) of bronchoalveolar lavage (BAL) fluid by Huang *et al*[11] identified immune pathways that correlated with progression-free survival (PFS) and microbial diversity. Increased abundance of *Streptococcus* operational taxonomic unit 1345 correlated with decreased immune response and poor PFS. Further, they demonstrated that host–microbiome interactions influence fibroblast responsiveness. *Streptococcus* was the most common genus, followed by *Prevotella* and *Veillonella* in BAL fluid of patients with IPF. Pneumolysin, a pore-forming toxin from *Streptococcus pneumoniae* was shown to exacerbate fibrosis in murine model.[12] However, the mechanism by which *S. pneumoniae* aggravates fibrosis was unclear.

In response to pathogen invasion, host cells undergo reprogramming, including activation of the glycolytic pathway. Disruption of glycolysis is also implicated in sepsis.[13] However, its role was unclear in fibrosis until recently when glycolytic reprogramming was reported to play a role in lung fibrosis pathology.[14] Glycolysis is a physiological process responsible for regulating glucose metabolism. Among various glucose transporters, glucose transporter (GLUT)1 is a key glucose transporter that is highly conserved in mammalian cells.

The importance of GLUT1-dependent glycolysis in fibrosis was demonstrated by Cho *et al*,[15] who reported that GLUT1-dependent glycolysis in the lungs of aged mice was significantly higher compared with young mice. Moreover, pharmacological inhibition of GLUT1 attenuated lung fibrosis. Following up on their study, Cho *et al* identified a novel mechanism where GLUT1-dependent inflammasome activation exacerbated lung fibrosis. In this study, the authors report that a secondary insult with *S. pneumoniae* infection in bleomycin-treated mice resulted in exacerbation of fibrosis. The authors chose *Streptococcus* for secondary infection because it is reported to be abundant in patients with IPF with acute worsening of their disease.[8] In this study, GLUT1 expression was selectively elevated in bleomycin+*S. pneumoniae*-treated lungs. Myeloid cell-specific deletion of GLUT1 using LysM-cre mice ameliorated glycolysis, fibrosis and morbidity.

Inflammasomes (NLRP1, NLRP3, IPAF1 and AIM2) are multiprotein complexes that assist in maturation of proinflammatory cytokines to participate in immune defence. Fluctuations in the glycolytic pathway signal inflammasome activation.[16] Exposure to pathogens, pathogen-associated molecular patterns or danger-associated molecular patterns activates NLRP3 inflammasome.[17] NLRP3 inflammasome is also activated in response to high extracellular glucose levels.[18] Conversely, Moon *et al*[19] reported that mammalian target of rapamycin complex 1 (mTORC1)-induced glycolysis activates the absent in melanoma 2 (AIM2) inflammasome, which is also a cytosolic dsDNA sensor. Because circulating cell-free mitochondrial DNA (mtDNA) is elevated in patients with IPF, Cho *et al* hypothesised that AIM2 inflammasome can potentially recognise mtDNA contributing to acute exacerbation of fibrosis. In the BAL samples of the bleomycin+*S. pneumoniae* group, increased inflammatory cells with augmented AIM2 inflammasome activation localised to alveolar macrophages. To determine the role of NLRP3 inflammasome, when NLRP3$^{-/-}$ mice were stimulated with bleomycin+*S. pneumoniae*, increased collagen deposition, inflammatory cells and mediators such as AIM2 and interleukin (IL)-1β were still observed. This indicates that these processes occur independent of NLRP3.

To understand the mechanism between GLUT1-dependent glycolysis and AIM2 inflammasome activation, siRNA-mediated knockdown of GLUT1 in bone marrow-derived macrophages from NLRP3$^{-/-}$ mice resulted in significant decrease in glycolysis. Treatment with poly (dA:dT), a potent inflammasome activator, decreased downstream activation of procaspase 1 and pro-IL-1β. To determine whether GLUT1-dependent glycolysis regulates AIM2 inflammasome activation, *LysMCre-GLUT1*$^{fl/fl}$ mice treated with bleomycin+*S. pneumoniae* abrogated activation of AIM2, and caspase 1 p20 complex activation was significantly

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diminished. These data confirm that GLUT1-dependent glycolysis indeed regulates AIM2 activation in macrophages. To confirm the role of AIM2 downstream GLUT1 activation, when AIM2 knockout mice were treated with bleomycin+*S. pneumoniae*, total collagen protein levels, caspase activation and IL-1β levels were decreased. This phenotype was not evident when Aim2 knockout mice were treated with bleomycin alone, indicating that AIM2-dependent regulation of fibrosis is specific to *S. pneumoniae* infection.

Accumulating evidence suggests that the microbial population is elevated in lungs of patients with IPF, compromising host defence and leading to heightened inflammation and risk of acute exacerbation. Metabolomic approaches in microbiome research has laid foundation to focus on specific genera which are otherwise not possible due to difficulty in culturing certain bacterial species. The findings in this study by Cho and colleagues have relevant clinical implications. They provide evidence of a plausible mechanistic link between bacterial infection, glycolysis, AIM2 inflammasome and progressive lung fibrosis. Limitations of this study include the use of LysM-cre mice, where cre is expressed not just in macrophages but neutrophils, dendritic cells and monocytes as well. Usage of macrophage-specific Cx3cr1-cre driver mice would strengthen that the findings are specific to macrophages. Despite using bone marrow-derived macrophages from AIM2 whole-body knockout, the use of macrophage-specific AIM2 deletion would make the study more affirmative. Finally, although AIM2 levels were found to be higher in IPF lungs, the cells expressing AIM2 need further identification.

Although the findings from the study by Cho *et al*[20] provide novel mechanistic insight regarding the relationship between bacterial infection and lung fibrosis, it also raises additional questions that remain to be answered. For example, are these mechanistic findings relevant to other microbes associated with IPF exacerbation? Are these findings relevant to bacterial load or bacterial subtypes? Do other GLUT transporters contribute to AIM2 inflammasome activation? Currently, there is no unified approach to treating acute exacerbation in IPF. Answering these questions will address whether GLUT1 and AIM2 activation are central mediators of acute exacerbation of IPF. Establishing broad relevance would support pursuing them as therapeutic targets.

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