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COMMENTARY

Influenza Casts a Lung Shadow

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Influenza A viruses (IAVs) are a major cause of infectious morbidity and mortality worldwide. IAV is highly prone to genomic change over time. Yearly epidemics of IAV result from accumulated point mutations in viral envelope proteins such that adaptive immune responses to prior strains are no longer protective. IAV pandemics result from acquisition of whole new genome segments from animal viruses (generally porcine or avian strains). Although most infected people recover uneventfully, mortality from IAV remains a challenge in certain vulnerable groups, including those at extremes of age, with lung disease, pregnancy, heart disease, or diabetes. However, IAV can also cause mortality in young, otherwise healthy adults. This has been more evident during pandemics of IAV, but also occurs during seasonal epidemics. IAV can kill by either causing diffuse lung injury or altering host defense against secondary bacterial infection. Often bacterial super-infections occur in patients partially recovered from the primary IAV infection.

Murine models have provided important insights into the causes of diffuse lung injury or secondary bacterial pneumonia. Despite having a limited genome (eight main gene segments encoding approximately ten to eleven proteins) as compared with DNA viruses, IAV causes a remarkably complex innate immune response.[1] One of the surprising features of IAV infection is that pathological and immunological changes in the lung persist long after clearance of the virus.[2] The article by Pociask et al[3] in this issue of *The American Journal of Pathology* provides important new insights into the mechanism of these prolonged effects.[3] Although there are clearly important differences between IAV infection of mice and humans, the bacterial super-infection model in mice has many parallels to human infections. In both cases the greatest period of vulnerability appears to be at some delay after the initial viral infection (approximately 7 days post viral infection) and to lead to marked worsening of the bacterial pneumonia. Alcorn et al (along with other groups) have made major contributions to our understanding of the causes of post IAV bacterial super-infection.[4–7] The breakdown in anti-bacterial defense is likely multifactorial, but virus-induced Type I interferon generation leading to suppression of Type 17 immunity is one critical mechanism.

The article by Pociask et al[3] shows that important changes in the lung persist up to two months after viral infection. Day 21 post viral infection was studied most extensively. At this time point, there is ongoing inflammation based on elevated inflammatory cell counts and increased CD4, CD8, and interferon-γ positive lymphocytes in bronchoalveolar lavage fluid. This ongoing inflammation is present despite recovery of weight by the mice and essentially undetectable viral loads in the lung. Significant histopathological changes in the lung are also present including epithelial metaplasia and airspace occlusion, and these changes persist up to 60 days post infection. Of interest, despite the varied changes present at day 21, the mice no longer show increased susceptibility to *Staphylococcus aureus* superinfection based on bacterial loads. Some of the immune changes seen during bacterial superinfection at earlier time points (eg, day 7 post viral infection) persist, including depression in IL-17 generation and neutrophil influx (as compared with mice infected with *S. aureus* without prior viral infection). However, the mice infected with *S. aureus* 21 days after viral infection have strongly increased IL-22 production and no longer have depressed Reg3b expression.

IL-22 has been shown to reduce inflammation and to protect against bacterial superinfection post IAV infection.[8,9] IL-22 promotes antimicrobial peptide production in the lung, which Dr. Alcorn's laboratory have shown to

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be depressed at day 7 post IAV infection.[10] Based on transcriptomic analysis in the current study, several anti-microbial peptides show increased expression at day 21 and 35, and this may contribute to the ability of these mice to control *S. aureus* super-infection despite reduced neutrophil influx. Another beneficial effect of IL-22 in the context of IAV infection is promotion of lung repair.[11]

Reg3b is an anti-microbial lectin which has been studied extensively for its role in protection against bacterial infection in the gut.[12] Reg3b can bind bacteria, but, unlike the related protein Reg3g, it has not been clearly demonstrated to work through direct bactericidal activity. Rather, it has been shown to inhibit bacterial translocation and to act as an alarmin, recruiting other inflammatory cells, including neutrophils. In an intestinal model of inflammation (dextran-induced colitis), Reg3b induced recruitment of IL-22−secreting neutrophils resulting in decreased inflammation and improved epithelial reconstitution.[12]

Another key finding of the paper by Pociask et al[3] include demonstration of marked lung transcriptomic changes at days 21 and 35 post IAV including genes associated with endoplasmic reticulum stress which was confirmed at the level of protein expression as well (ie, increased CCAAT-enhancer binding protein homologous protein and activating transcription factor staining in the lung). Other up-regulated genes at days 21 and 35 include cytokines and other inflammatory mediators, antimicrobial peptides, but also genes associated with tissue remodeling and repair. These findings correlate with the bronchoalveolar lavage and histological findings.[3] The overall picture derived from the transcriptomic findings is one of prolonged inflammation and epithelial injury long after viral clearance.

Finally, important findings were obtained by use of miRNA microarray. Specifically, miR-155 expression was increased, and expression of 26 known target genes for miR-155 were differentially modulated (some increased and others decreased) post IAV infection. miR-155 has previously been shown to be increased post IAV infection in mice and to worsen outcome of post-IAV bacterial super-infection.[13] miR-155 promotes lung inflammation in response to LPS in part by inhibiting SOCs expression,[14] to promote lung fibrosis[15] and lung cancer progression.[16] In the present study,[3] miR-155 was found to worsen the extent of weight loss, and the degree sustained lung injury (histology) and ER stress at day 21. This was demonstrated using miR-155 knockout mice. Absence of miR-155 did not affect viral loads, inflammatory cell infiltrates or most cytokines at day 7 or day 21. Hence, miR-155 seemed to be mainly inhibiting lung reparative processes at the later stage post infection.

Overall, the paper by Posiask et al[3] shows that IAV infection leads to prolonged changes in the lung well after apparent viral clearance and specifically highlights the potential role of lung repair mechanisms in successful recovery from IAV infection. It is tempting to speculate on potential treatment of severe IAV infection with combinations of antivirals and measures to promote lung repair including IL-22 or Reg3b or inhibitors of miR-155. A potential caveat to such an approach include the reports that miR-155 is protective versus *Mycobacterium tuberculosis* infection in mice.[17] In addition, proteins known to accelerate lung epithelial proliferation might increase viral replication.

Another possible implication of the results is the consideration that the sustained changes seen after IAV infection could relate to its known ability to cause sometimes sustained worsening of lung function in patients with cystic fibrosis[18] or chronic obstructive pulmonary disease.[19,20] It must be noted that the findings by Pociask et al[3] are in a well-defined mouse model using a highly mouse adapted IAV strain, hence, ultimately their relevance in models more closely resembling human infection (or in human infection) will need to be determined. An additional line of inquiry will be to determine the effect of other viral strains or specific viral components on the sustained lung injuries caused by IAV.

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