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RESEARCH HIGHLIGHT

Seedy CD8+ T_{RM} cells in aging lungs drive susceptibility to pneumonia and sequelae

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Aging is a leading risk factor for pneumonia and subsequent comorbidities, including chronic pulmonary diseases.[1] The underlying causes of age-related susceptibility to pneumonia-related morbidity and mortality are complex and poorly understood. While aging has been associated with many changes in diverse immune compartments, if and how aging affects resident immune cells in the lung have not been examined. A recent study by Goplen et al.[2] made seminal contributions to this knowledge gap by demonstrating that aging lungs have a propensity to recruit excessive and aberrant numbers of $CD8^+$ resident memory T (T_{RM}) cells after viral pneumonia, and then, these T_{RM} cells drive chronic inflammatory processes in the lung and exacerbate fibrotic lung disease (Fig. 1). By fostering comorbidities that predispose patients to pneumonia, this deleterious immune response to pneumonia may contribute to a positive feedback loop of unhealthy aging.[1]

After infection, older lungs recover poorly. Goplen et al. compared infections of younger (2-month old) and older (22-month old) mice with a mouse-adapted strain of influenza A virus (IAV) known as PR8. All the younger mice survived the infection, whereas half of the older mice did not. The older mice exhibited slightly more inflammation (shown by increased numbers of monocytes and neutrophils) during the infection, despite comparable viral elimination by day 15 in both the younger and surviving older mice. Recovery from PR8 infection is notoriously difficult for mice and results in aberrant epithelial and tertiary lymphoid structures.[3,4] Goplen et al. observed that older mice struggled more intensely. After 60 days, pulmonary inflammation was more widespread throughout the lungs of the older mice, which continued to exhibit elevated numbers of monocytes and neutrophils. Using Nanostring to compare the pulmonary transcripts of >500 immune-related genes, ~10% of the genes were differentially expressed in younger mice 60 days after infection, whereas >70% of the genes were differentially expressed in older mice. Bulk lung transcriptome analyses at day 60 confirmed that older lungs had excessive signals for inflammation and adaptive immunity compared to younger lungs. Of note, younger lungs (compared to the older lungs) displayed enrichment for gene signatures associated with tissue repair, suggesting that aged lungs might also have poorer tissue recovery. The changes in adaptive immune genes in aged mice reflected the elevated numbers of B cells, $CD4^+$ T cells, and $CD8^+$ T cells in their lungs compared to those in the lungs of younger mice. These lung lymphocytes included excessive numbers of IAV-specific $CD8^+$ T cells in older mice, which were identified as T_{RM} cells based on surface marker phenotype analysis and parabiosis experiments. Thus, after IAV infection, older lungs exhibit prolonged inflammation and excessive numbers of $CD8^+$ T_{RM} cells.

Excessive $CD8^+$ T_{RM}-cell accumulation is driven by old lungs rather than by old lymphocytes. To determine whether the excessive accumulation of $CD8^+$ T_{RM} cells in older lungs was due to aging lymphocytes or to T cell-extrinsic causes (such as aging lungs), Goplen et al. performed adoptive transfer experiments in which antigen-specific $CD8^+$ T cells from young mice were transferred into younger or older host mice that were then infected with PR8 viruses expressing the relevant antigens. Even younger $CD8^+$ T_{RM} cells accumulated excessively in older lungs. Thus, the aging lung or some lymphocyte-extrinsic factors mediate this excessive T_{RM}-cell accumulation. The older lungs expressed greater levels of TGF-β mRNA than the younger lungs after recovery from IAV infection, and $CD8^+$ T cells without TGF-β receptor II signaling did not accumulate in older lungs. Altogether, these studies suggest that changes in older lung tissues, which include but may not be limited to overabundant TGF-β expression, cause an excessive accumulation of $CD8^+$ T_{RM} cells after the resolution of pneumonia.

$CD8^+$ T_{RM} cells in older lungs are not only numerous but also dysfunctional. Resident memory lymphocytes in the lung are important for protection against heterotypic infections.[1,5] To test if the enhanced accumulation of $CD8^+$ T_{RM} cells in aged lungs confers protection against heterosubtypic IAV, Goplen et al. challenged older and younger mice with a lethal dose of the X31 strain of IAV 60 days after PR8 infection. While all the younger mice achieved immune protection due to PR8 exposure and survived X31 infection, most of the aged mice succumbed to the heterotypic infection despite the increased density of $CD8^+$ T_{RM} cells in their lungs. Single-cell RNA sequencing of the IAV-specific $CD8^+$ T_{RM} cells from the lungs of the older and younger mice revealed different clustering of cells at the different ages, and both the sequencing data and antigen stimulation assays suggested that aged $CD8^+$ T_{RM} cells were characterized by deficient IFN-γ production. In contrast to their aberrant accumulation, which was cell extrinsic, aberrant IFN-γ expression was cell intrinsic and reflected defects in the older lymphocytes rather than in the older lungs. Thus, aged lungs excessively accumulate $CD8^+$ T_{RM} cells that are intrinsically hyporesponsive to antigen challenge and ineffective at protecting against respiratory infection.

Chronic inflammation and fibrosis are products of $CD8^+$ T_{RM} cells in older lungs recovering from pneumonia. Although they are

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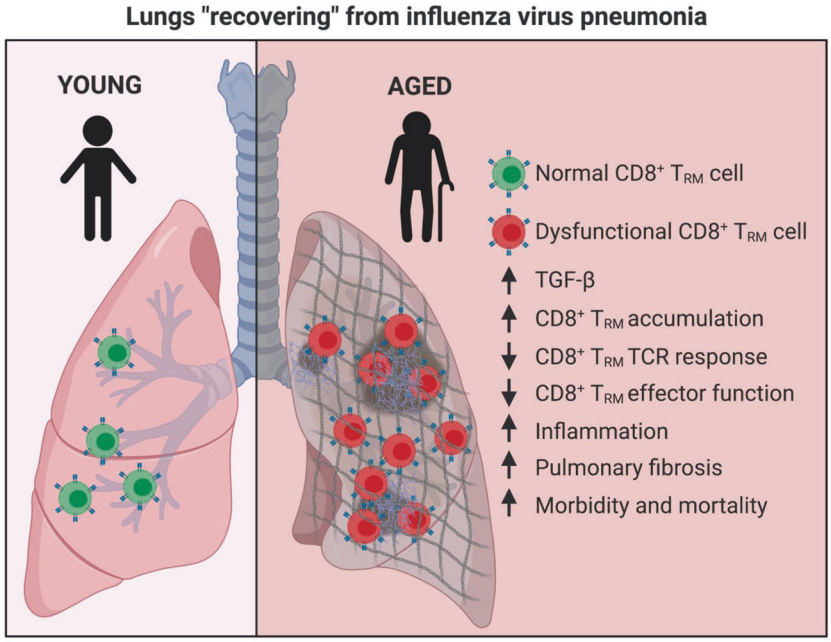

Fig. 1 $CD8^+$ T_{RM} cell-dependent chronic lung sequelae after viral pneumonia in aged mice. After influenza infection, the lungs of aged mice produce elevated levels of TGF-β, leading to hyperaccumulation of intrinsically dysfunctional $CD8^+$ T_{RM} cells. These cells are less responsive to TCR stimulation and are weak antiviral effectors but drive persistent pulmonary inflammation and fibrosis. This figure was created using BioRender.com

poor responders that do not protect against infection, aged $CD8^+$ T_{RM} cells are immunologically active in the lungs, promoting chronic pulmonary inflammation and fibrotic damage. The depletion of $CD8^+$ cells at day 21 after PR8 infection was sufficient to diminish the chronic inflammation observed at day 60, including the percentage of the lung that was inflamed, the numbers of extravascular monocytes and neutrophils, and the transcripts for proinflammatory mediators. Collagen deposition was also reduced by $CD8^+$ cell depletion, suggesting that the fibrosis in the aged lungs was driven by excessive and aberrant T_{RM} cells. The cellular and molecular mechanisms by which $CD8^+$ cells drive chronic inflammation and fibrosis remain undetermined and exciting areas for future investigation. Since $CD4^+$ T_{RM} cells can enhance acute pulmonary inflammation by posttranscriptional regulation of neutrophil-regulated chemokine genes in lung epithelial cells,[6] it will be interesting to see if $CD8^+$ T_{RM} cells exploit a similar type of signaling axis to enhance persistent inflammation in aged lungs.

Although this study contributes to the advancement of research, this study has limitations. The research was performed in mice, and human correlates have yet to be explored. Infections of mice with PR8 cause long-term tissue remodeling that includes a degree of lymphoid aggregates and epithelial anomalies that may not be typical of respiratory infections with influenza viruses or other microbes,[3,4] so extrapolating to other respiratory pathogens requires caution. Goplen et al. studied older mice that were naive to IAV, which does not account for the naturally acquired heterotypic immune memory that typifies adult mammals in the wild. Microbial histories change systemic and tissue immunity and make the immune responses of laboratory mice more closely resemble those of adult humans.[1,7–9] The lack of infectious disease and microbiome histories in these mouse studies may influence the excessive and aberrant T_{RM}-cell responses in the aged lungs that were observed here. Despite these limitations, the studies by Goplen et al. are ground-breaking due to their compelling discovery that aging lungs recruit excessive numbers of $CD8^+$ T_{RM} cells, that aging $CD8^+$ T_{RM} cells are ineffective for preventing heterotypic infection, and that lung $CD8^+$ T_{RM} cells promote chronic inflammation and pulmonary fibrosis after recovery from influenza infection.

Pneumonia is a key feature of aging, since older people are more susceptible to pneumonia and older people suffer more greatly from pneumonia.[1] As ~20% of the global population approaches ≥65 years of age,[10] understanding the disorders of pulmonary immunity that are causes or consequences of pneumonia has become increasingly necessary. The COVID-19 pandemic and its pronounced impact on the elderly population further emphasize these needs. The study by Goplen et al. highlights a specific immunological defect in aging lungs that both increases susceptibility to pneumonia and exacerbates downstream pneumonia sequelae. Future studies should further investigate when and how $CD8^+$ T_{RM} cells accumulate excessively or behave inappropriately. Measures or correlates of $CD8^+$ T_{RM}-cell function in humans have become an important goal and might be useful for identifying individuals with increased susceptibility to pneumonia or with a greater propensity for chronic inflammatory responses, fibrotic sequalae, or other long-term outcomes after pneumonia. For those in whom deleterious $CD8^+$ T_{RM}-cell activities mediate long-term sequelae of pneumonia, inhibiting $CD8^+$ T-cell activities may be an avenue worth exploring for therapeutic approaches. Insights into age-associated dysfunctions in lung immunity against infection, such as those from Goplen et al., are helpful for developing precision medicine strategies for preventing and treating pneumonia and its disastrous consequences in this most relevant population of pneumonia-susceptible subjects.

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AUTHOR CONTRIBUTIONS

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ADDITIONAL INFORMATION

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