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PP2A: A Novel Target to Prevent Cathepsin S–mediated Damage in Smoking-induced Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is a recognized global health crisis, with smoking being the most important and well-studied risk factor for disease development and progression (1). The World Health Organization estimates that 80 million individuals live with moderate to severe COPD, and this disease will become the third leading cause of death worldwide by 2030 (2). COPD is characterized by chronic inflammation and obstructed airflow, usually originating from long-term exposure to particulates, with the most egregious offender being cigarette smoke. Various signaling pathways are implicated in the induction of lung inflammation associated with COPD pathogenesis. Dysregulation of phosphatases such as PP2A (protein phosphatase 2A), protein tyrosine phosphatase 1B, and pTEN (phosphatase and tensin homolog) are known to occur (3, 4). Imbalances in the activities of proteases, including serine, aspartic, metal-activated, and cysteine proteases, are also linked to the severity and progression of COPD (5).

CTSS (cathepsin S) is an endopeptidase member of the C1 family of cysteine proteases. Unlike most cathepsin proteases, which exhibit maximal activity at acidic pH, it has a relatively unusual ability to exhibit activity across a wide range of pH values. Accordingly, CTSS plays diverse physiological roles, including participation in immune responses, lysosomal protein catabolism, and extracellular matrix remodeling (6). It is particularly important in inflammation and immunity, participating in antigen presentation by cleaving invariant chain (Ii) to CLIP, which permits associated major histocompatibility complex II protein to load and present antigen. CTSS activity is implicated in many pulmonary diseases, including asthma and allergic inflammation (7), as well as alveolar remodeling and pulmonary emphysema in COPD (8, 9).

In this issue of the *Journal*, Doherty and colleagues (pp. 51–62) report two novel and interrelated findings obtained using a mouse model of chronic exposure to cigarette smoke (10). First, they establish that CTSS gene and protein expression is induced by

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cigarette smoke in the lung, leading to high enzymatic activities in lung tissue and BAL fluid (BALF), and furthermore that this elevated activity is directly implicated in smoke-induced loss of lung function. They do so by comparing the effects of cigarette smoke on immune cell infiltration and loss of lung function in wild-type versus $Ctss^{-/-}$ mice. Total immune cell counts in the BALF, as well as infiltrating alveolar macrophages and neutrophils, were reduced in the lungs of knockout mice exposed to smoke relative to smoke-exposed wild-type mice, although T-cell, B-cell, and eosinophil numbers were constant between the strains. Moreover, functional measures suggest that suppression of emphysematous changes accompany knockout of CTSS. These findings are suggestive of a driving role for CTSS in smoke-induced COPD in this murine model, although reduced functioning of immune pathways associated with global CTSS insufficiency may contribute to this protective phenotype.

The second novel finding is the authors' implication of PP2A activity as being protective against the pathological consequences of CTSS induction in COPD. PP2A is known to be reduced in bronchial tissue of subjects with COPD (11). In this study, Doherty and colleagues found that human bronchial epithelial cells isolated from subjects with COPD showed enhanced expression and secretion of CTSS compared with those cultured from healthy control subjects. Transfection of PP2A or SMAP, which activates PP2A, had protective effects against induction of CTSS activity, whereas introduction of siRNA to PP2A potentiated induction of CTSS. Furthermore, SMAP treatment of mice exposed to acute or chronic smoke exposure prevented CTSS induction and smoke-induced loss of lung function. The authors conclude by suggesting that PP2A activation represents a novel new target for the treatment of COPD.

The findings of this elegant study are compelling and should energize translational efforts in this much-needed area. CTSS regulation is, however, complicated by findings that biologically elevated CTSS activity is due as much to a decreased abundance of endogenous CTSS inhibitors, such as cystatin C, as it is to actual increases in CTSS protein (12, 13). The findings by Doherty and colleagues show that the increased CTSS activity elicited by cigarette smoke in BALF exceeded the actual increase in protein abundance, suggesting that additional factors that modulate CTSS activity may also participate here in the disease process by failing to inhibit CTSS. These factors may or may not be responsive to regulation by PP2A. In addition, the current study addresses only targets associated with smoking-induced COPD. A number of individuals with COPD may be nonsmokers, suggesting that other risk factors (e.g., genetics, asthma, air pollution, biomass gases, and other environmental factors) also play a role in its induction (14). Next, it will be important to determine whether CTSS and PP2A are similarly induced in cigarette smoke–independent COPD, and whether other proteases and phosphatases that are also implicated in chronic inflammation and emphysema represent additional targets.

One possible side effect of using exogenous CTSS inhibitors for prolonged periods is that the body's feedback loop may kick in to maintain CTSS activity by increasing CTSS production/activation, as was shown in a recent phase I clinical dose escalation study of a cathepsin inhibitor, LY3000328 (15). Therapeutic modulation of CTSS activity through multiple regulatory avenues, such as boosting PP2A activity, as an approach to treat COPD could thus represent a viable alternative or additional approach. However, activation of PP2A alone for an extended period may weaken the immune system and increase the risk of secondary infection (16). Agents that affect the pathways identified in this novel study may thus have a lower therapeutic index, as is the case with other potent immunomodulatory agents such as rapamycin and cyclosporine A, and require more in the way of therapeutic monitoring during development. However, many of the CTSS inhibitors that are currently in clinical development are for systemic autoimmune diseases such as psoriasis (https://www.clinicaltrials.gov, identifier: NCT00396422), primary Sjögren's syndrome (https://www.clinicaltrials.gov, identifier: NCT02701985), and rheumatoid arthritis (https://www.clinicaltrials.gov, identifier: NCT00425321) and other related diseases, and use oral delivery approaches that are more likely to elicit systemic side effects. The ability to treat COPD via local targeting of both CTSS and PP2A through pulmonary delivery modalities could be advantageous for developing formulations to minimize exposure of the rest of the body to unwanted side effects.

In summary, the current study elegantly links the various mechanisms involved in smoking-induced pathogenesis of COPD, with direct potential to guide new translational applications for treatment of this pervasive disease. ■

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Herpesviruses: Silent Instigators of Lung Injury after Hematopoietic Cell Transplant

Although recent advances in the care of allogeneic hematopoietic cell transplantation (allo-HCT) recipients have improved outcomes for this population (1), the overall success of allo-HCT continues to be tempered by noninfectious pulmonary complications. Mortality from idiopathic pneumonia syndrome (IPS) occurring early after transplant remains unacceptably high (2). Bronchiolitis obliterans syndrome (BOS) is a devastating diagnosis associated with diminished quality of life and increased nonrelapse mortality (3). The ability to prevent and treat these conditions has been hampered by a limited understanding of the clinical and biological factors that contribute to their development. In this issue of the *Journal*, Zhou and colleagues (pp. 63–74) build on accumulating epidemiologic evidence of the role of viruses in noninfectious lung disease and provide new insight into the pathogenesis of alloimmune-mediated lung injury after HCT (4).

Reactivation of herpesviruses is common in the first 100 days after allo-HCT and is associated with overall and nonrelapse mortality (5). Using a cohort of over 700 allo-HCT recipients, Zhou and colleagues demonstrate that first infection with human herpesvirus 6 (HHV-6) or Epstein-Barr virus (EBV) is an independent risk factor for IPS, and first post-transplant cytomegalovirus (CMV) infection increases the risk of BOS. The authors applied rigorous statistical methods that considered first viral infection as a time-dependent covariate, adjusted for confounding variables, and accounted for variable follow-up as well as the competing risks for death or disease relapse. Zhou and colleagues then recapitulated these epidemiologic observations in a novel murine model in which mice were infected with an HHV-6 homolog and allowed to develop latent infection before mismatched HCT. Six weeks after HCT, lungs from these preinfected mice demonstrated increased interstitial and peribronchiolar inflammation and BAL fluid TNF-α protein as compared with control mice. These mice also developed skin and gut pathology consistent with acute graft-versus-host disease (GVHD). Notably, the preinfected mice had evidence of viral reactivation by the presence of lung tissue viral polymerase expression but undetectable viral DNA in the BAL fluid (4).

The striking observation here is that reactivation of viruses that are not specific to the lung may be causal for pulmonary injury. Although CMV is a well-recognized cause of pneumonitis in the immunocompromised population, the roles of HHV-6 and EBV are less clear. A provocative study demonstrated detection of HHV-6 DNA in BAL fluid from 20% of patients who were previously diagnosed with IPS (6), suggesting a possible role of HHV-6 in mediating this condition or as an unrecognized cause of pneumonitis. Zhou and colleagues now provide compelling evidence that HHV-6 reactivation is not merely a bystander but may directly contribute to the development of acute lung injury after allo-HCT. In the current study, first post-transplant infections with herpesviruses were also associated with acute GVHD, and a recent meta-analysis further supports this link (7). Taken together, these findings challenge the paradigm of IPS, which is considered noninfectious by definition, and suggest that acute lung injury occurring early after allo-HCT can be a manifestation of an alloimmune response triggered by latent herpesvirus reactivation. Interestingly, first-onset viral infection with CMV was associated with BOS. Although this observation may be surprising given the significant time lapse between infection and disease manifestation, it is consistent with findings in lung transplant recipients, in whom BOS is a more frequent complication (8).

The hypothesis that arises from these findings is that viral infections alter the host immunologic profile in a way that precipitates a proinflammatory and subsequent profibrotic milieu that contributes to acute and chronic organ injury. Furthermore, these results suggest that the immunological sequelae of viral reactivation develop even

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