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C-rac-king the Code of Smoke-induced Pneumonia Susceptibility

Pneumonia causes a tremendous burden of disease, impacting more individuals than HIV, cancer, diabetes, and many other leading public health priorities (1). Although impressive, these burdens are underestimates, because respiratory infections and their resulting host countermeasures influence the occurrence and/or severity of virtually all lung diseases including cancer and chronic obstructive pulmonary disease, conditions with well-established ties to tobacco smoking (1, 2). The threat of pneumonia is also much greater among smokers (3, 4). As many as one in three pneumonia cases has been attributed to smoking (3), which represents pneumonia's most significant modifiable risk factor (4). Given the importance of lung infections and the degree to which they coalesce with other diseases (5), discovering mechanistic connections between cigarette smoke and pneumonia has important and broad clinical implications.

Pneumonia outcome is dictated by integrated signals controlling immune resistance and tissue resilience, with the former relying on contributions from numerous cellular sources such as lung epithelium and the resident and recruited leukocyte pools (5). Many of these cell types can be adversely affected by cigarette smoke (2), but precisely how this drives pneumonia susceptibility remains unclear. In this issue of the Journal, Larson-Casey and colleagues (pp. 1288–1301) reveal a compelling connection between macrophage NOX2 (NADPH

oxidase) activity and heightened pneumonia vulnerability in the setting of cigarette smoke (6). Knowing that macrophages are essential for pulmonary host defense (7), due in part to NOX2-derived reactive oxygen species (ROS) (8), the authors investigated the effects of smoking on macrophage responses and host outcome in a mouse model of pneumococcal pneumonia. Cigarette smoke exposure markedly impaired clearance of Streptococcus pneumoniae from the lungs, and this finding was associated with a complete loss of infection-induced ROS in BAL cell membrane fractions. Importantly, pneumonia-induced cytokines and BAL leukocyte accumulation were unaffected by cigarette smoke, suggesting that immunodeficiency was likely a consequence of diminished cellular function rather than limited recruitment.

While investigating potential effects of cigarette smoke on the NOX2 complex, the authors found that $p67^{phox}$ and Rac2 (Rac family small GTPase 2), which is required for the former's membrane translocation, were both reduced after smoke exposure. Substantial in vitro and in vivo evidence supports their conclusion that abrogated Rac2 responses are causally linked to impaired defense in this setting. Pneumococcal infections in $Rac2^{-/-}$ mice phenocopied the effects of cigarette smoke to a remarkable degree, eliminating BAL cell membrane ROS generation, and reducing bacterial killing and survival with no appreciable changes in leukocyte recruitment or inflammatory cytokine induction. This finding alone does not firmly establish Rac2 as the functional liaison between smoke and immunodeficiency. However, the fact that cigarette smoke exposure failed to elicit measurable effect in $Rac2^{-/-}$ mice

lends strong support to this hypothesis, as does their finding that Rac2 overexpression in mouse lungs (predominantly macrophages) completely reversed immunodeficiency due to either smoke exposure or Rac2 deficiency. To begin addressing mechanisms whereby cigarette smoke promotes the loss of Rac2, the authors measured a panel of smoke-associated metals in mouse BAL fluid and identified cadmium as the sole elevated representative. Impressively, cadmium administration, like Rac2 deficiency, recapitulated the effects of smoke during pneumonia, and mechanistically, the authors' data suggest that both cigarette smoke and cadmium target Rac2 by limiting isoprenylation at C-189, a requisite post-translational modification for Rac2 function.

Overall, the use of multiple complementary approaches elegantly supports mechanistic relationships among smoking, cadmium, Rac2 deficiency, and pneumonia susceptibility, but whether this finding is applicable to human disease is a critical albeit more difficult question to address. To this end, the authors found that cadmium concentrations were significantly and selectively (vs. other metals) elevated in BAL cells from human smokers, and this finding again correlated with substantial loss of membrane Rac2 and $p67^{phox}$ localization and ROS synthesis. The proposed importance of Rac2-mediated immunity is consistent with rare observations in children bearing dominant negative Rac2 mutations, which is associated with immunodeficiency similar to that observed in patients with leukocyte adhesion deficiency and chronic granulomatous disease (9). The current investigations raise exciting possibilities for new research directions aimed at unveiling the functional relationship between Rac2 and pneumonia in smokers.

Another remaining question pertains to the macrophage-specific nature of the authors' findings. Their data implicate macrophages and not neutrophils as the predominant site of Rac2 dysfunction after smoke exposure, because depletion of the former but not the latter diminished ROS formation and antibacterial defense. This is surprising given the long-established role of neutrophils in lung immunity, both clinically and experimentally (10, 11). Indeed, Rac2 itself has been linked to neutrophil-derived IFN-γ and antibacterial defense in the lungs of mice challenged with pneumococcal pneumonia (12), whereas the experimental circumstances of the present study do not indicate an essential role for this cell type. It will be interesting and important to determine whether these findings in macrophages extend to other cell types, including but not limited to neutrophils, as experimental conditions vary. Even among macrophages themselves, both resident and recruited, functional heterogeneity (13) demands a more comprehensive interrogation of macrophage subsets. Last, and notably, others have reported increased oxidative stress in macrophages exposed to cigarette smoke (14, 15), possibly as a source of lung tissue damage. Although this seemingly contradicts the results of the current study by Larson-Casey and colleagues, differences could certainly be attributable to where (membrane vs. total) and/or how such observations were made, with responses to live bacteria after smoke exposure being entirely different from those recorded under alternative conditions.

This work represents a significant advance for a staggeringly harmful relationship that has long frustrated lung researchers and clinicians. The mechanistic basis of pneumonia susceptibility in smokers is surely more complex than the effect of one metal (cadmium) on one biological process (NOX2 activity) in one cell type (macrophages). Yet, the authors have provided a compelling piece of the puzzle and an interesting platform for future investigations. ∎

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Renewed Promise of Nonionizing Radiation Imaging for Chronic Lung Disease in Preterm Infants

Preterm birth remains a significant cause of morbidity and mortality. Low birth weight accounts for up to 34% of infant deaths nationally (1). More than 40% of postnatal deaths are attributable to lung disease, and almost 40% of deaths in preterm infants at least 28 days old are the result of chronic lung disease of prematurity (CLDP) or bronchopulmonary dysplasia (BPD), a common lung disease second only to asthma.

BPD is variably diagnosed at 28 days after preterm birth or at 36 or 40 weeks' postmenstrual age (PMA), based on physician-determined need for supplemental oxygen (2). Although the diagnosis of BPD reproducibly predicts persistent respiratory disease (PRD) during the early years of infancy and childhood, many children with moderate to severe BPD do not have persistent symptoms, whereas others not qualifying for the diagnosis do require ongoing or intermittent medical care for pulmonary disease. The contemporary Prematurity and Respiratory Outcomes Program found that more than 75% of 721 infants born younger than 29 weeks PMA with moderate and severe BPD had first-year PRD, marked by respiratory hospitalizations, home respiratory support, medications, and/or symptoms of cough and wheeze, yet PRD was absent in 21%, whereas 60% of those with no or mild BPD had PRD compared with approximately 30% of normal full-term infants, demonstrating a need to improve methods for prediction of lung disease in infants (3). Histopathologically, with improvements in ventilators, oxygen therapy, and nutrition, CLDP is less dominated by inflammatory infiltrates, epithelial metaplasia, and fibrosis, and more typically by a bland inflammation with alveolar developmental arrest, small airway and vascular abnormalities, interstitial edema, and scattered fibrosis, resulting in a heterogenous disease (4). Pulmonary function tests of infants with severe BPD, studied at 52 weeks' PMA, showed predominantly obstructive disease, but restrictive (9%) or mixed (40%) restrictive and obstructive phenotypes also occurred, all with varying responses to bronchodilators (5). Improvements are needed to monitor these and other confounders of CLDP, including chronic aspiration and respiratory mechanics compromised by intestinal dysfunction, poor linear growth, and osteoporosis.

In the neonatal intensive care unit, this multifactorial, multidimensional CLDP is monitored largely by two-dimensional and toxic applications of ionizing radiation delivered in frequent chest X-rays and less frequent, but no less toxic, cardiac catheterizations and computed tomography scans. Treatment consists of disappointingly arbitrary use of oxygen, methylxanthines and other bronchodilators, steroids, antibiotics, and diuretics. For those requiring chronic ventilation, a transition occurs from reduced compliance and short time constants managed with short inspiratory times and rapid respiratory rates, to obstructive chronic disease requiring longer inspiratory and expiratory times for equilibration. Yet when that transition occurs is unclear (6). Worse, heterogeneity and V̇/Q̇ mismatch characterize the disease, especially when complicated by viral infection or environmental triggers of mucus production and airway reactivity. Routine chest X-rays demonstrate areas of atelectasis, subjectively distinguished from infiltration and scarring, scattered in overall hyperinflated lungs with little insight into presence of airway or vascular abnormalities, such that application of focused and personalized therapy and prognostication is markedly imprecise. Computed tomography imaging in pediatrics, although improved in recent years to reduce radiation dose and diagnostic efficacy, remains reserved for complex patients and is rarely performed serially because of the associated need for intubation, sedation, and ionizing radiation (7).

In this issue of the Journal, Higano and colleagues (pp. 1302–1311) demonstrate significant advances in image processing with the potential to improve on the diagnosis of BPD and to identify heterogeneity in sufficient detail to direct specific therapy and to monitor progression, persistence, or improvement of disease with therapy without ionizing radiation (8). Expanding on previous comparisons in term and preterm infants (9), the current study examines 42 free-breathing neonates, including 20 with severe BPD and nine control patients, all at "term" PMA (40 ± 3 wk), with proton-based magnetic resonance imaging (MRI), without intravenous contrast, using gradient echo and ultrashort echo-time, spiral acquisition sequences, and modified Ochiai scoring (10). The imaging distinguished CLDP severity and predicted short-term respiratory outcomes better than the usual clinical measures, including the 36-week, oxygen-use-dependent diagnosis of BPD. Overall, mean MRI scores directly correlated with respiratory disease severity as none (term), none (preterm), mild, moderate, severe, and death because of BPD. The scores also predicted respiratory support at neonatal intensive care unit discharge, suggesting the potential to guide interventions such as antiviral prophylaxis and chronic ventilation, as well as to optimize therapy to reduce airway dysfunction.

Advantages of the MRI approach are first and foremost nonionizing radiation, such that longitudinal scans are acceptable. Such serial assessments in neonates uniquely could provide four-dimensional evaluation of disease status, improving differential diagnosis (11), prognostication, measurements of therapeutic efficacy and outcomes, and determination of pathogenesis. Anomalies of airways and vascular structures that contribute to variable severity of the disease could be detected without invasive bronchoscopy and radiographic contrast. Advances in respiratory-gated MR image processing, cardiac MRI, cardiorespiratory