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COMMENTARY

Why Do We Need a Nonhuman Primate Model of Smoking-Induced COPD?

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The accelerating worldwide use of tobacco products causes immense human suffering. Smoking tobacco products ultimately kills up to half of its estimated 1 billion worldwide users: on average, one death every 6 seconds or 5.4 million per year (World Health Organization, *http://www.who.int/tobacco/mpower/tobacco_facts/en*, last accessed on December 10, 2014). Besides its role in cardiovascular and cerebrovascular diseases and a host of malignancies, tobacco smoking is the principal cause of chronic obstructive pulmonary disease (COPD) in the industrialized world. COPD is the only one of the top 10 leading causes of death that is increasing in incidence, now occupying the third position in the United States, and projected by the World Health Organization to be the leading cause of death worldwide within this century. With the proliferation of new forms of nicotine delivery[1,2] that challenge efforts to control tobacco use, there is an increasing urgency to understand COPD pathogenesis.

In this issue of *The American Journal of Pathology*, Polverino et al[3] report their development of an experimental COPD model in cigarette smoke (CS)-exposed nonhuman primates (NHPs). This collaboration between research groups at Brigham & Women's Hospital (Boston, MA) and the Lovelace Respiratory Research Institute (Albuquerque, NM) combines pathological, physiological, and immunological analyses to produce a comprehensive and interesting report. But even acknowledging the global importance of understanding COPD pathogenesis, readers might question whether the expense, requirement for highly specialized research facilities, and ethical issues surrounding NHP research justify this undertaking. We attempt to explain why they do and to highlight key features of this novel model that fill gaps in existing animal models.

The Accelerating Global Disease Burden of COPD

COPD comprises a spectrum of conditions characterized by incompletely reversible airflow obstruction and chronic respiratory symptoms, but also by significant systemic components. It encompasses a complex range of lung pathologies, including emphysema, airway fibrosis, mucus hypersecretion, and the disappearance of small airways.[4] The variable coexistence of these processes in individuals contributes to specific and pleomorphic phenotypes. Once these pathological process are initiated in susceptible smokers, they generally progress even after the cessation of active smoking. Although COPD is manifestly an inflammatory condition, it responds poorly, if at all, to current anti-inflammatory agents. This inexorable progression means that even if every smoker in the world quit tomorrow, COPD prevalence would continue to increase for 20 to 40 years. Despite progress in smoking cessation in most industrialized nations, tobacco use is increasing rapidly in developing nations.

Worldwide growth of COPD is also driven by air pollution, especially indoor inhalation of biomass fuel. Inadequate ventilation of fumes from wood, dung, and lignite used in cooking and heating results in toxic exposure of an estimated 3 billion individuals, disproportionately women and children.[5] Verifying whether biomass fuel inhalation and other forms of non−tobacco-related toxic exposures induce identical pathology and the full range of COPD phenotypes as induced by CS is an important area of ongoing investigation. Because existing data support the strong similarity

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of the processes, it is likely that therapeutic advances in smoking-induced COPD could also benefit the millions in the developing world who will develop disease from exposures other than CS.

Importance and Limitations of Current Animal Models of COPD

The inability of current therapies to stop COPD progression mandates the development of new drugs, a process that necessarily uses animal models for preclinical testing. Animal models are also crucial for the bidirectional process of target discovery: They permit the validation of the molecular mechanisms identified as potentially involved in human disease, and they allow for the generation of novel hypotheses that can be tested for relevance in human pathological specimens.

Models of COPD already exist in multiple animal species.[6] Those using mice enjoy the many advantages of that species: small size, high fecundity, abundant and well-defined genetic variation between strains, robust transgenic technology, and unparalleled immunological reagents.[7–9] The small size of mice presented initial challenges for the imaging and measurement of pulmonary function; however, technological advances including micro computed tomography[10] now permit sophisticated serial nonlethal end points. Murine CS-exposure models have been highly useful in defining the effects of smoking on pulmonary immunity[11] and the responses to relevant lung pathogens.[12–14] However, without the addition of microbe challenge, these models tend to produce rather modest pathological changes, and without additional manipulations, they do not induce chronic bronchitic changes. Such deficiencies have motivated the development of murine models more closely mimicking the full range of pathological changes found in advanced COPD in humans. Several groups have done so by combining intrapulmonary elastase and lipopolysaccharide,[10,15] whereas others have succeeded via transgene expression[16] or by combining CS exposure with innate immune stimulation.[17]

But murine models of CS-induced COPD will always have significant, inescapable limitations due to vast differences in anatomy between rodents and humans. For pathogenesis studies, the most glaring are the absence of bronchial submucosal glands, of dichotomously dividing airways or distinct respiratory bronchioles, and of well-defined lobular architecture. Moreover, laboratory mice have defined microbiomes that differ markedly from ours, resulting in immune responses that similarly differ from those of humans. And, of course, murine pharmacodynamics and pharmacokinetics, although very well understood, are quite disparate from those of humans.

To date, no COPD model in larger species has sufficient advantages imparted by physical size or similarity to human respiratory anatomy to overcome the deficits of lack of robust transgenic technology and limitations of immunological reagents. However, after the meritorious features of NHPs to model obstructive airway diseases were reviewed in 2008,[18] it was perhaps inevitable that someone would take up the challenge of developing one via CS exposure.

Development of an NHP Model of CS-Induced COPD

Polverino et al[3] describe two series of experiments in female cynomolgus macaques that realize the promise of an NHP model of COPD.[3] In the first, they exposed these NHPs to CS (or air) for up to 4 weeks, with a goal of detecting the early changes in lung inflammation and mucus metaplasia that precede overt development of COPD in humans. In the second series, exposures were lengthened to 12 weeks, searching for remodeling of small airways, development of emphysema or intrapulmonary lymphoid aggregates, and evidence of systemic inflammation.

This study used whole-body exposures, the only practical approach with NHPs, but also one that minimizes stress due to immobilization and avoidance behaviors that complicate nose-only exposures in rodents. The exposures at the Lovelace facility were substantial, which may have contributed to their success. Estimated doses, calculated based on pulmonary total suspended particulate matter, approximated humans' smoking 1.8 and 4 packs per day in the acclimation and target exposure periods, respectively.

Results show the development of multiple pathological changes characteristic of human COPD, including mucus metaplasia in both large (cartilaginous) and smaller airways. Lung inflammation was documented by greater absolute numbers of neutrophils and macrophages in the bronchoalveolar lavage fluid, which at 12 weeks' exposure also showed elevated concentrations of CCL2 and CXCL8, the key matrix metalloproteinase MMP-9, and a trend toward increased IL-6. Lung sections supported these inflammatory changes, both by the accumulation of myeloperoxidase-positive neutrophils and CD68-positive mononuclear phagocytes in distal lung and by the detection of parenchymal lymphoid aggregates containing germinal centers. Increased apoptosis of alveolar septal cells was documented by both TUNEL and cleaved caspase-3 assays.

What This Article Adds

This new experimental model complements rodent models in several important manners. Significant and widespread bronchial submucosal glandular hypertrophy was achieved. A particularly interesting finding is the development of robust small airway remodeling, an important pathological change that has been elusive in rodent CS-exposure models in the absence of additional transgene expression. The physical size of NHP permits serial testing, including repeated bronchoalveolar lavage for the analysis of cells and inflammatory mediators, before euthanasia to obtain

pathological specimens. Such paired analyses reduce sample sizes while increasing statistical power. Polverino et al[3] were able to exploit an important advantage of NHP, the cross-reactivity of many antibodies with antihuman reagents, and the model will almost certainly more closely mimic human physiology than most other nonprimate species. The species they studied, *Macaca fascicularis,* is one whose genome is being characterized by the Nonhuman Primate Reference Transcriptome Resource (*http://nhprtr.masonlab.net/website2.html*, last accessed December 10, 2014), which should facilitate eventual comparative studies in patients with COPD.

Limitations and Unanswered Issues

In considering the experimental use of animals for understanding human diseases, it is worth distinguishing the analysis of an animal disease in its natural host (eg, simian immunodeficiency virus in NHPs) versus a simplified model system (eg, the smoke-exposure system used by Polverino et al[3]). The latter generally does not perfectly capture all aspects of the corresponding human disease. Hence, it is not surprising that this model has some limitations. There were no significant differences in pulmonary function, although at 12 weeks there was a nonsignificant trend toward lower forced expiratory volume in 0.1 seconds in CS-exposed NHPs ($P = 0.088$). NHPs did not develop significant emphysema in this model. Emphysema was once thought to develop in only a minority of COPD patients, generally as a late manifestation of advanced disease. However, this area is one of several in which the appreciation of the range of human COPD phenotypes has increased due to newer technologies and the analysis of cohorts less biased toward severe symptoms. Greater use of high-resolution computed tomography has disclosed that emphysema is more common than previously suspected and can be substantial in some subjects with little or no airflow obstruction.[19,20] Fortunately, emphysema is a pathological feature well represented in rodent and especially murine models.

Additionally, Polverino et al[3] studied only female NHPs. Given the important role of sex-specific differences in COPD pathologies,[21] these experiments should be repeated in male NHPs. Doing so, perhaps for somewhat longer durations, might uncover emphysema in male NHPs, as male human smokers are generally more likely than are female smokers to develop emphysema.[20,22]

Probably the greatest limitation of this interesting model is shared with all CS-exposure models of COPD of which we are aware. Despite the use of outbred NHPs, pathological changes appear to be reasonably uniform between the individual animals. For this reason, it remains unclear whether this model system will aid in elucidating the most puzzling issue in COPD pathogenesis: Why do only certain human smokers develop pathology leading to accelerated loss of lung function? To date, even large human genome−wide association studies have not shown that specific genetic differences explain the majority of this susceptibility. Exploration of additional genetic factors, such as noncoding sequences and epigenetic effects from exposures in previous generations, is admittedly only beginning, and may ultimately explain this missing heritability. An intriguing alternative answer to this central mystery of COPD pathogenesis is that it results from complex interactions between the particular immune repertoire of individual smokers (stochastically molded by previous infections) and their total microbiome (especially in the gut, the site of greatest contact between our microbes and our immune systems).

The Future

The laboratories of Dr. Yohannes Tesfaigzi (Lovelace Respiratory Research Institute, Albuquerque, NM) and Dr. Caroline A. Owen (Brigham and Women's Hospital, Boston, MA) are to be congratulated for producing a model that fills crucial gaps in the armamentarium of researchers interested in COPD pathophysiology and treatment. Although predictions are risky, we anticipate that the requirement for specialized primate centers and the current lack of transgenic technology in NHPs may restrict its widespread use in defining pathophysiological mechanisms. Instead, we would envision this new model to be most useful in at least three situations. First, the pharmaceutical industry might employ it for final preclinical testing of novel therapeutics, especially biologics, when there is serious concern that the many disparities between humans and mice could undermine success. Second, this model might be exceedingly useful in deconvoluting the effects of comorbid conditions on COPD phenotypes. Third, the NHP model might be particularly well suited for the study of microbiome−immune interactions, mentioned in *Limitations and Unanswered Issues*. We feel more secure predicting that murine CS-exposure models will continue to be used productively. And that is a good thing, as researchers worldwide need every advantage they can get to combat the scourge of CS-induced diseases.

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