EDITORIALS

Vulnerability and Genetic Susceptibility to Cigarette Smoke–Induced Emphysema in Mice

Cigarette smoke (CS) has been identified as the most important risk factor for the development of chronic obstructive pulmonary disease (COPD). Studies of cigarette/tobacco smoke–induced COPD have primarily utilized small laboratory animals, particularly inbred laboratory mice, as the model of choice. The first comprehensive review of animal models of emphysema relating to the pathogenesis of COPD was published in 2002 (1), and a more recent review provided updates on this topic (2).

The use of this species has several advantages, including a relatively low cost, a rapid reproductive cycle, large litter sizes, and the availability of antibodies and molecular probes developed specifically for mouse research. In addition, many inbred strains and their mutants (transgenic and knockout) are available and a high overall genetic homology exists between the mouse and human genomes. Finally, mouse genes can be manipulated to create global as well as conditional mutants (using CRISPR/Cas and tissue-specific Cre transgenes). However, the mouse has limitations as an animal model of COPD in that it does not fully recapitulate the human disease. One should keep this limitation in mind when planning experiments and interpreting results obtained in this species (3).

Importantly, mice are obligatory nose breathers, so they exhibit a different pattern of particle filtration in the nares and upper respiratory tract compared with humans. However, this does not invalidate the methods commonly used to expose mice to cigarette/tobacco smoke, i.e., by nose only and by whole body, because both approaches model second-hand smoke exposure. Moreover, mouse submucosal glands are restricted to the trachea, and mice lack a significant population of goblet cells in their bronchi and bronchioles. The appearance of clusters of goblet cells in their bronchi/bronchioles after cigarette/tobacco smoke exposure is likely due to metaplasia and not hyperplasia. Additionally, mice do not have respiratory bronchioles and therefore lack the anatomical basis for the development of centrilobular emphysema, a lesion of respiratory bronchioles. Instead, they develop patchy emphysema.

Mice can be exposed to CS delivered by a smoking machine via nose-only (4) or whole-body (5) exposure. The types of cigarettes, chemical emissions, and particulates used, the number of cigarettes smoked per day, and the duration of exposure (in months) have not been standardized, so these aspects differ among laboratories. Generally, either reference (R series) cigarettes from the Kentucky Tobacco Research and Development Center at the University of Kentucky (Lexington, KY) or the commercially available Marlboro Red cigarettes are used. The latter have the advantage of being widely available and commonly used by smokers. CS studies have provided a fundamental understanding of the complex pathogenic cellular and molecular processes, including genetic/epigenetic mechanisms, that are involved in the development of COPD and the testing of potential therapeutics.

Not all strains of mice develop emphysema after chronic CS exposure. In susceptible strains, the severity of emphysema is limited and does not reach the extent seen in human smokers or in other murine models of emphysema. The different susceptibilities to emphysema observed in three strains of mice have been linked to strain-dependent differences in antioxidant defenses in response to CS (6). ICR mice were shown to have increased lung antioxidant defenses (possibly Nrf2, glutathione, glutaredoxin-1, and peroxiredoxin-6) when acutely exposed to CS, whereas C57BL/6J and DBA2 mice did not (6). Of interest, C57BL/6J and DBA2 mice developed significant emphysema when exposed to CS for 6–7 months, whereas ICR mice did not (6). Chronic CS exposure causes other cellular alterations; for example, impaired ciliated epithelial cell function was observed in C57BL/6J mice exposed to whole-body CS for up to 1 year (7). Another study of several mouse strains (i.e., NZWLac/J, A/J, SJ/L, and AKR/J) confirmed the strain dependency response to CS and identified other emphysema-resistant or susceptible strains (8). However, the genetic basis for that variability has not been studied. Recently, a recombinant inbred strain of mice, referred to as the Collaborative Cross (CC), was derived from random mixing of eight different founder strains (9). This CC strain has a high phenotypic diversity that closely mimics human disparity/diversity, thus enhancing investigators' ability to map the causative loci underlying complex disease-related traits (9). Future studies using the CC strain will provide a better understanding of tobacco/CS-induced COPD/emphysema models at the level of systems genetics.

In this issue of the *Journal*, Radder and colleagues (pp. 367–375) show that susceptibility to CS-induced emphysema as measured by alveolar chord length is a variable and continuous trait in 34 inbred strains of mice (10). They demonstrate various degrees of susceptibility, ranging from no response (resistant CBA/J) to extremely susceptible (A/J). The C57BL/6J mice were the second most susceptible to CS. By testing the association of this quantitative trait across the genome and then integrating mouse and human genome-wide scans, Radder and colleagues identified *Abi3bp* as a novel candidate gene contributing to emphysema susceptibility. This gene encodes for ABI family member 3 binding protein (also known as TARSH or eratin), a putative tumor suppressor that is predominantly expressed in the lung. Intriguingly, this protein controls the growth and differentiation of stem and tumor cells while it also promotes senescence in some cells via a p21-dependent pathway (11). Cellular senescence is one of several molecular mechanisms that have been shown to mediate COPD pathogenesis (12). The potential role of the *Abi3bp* gene in pulmonary emphysema requires more investigation in humans and laboratory animals (i.e., knockout mice for *Abi3bp*) to establish the mechanistic contribution of this gene to emphysema susceptibility.

In addition to the study limitations identified by the authors, only female mice were used, and sex-specific differences may be important (13). Also, the authors were unable to detect any regions that strictly met multiple testing correction thresholds. Importantly, alveolar chord length is not a correlate/measure for emphysema, and emphysema is only one pathological entity of COPD. The moderate functional and anatomical responses to

chronic CS exposure in animals are certainly another important limiting factor. There is a lack of information about various lung mechanical properties (e.g., compliance, resistance, and pressure-volume), although the authors used the flexiVent system (SCIREQ, Montreal, Quebec, Canada) for forced oscillatory mechanics measurements and provided the tissue elastance H data. Nevertheless, the findings in this study point to a novel intrinsic factor that may be an important determinant of the disease course.

COPD is a collection of conditions, including emphysema, chronic bronchitis, mucus hypersecretion, bronchiolar and vascular remodeling, and sometimes the development of fibrotic areas scattered throughout the parenchyma, that frequently coexist in humans with this disease. Remarkable variation in the manifestation of COPD is observed among individuals with regard to the severity of symptoms and the rate of decline in FEV_1 (14). Understandably, it is challenging to expect experimental rodent models to fully recapitulate the diverse aspects of the disease in humans.

Future research should seek to identify the basis of COPD exacerbations and clarify the pathophysiological processes that contribute to the multiple phenotypes (susceptibility, severity, rate of progression, propensity to experience exacerbations, and emphysema versus chronic bronchitis) caused by CS or by flavored tobacco products such as electronic nicotine delivery systems, cigars, and waterpipes/hookahs. Different genetic loci may contribute to different aspects of the disease, and may or may not be the same as those that determine susceptibility. Animal models constitute essential tools because they can be used to study a defined phenotype and to identify novel candidate genes. In that regard, mouse strains that develop CS-induced emphysema with or without goblet cell metaplasia, or that develop emphysema with pulmonary fibrosis or emphysema associated with pulmonary hypertension are currently available. The use of relevant mouse strains may thus help to identify candidate genes of probable biological relevance for the pathophysiology of a defined phenotype, and to inform human studies seeking to identify gene linkage quantitative trait locus with COPD (9, 10). ■

Irfan Rahman, Ph.D.
Department of Environmental Medicine
University of Rochester Medical Center
Rochester, New York

Giovanna De Cunto, Ph.D.
Department of Molecular and Developmental Medicine
University of Siena
Siena, Italy

Isaac K. Sundar, Ph.D.
Department of Environmental Medicine
University of Rochester Medical Center
Rochester, New York

Giuseppe Lungarella, M.D.
Department of Molecular and Developmental Medicine
University of Siena
Siena, Italy

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