A new short-term mouse model of chronic obstructive pulmonary disease identifies a role for mast cell tryptase in pathogenesis

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Background: Cigarette smoke–induced chronic obstructive pulmonary disease (COPD) is a life-threatening inflammatory disorder of the lung. The development of effective therapies for COPD has been hampered by the lack of an animal model that mimics the human disease in a short timeframe.

Objectives: We sought to create an early-onset mouse model of cigarette smoke–induced COPD that develops the hallmark features of the human condition in a short time-frame. We also sought to use this model to better understand pathogenesis and the roles of macrophages and mast cells (MCs) in patients with COPD.

Methods: Tightly controlled amounts of cigarette smoke were delivered to the airways of mice, and the development of the pathologic features of COPD was assessed. The roles of macrophages and MC tryptase in pathogenesis were evaluated by using depletion and *in vitro* studies and MC protease 6–deficient mice.

Results: After just 8 weeks of smoke exposure, wild-type mice had chronic inflammation, mucus hypersecretion, airway remodeling, emphysema, and reduced lung function. These characteristic features of COPD were glucocorticoid resistant and did not spontaneously resolve. Systemic effects on skeletal muscle and the heart and increased susceptibility to respiratory tract infections also were observed. Macrophages and tryptase-expressing MCs were required for the development of COPD. Recombinant MC tryptase induced proinflammatory responses from cultured macrophages.

Conclusion: A short-term mouse model of cigarette smoke–induced COPD was developed in which the characteristic features of the disease were induced more rapidly than in existing models. The model can be used to better understand COPD pathogenesis, and we show a requirement for macrophages and tryptase-expressing MCs. (J Allergy Clin Immunol 2013;131:752-62.)

Key words: *Cigarette smoke, chronic obstructive pulmonary disease, inflammation, emphysema, airway remodeling, lung function, macrophage, mast cell, protease, mouse mast cell protease 6, htryptase-β*

Cigarette smoke–induced chronic obstructive pulmonary disease (COPD) is a debilitating disorder of the lung. It is the fourth-leading cause of chronic morbidity and death worldwide, and its prevalence is increasing.[1] The disease is characterized by chronic airway inflammation, mucus hypersecretion, airway remodeling, and emphysema, which lead to reduced lung function and breathlessness.[2,3] Systemic effects also are observed in the skeletal muscle, heart, and other organs. Moreover, patients with COPD are more susceptible to respiratory tract infections. Because the mechanisms that lead to COPD and its sequelae are poorly understood at the molecular level, there are no effective treatments.

A major factor that has hampered the study of COPD is the lack of a small-animal model that recapitulates the hallmark features of the disease in a reasonable time frame. Although LPS and elastase have been used to induce lung damage in rodents that somewhat resemble COPD in human subjects, such single-factor approaches are not representative of the complex pathology that occurs in those patients who smoke for many years.[3] Current models of smoke-induced COPD involve whole-body or nose-only exposure of mice to cigarette smoke.[3] Acute models of 4 days' to 4 weeks' duration have been valuable for evaluating the early smoke-induced inflammatory responses in the lung. However, the smoke-exposed mice in these models do not have emphysema or diminished lung function.[4-8] Chronic models of more than 6 months' duration result in airway remodeling and emphysema but only induce mild alterations in lung function,[4,9-12] and the prolonged time needed to induce these features greatly restricts the use of these models for extensive therapeutic and mechanistic research. This is especially relevant considering the short lifespan of the mouse and the substantial animal and labor expenses needed for 6-month experiments. Thus there is a need

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Abbreviations used
B6: C57BL/6
BALF: Bronchoalveolar lavage fluid
COPD: Chronic obstructive pulmonary disease
FEV_{100}: Forced expiratory volume in 100 ms
FRC: Functional residual capacity
FVC: Forced vital capacity
htryptase-β: Human tryptase-β
MC: Mast cell
mMCP: Mouse mast cell protease
qPCR: Quantitative PCR
TLC: Total lung capacity
WT: Wild-type

for a mouse model of cigarette smoke–induced COPD that has all of the major features of the human condition that are induced in a shorter time frame.

Although mast cells (MCs) have been casually linked to the pathogenesis of COPD, the relevant factors exocytosed from these immune cells have not been identified.[13] MC numbers are increased in inflammatory infiltrates in patients with COPD, which is associated with reduced lung function, airway remodeling, and emphysema.[14,15] MC-derived human (h)tryptase-β levels in sputum correlated with the severity of COPD in one study,[16] and the exposure of IL-3–dependent MCs to cigarette smoke–treated culture medium resulted in increased expression of mouse mast cell protease 6 (mMCP-6).[17] mMCP-6 is also known to promote inflammation, chemokine expression, and macrophage and neutrophil chemotaxis,[18] which are all hallmark features of COPD. Nevertheless, the roles of htryptase-β and mMCP-6 in COPD pathogenesis have not been investigated in depth.

Here we report the development of a mouse model of COPD in which we deliver tightly controlled amounts of cigarette smoke directly into the airways. The exposed mice exhibit the major characteristic features of COPD observed in human subjects after only 8 weeks of smoke exposure, thereby facilitating the discovery, testing, or both of the efficacy of new therapeutics. The model also enables us to elucidate the cellular, biochemical, and molecular mechanisms that underpin the pathogenesis of COPD. In that regard we now show, for the first time, detrimental roles for an MC-restricted tetramer-forming tryptase in experimental COPD.

METHODS

Additional details are described in the Methods section in this article's Online Repository at www.jacionline.org.

Smoke exposure

Wild-type (WT) BALB/c, WT C57BL/6 (B6), and mMCP-6$^{-/-}$ B6 mice[18] were used in the study. In each experiment 12 mice were simultaneously exposed to cigarette smoke (twelve 3R4F reference cigarettes [University of Kentucky, Lexington, Ky] twice per day and 5 times per week for 1 to 12 weeks) by using a custom-designed and purpose-built nose-only, directed-flow inhalation and smoke-exposure system (CH Technologies, Westwood, NJ) housed in a fume and laminar flow hood (see Fig E1 in this article's Online Repository at www.jacionline.org). Each exposure lasted 75 minutes. All experiments were approved by our institutional animal ethics committee.

Airway and lung inflammation, airway remodeling, and emphysema

Airway inflammation was assessed by means of differential enumeration of inflammatory cells in bronchoalveolar lavage fluid (BALF).[19-22] Parenchymal inflammation was assessed by counting the inflammatory cells in 10 randomized fields (×100 magnification) of whole lung sections.[23] RNA was extracted, and transcript levels were assessed by using standard real-time quantitative PCR (qPCR) assays[24] with the primers described in Table E1 in this article's Online Repository at www.jacionline.org.

Macrophage and MC numbers in lung homogenates were assessed by using flow cytometry and histochemistry.[25-27] Airway remodeling was determined by measuring the number of mucus-expressing goblet cells around the airways and by assessing airway epithelial thickening.[22,23,28-30] Emphysema was assessed by using the mean linear intercept technique, which is a standard method for assessing alveolar diameter and emphysema in mice.[28]

Lung function

Forced oscillation and forced maneuver techniques were used to assess lung function parameters.[25,31]

Glucocorticoid treatment

Dexamethasone (1 mg/kg in 50 μL of sterile water; Sigma, St Louis, Mo) was administered intranasally 3 times per week.[32] Control animals were sham treated with sterile water.

Respiratory tract infections

Mice were infected with mouse-adapted strains of *Streptococcus pneumoniae* intratracheally or influenza virus intranasally. Pathogen load was determined by using culture or plaque assays of lung homogenates, respectively.[33-36]

Macrophage and neutrophil depletion

Lung macrophages and neutrophils were depleted by means of intranasal administration of liposome-encapsulated clodronate or intraperitoneal injection of anti-Ly6G antibody (1A8; BioXCell, Lebanon, NH), respectively, 3 times per week.[25,37]

Transcript expression in tryptase-treated macrophages

B6 mouse bone marrow–derived macrophages were cultured in the absence or presence of recombinant htryptase-β (0.8 μg/mL, 25 nmol/L; Promega, Madison, Wis). RNA was isolated, and qPCR assays were used to evaluate the levels of TNF-α, Cxcl1/keratinocyte chemokine, and IL-1β transcripts.

Statistical analyses

Data are presented as means ± SEMs (n = 6-8). Comparisons between 2 groups were made by using a 2-tailed Mann-Whitney test. Multiple comparisons were made by using 1-way ANOVA with the Tukey post-test or Kruskal-Wallis analysis with the Dunn post-test, in which nonparametric analyses were appropriate. Weights were assessed by using 1-way ANOVA (repeated measures). Analyses used GraphPad Prism Software (San Diego, Calif).

RESULTS

Nose-only exposure of WT BALB/c mice to cigarette smoke induces the hallmark features of COPD

We delivered tightly controlled amounts of cigarette smoke into the nares of WT BALB/c mice for 1 to 12 weeks and assessed the hallmark features of COPD. Weight loss was evident within the first week of exposure (Fig 1, *A*). Animals lost 10% of their initial weight after 3 weeks and only regained 5% of this initial weight over the remaining exposure period. In contrast, age-matched nonexposed mice steadily gained weight. Four days of exposure to cigarette smoke led to acute inflammation in the airways characterized by increased macrophage and neutrophil numbers in the BALF (Fig 1, *B*). Inflammation persisted and increased with the additional