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Animal Models of Chronic Obstructive Pulmonary Disease

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Abstract

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the USA and currently there are minimal therapies specific for the treatment of COPD. To advance our knowledge on COPD pathogenesis and develop new therapeutics, animal models are needed that represent key clinical and pathologic features of the human disease. The primary animal models utilized to study COPD rely on several factors associated with disease progression, i.e. genetic and epigenetic changes, environmental exposures and the microbial flora of the lungs. Here, a systematic approach was taken to summarize and evaluate the current animal models employed to study COPD pathogenesis, comorbidities and exacerbations. The strengths and limitations of these disease models are also delineated. The rodent COPD models have been extensively utilized but several studies have highlighted the potential of larger animals as an additional approach. Due to the inherent heterogeneity of COPD, the usefulness of certain animal models may be limiting but still represent helpful means to explore gene functional studies, testing new therapeutics and the exploring the significance of microbial floral changes. Therefore, interpreting the findings from animal models for the study of COPD represents a critical approach in deciding possible future human therapeutics.

Keywords: disease models, COPD, animal, cigarette smoke, lung remodeling, comorbidities, exacerbation

1. Introduction

Chronic obstructive pulmonary disease (COPD) is one of the major cause of morbidity and mortality worldwide and a significant economic and social burden worldwide [1, 2]. COPD

is defined as a disease state characterized by airflow limitation that is not fully reversible, associated with an abnormal inflammatory response of the lungs to noxious particles or gases [3]. Airflow is limited by airway inflammation [4], loss of lung elasticity [5], lung tissue destruction [6] and the narrowing of small airways [7, 8]. Most pulmonologists believe that parenchymal destruction (emphysema) and small-airway obstruction (chronic bronchitis) are the most significant phenotypes of COPD, as they both contribute to airflow limitation [9]. Therefore, when designing models of disease these two phenotypes are crucial when interrelating data to clinical significance. Emphysema is further characterized by augmented inflammation, irreversible destruction of alveolar walls leading to airspace enlargement, loss of elastic recoil and hyperinflation [10]. Most COPD models investigate emphysema characteristics.

Cigarette smoke (CS) inhalation is the primary etiologic risk factor associated with COPD. The age-adjusted mortality for COPD has increased over the past three decades [11] highlighting the need for better therapies [12, 13], increased COPD research and enhanced utilization of research models. In the US, the CS prevalence is estimated to be 15–19% of the US population (age 18 or older) [14] and smoking will remain a major public health issue in the coming years. It is estimated that 16% of all 8th graders have tried smoking and 17% of high school students continue smoking beyond graduation [15]. The smoking prevalence globally remains high, with CS rates at 28% in China [16], 27% in Germany, and 36% in Russia [17, 18]. These statistics will ensure that this disease continues to be a major public health issue for the foreseeable future and CS-induced COPD will likely continue to rise over the next decades. Projects predict COPD to be the third leading cause of death globally by 2030 [19]. Beyond smoking cessation, very limited options currently exist for therapeutic intervention to halt COPD progression. The mechanistic basis underlying the pathogenesis of both emphysema and chronic bronchitis is very complex, involving a combination of recurrent inflammation, enhanced autophagy, oxidative stress, protease/antiprotease imbalance, tissue injury, repair and cell death [20]. These changes are all modulated by environmental exposures and host genetics [21]. Clinical studies demonstrate that all smokers experience a range of pulmonary inflammation but only 15–20% of smokers develop severe progressive emphysema [22]. This disparity underscores the importance of susceptibility factors, a subset of which are almost certainly controlled by host genetics in addition to environmental exposures. Chronic bronchitis is almost three times more frequently diagnosed compared to emphysema in the COPD population in the US but emphysema is associated with a greater frequency of patient deaths [23]. To this extent, new research approaches are required to advance our understanding of the key players involved in COPD development and to identify new therapeutics for future treatment of both emphysema and chronic bronchitis.

To better understand this complex disease, multiple research model approaches are needed. Much of our current understanding of the normal functioning of the lung and mechanisms of lung disease comes from these studies utilizing animals. A model is defined as a simplified system that is accessible and easily manipulated. The natural course of COPD in humans can take decades. However, the ideal models of COPD would involve inexpensive species which

could grow quickly and in which disease induction occurs over a short period of time while still mimicking the human condition. There are other factors that must be discussed as well when considering a model of COPD, such as sex, age, animal strain/background, exposure dosing and frequency which can all significantly impact on data interpretation. In this chapter, we will outline several animal models that have been used to study the biological processes believed to play major roles in the pathogenesis of COPD. Currently, the majority of COPD research is conducted in rodents. We will also discuss whether data obtained from rodents can shed light on COPD in humans.

2. Models of COPD

While cigarette consumption is the main risk factor for COPD, up to 10–15% of COPD cases are not related to CS exposure. Therefore, other factors can contribute to COPD initiation and progression, and multiple models are required to decipher the key mechanisms driving the disease. At this time, the majority of animal models for COPD research focus on emphysematous characteristics. Three major experimental approaches are commonly employed for the induction of emphysema-like symptoms in animals: elastase lung instillation, CS exposure and genetic manipulation. These three approaches are employed because CS is the primary etiologic risk factor associated with COPD and its comorbidities, an imbalance between elastase and anti-elastase activity results in enzymatic degradation of elastin and emphysema formation [24], and predisposing genetic factors are associated with COPD initiation and progression [25–27]. It is also known that CS has a major impact on elastase imbalance [28] and can also modulate gene expression [29]. Therefore, CS inhalation is the preferred approach and the following sections will outline these exposure models. Several other models of chronic bronchitis will also be discussed in addition to the emphysema models. The primary approaches to establish a model of COPD in animals is summarized in **Figure 1**.

2.1. Elastase model

In 1963, Laurell and Eriksson reported that patients deficient for α_1 -antitrypsin developed emphysema at an earlier time in life due to an abundance of elastase [30]. This discovery is the primary clinical observation that influenced the development of the elastase model of emphysema. In 1965, Gross et al. [31] developed the first animal model of protease-induced induction of emphysema in rats by instillation of the plant protease, papain, into the lung. Subsequently, numerous other proteases have been utilized to induce emphysema [32–35], including cathepsin B and proteinase 3. The most common protease utilized currently in animal models is elastase, primarily isolated from porcine. Porcine pancreatic elastase (PPE) is inexpensive and administration of PPE has been shown to induce features that resemble pan-acinar emphysema and lung damage throughout the organ. This approach has been utilized in many species of animals for several decades. The most frequently documented species, administration routes of PPE and exposure outcomes are summarized in **Table 1**, and will be discussed here in depth.

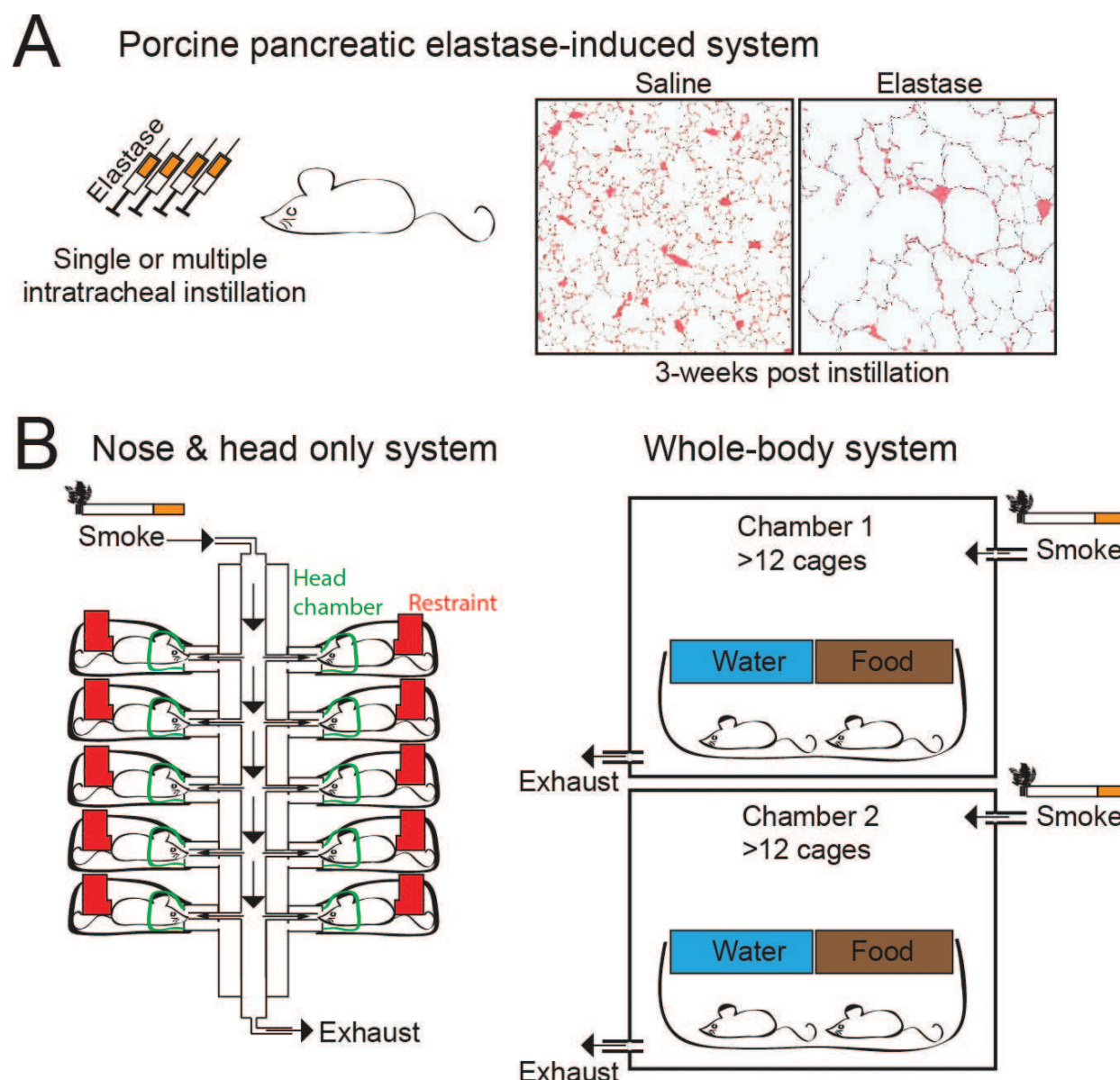


Figure 1. Typical mouse models for COPD. (A) Mice are administered a single or multiple intratracheal doses of PPE to induce an emphysema phenotype. One weeks onwards, there is significant evidence for airspace enlargements. Images show a typical profile following a single 1.2 Unit intranasal dose of PPE or saline and examination of the airways 3-weeks later by H&E staining. (B) A nose or head only (NHO) cigarette smoke exposure system requires animals to be restrained while they inhale the cigarette smoke. The whole-body system streams CS into a larger exposure system chamber and allows animals access to food and water without restraint.

As mentioned already, well-established genetic rodent background strains that utilize PPE-induced emphysema have been widely used as a model of COPD. Employing these strains/backgrounds, data from multiple studies are more reproducible and can be compared between groups. The animal genetic background is also critical for an appropriate control. Each strain has unique background alleles that may interact with and modify the expression of other genes upon establishment of a disease model or upon a stimulus, such as CS or elastase. Mice with different genetic backgrounds show disparate susceptibility to the development of

Animal	Reference	Strain	Administration	Outcomes
Mouse	[41, 44–49]	C57BL/6 and C57BL/6J	Intratracheal PPE	Enhanced airspace enlargements, lung volume and capacity, lung dendritic cells, MMPs, fibronectin degradation, epithelial cell apoptosis, alveolar epithelial damage, macrophages, neutrophils, lymphocytes, eosinophils, NK cells, CD8+ cells, A2M, HMOX1, MMP2, TIMP1, TNF- α , IL-1 β , IL-4, IL-5, IL-6, IFN- γ and TGF- β . Reduced α 1-antitrypsin, VEGF and DFCO
	[39, 41, 50]	BALB/c and BALB/cJ	Intratracheal PPE	Enhanced airspace enlargements, lung volume and capacity, compliance, hyperinflation, elastic and collagen fiber content, BALF hemoglobin content, M1 macrophages, neutrophils, lymphocytes, eosinophils, NK and NKT cells, CD4+ and CD8+ cells, NRF2, A2M, HMOX1, Arg1, Fizz1, MMP12, MMP2, TIMP1, SLPI, PrxI, HO-1, GST-Yc, NQO1, TNF- α , IL-1 β , IL-4, IL-5, IL-6, IL-18, iNOS, IL-17A, IFN- γ and TGF- β . Reduced α 1-antitrypsin, VEGF and DFCO
	[51, 52]	A/J	Endotracheal PPE	Enhanced airspace enlargements and lesions in the lung parenchyma distal to the terminal bronchioles
	[53, 54]	Swiss	Intratracheal PPE	Enhanced airspace enlargements, hyperinflation of the alveoli, alveolar collapse, MMP9 and TNF- α . Reduced VEGF and lung elastance
	[55, 56]	FVB/J	Intratracheal PPE	Enhanced airspace enlargements, lung volume, BALF hemoglobin content and alveolar surface area. Increased TGF- β , FGF-2 and GAG in BALF
Rat	[57–59]	Wistar	Intratracheal PPE	Enhanced airspace enlargements, NO release and leukocyte infiltration
	[60]	Sprague-Dawley	Intratracheal PPE	Enhanced airspace enlargements and increased dynamic compliance
Hamster	[61, 62]	–	Intratracheal PPE	Increased secretory cell metaplasia
Guinea pig	[63–65]	–	Intratracheal PPE	Evidence of interstitial edema, degradation of fibrous tissue, elastin degradation and enhanced alveolar enlargements. Loss of epithelial cilia and detachment of epithelial cells from the basement membrane. Increase tracheal hyperresponses to histamine, diaphragm fatigue, PMNs in tracheal submucosa blood vessels and infiltration of macrophages into the parenchyma

Animal	Reference	Strain	Administration	Outcomes
Rabbit	[62, 66, 67]	–	Intratracheal PPE	Enhanced airspace enlargements, apoptosis and 8-OHdG in lung tissues and reduced DLCO
	[68, 69]	New Zealand White	Endotracheal aerosolized PPE	Enhanced airspace enlargements, static compliance increase and decrease in expiratory flow and conductance
Pig	[70]	Yorkshire	PPE instilled into left lower lobe bronchus	Evidence of early edema, panlobular, centrilobular and paraseptal emphysema
Dog	[33, 71]	–	Endotracheal PPE	Enhanced airspace and septal destruction and enhanced airway enlargements
	[72]	Beagle	PPE injected through bronchi	Emphysema determined by chest computed tomography and histology
Sheep	[73–75]	–	Endotracheal and intrabronchial PPE	Enhanced lung resistance, bronchoconstriction and BALF levels of tissue kallikrein. Decreased tracheal mucus velocity

Abbreviations: GAG, glycosaminoglycan; NO, nitric oxide; PMNs, polymorphonuclear leukocytes; 8-OHdG, 8-hydroxy-deoxyguanosine; MMP, matrix metalloproteinases; NK, natural killer; IL, interleukin; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor; IFN, interferon; TGF, transforming growth factor; NO, nitric oxide; SLPI, secretory leukocyte peptidase inhibitor; iNOS, nitric oxide synthases; VEGF, vascular endothelial growth factor; DLCO, diffusing capacity of the lungs for carbon monoxide; FGF, fibroblast growth factors; GAG, glycosaminoglycans.

Table 1. Animal models of COPD induced by elastase.

emphysema, when utilizing the elastase model of emphysema [36]. Globally, C56BL/6 mice are the primary background strain used to generate many types of genetically engineered mice, but they are one of many strains. Other strains, including BALB/c mice, are documented to be more sensitive to dose and time dependent to PPE injury, as demonstrated by significantly greater mortality, weight loss, decline in lung function, immune cell infiltration and loss of alveolar tissue [36]. This may be due to several genetic differences between strains. BALB/c and C57BL/6J mice have differing type 1 and type 2 cytokine-mediated inflammation responses that could play key roles in determining the resistance or susceptibility to many diseases. Altered allergy responses in subgroups of patients is frequently observed in COPD, frequently coinciding with eosinophilia and enhanced allergy responses or independent of allergies [37, 38]. Therefore, it is critical to select the appropriate mouse background when utilizing mice for COPD studies and the correct controls.

Most PPE-induced emphysema studies highlight the proteolytic activity of elastase on lung structural changes, such as higher airspace enlargements (measured by mean linear intercept) both in mice and in rats (**Table 1**). Furthermore, several studies report changes in extracellular matrix (ECM) composition, such as disorganized elastin, degradation of proteoglycans, and abnormal collagen remodeling [39]. However, these effects are dependent on several factors, including animal strain, PPE dose at each instillation, and number of PPE challenges. Mice subjected to repeat PPE administrations within 1-week developed a more severe phenotype, with

enhanced alveolar destruction, weight loss, diaphragmatic dysfunction, exercise intolerance, and pulmonary arterial hypertension observed [40]. The pulmonary changes observed in PPE-induced emphysema in mice persisted for 6 months after injury induction [40] and therefore make this exposure model a useful tool in studying lung repair. Limjunyawong and colleagues [41] performed the most extensive study on PPE-induced emphysema in mice, utilizing two mouse strains, multiple doses of PPE over several weeks and extensive pulmonary function analysis. This study found strain, time and dose dependence on PPE-induced mortality, body weight loss, decline in lung function, lung inflammation and loss of alveolar tissue [41]. They also found heterogeneity in signaling associated with emphysema in each mouse strain.

Over the past few decades, other rodents have been utilized in models of PPE-induced COPD, such as rat, guinea pig, and hamster. These and other animal models are utilized as the mouse lung differs to human lungs in total lung capacity, left lung lobe number, lung pleural thickness, parenchyma percentage of the lung, alveoli size, blood-gas barrier thickness, trachea cartilage structure, number of respiratory bronchioles and airway generations, epithelium thickness and airway lumen size [42]. These other rodent models have several phenotypes that more closely reflect the human lung than the mouse. The lung volume and alveolar size have a closer resemblance to human lungs [42]. The airway of most rodents (except guinea pig) do not respond to leukotrienes, important mediators that cause bronchoconstriction in humans [43]. Depending on the species utilized, several confounding factors may not be functional in the model. Larger animal models of PPE-induced emphysema, utilizing rabbits, dogs, pigs and sheep, have observed enhanced airspace and septal destruction, and enhanced airway enlargements (see **Table 1**). Despite the many advantages of these larger species as an animal model of COPD, these species have not been widely used, probably due to limitations in terms of cost and reagent availability. Larger animal models do offer better understanding of lung structure than smaller animal models but most PPE-induced studies in these species were performed over 40 years ago, and the imaging and pulmonary function equipment was limited to address many parameters. Revisiting this approach in these species may be beneficial.

Overall, the intrapulmonary administration of tissue degrading enzymes, such as elastase, represents a useful approach to studying emphysema, especially when focusing on mechanisms to repair. PPE-based models are an attractive approach, since it is a simple exposure protocol with a single (or multiple) lung administration leading to significant and rapid changes. However, comparing this rapid method to the lifetime development of human COPD is very difficult since this method bypasses a large number of biological mediators. Equally, this method may reflect one subtype of emphysema and may not represent other subgroups of the disease. Therefore, the protease based models encompass several important features of human COPD but other methods, such as inhalation exposures, may also be required to better represent the phenotype of the human disease. The elastase model may be suited to tissue repair research.

2.2. Smoke exposure models

Like PPE-induced emphysema, a variety of animal species have been exposed to CS to mimic the human disease. Rodents (mice, rats, guinea pigs and hamsters) are the most commonly utilized species in CS exposure systems, but rabbits, ferrets, pigs, sheep and dogs have also been

used (see **Table 2** for a summary of multiple studies). In parallel to PPE models, the severity of emphysema induced by the CS exposure model can be influenced by a variety of factors, such as differences in animal strains, smoke concentration, and duration of exposure, and the sex of the animal. The background strain of a mouse is an important factor in CS exposure with differing strains having varying susceptibilities or resistances to the development of CS-induced emphysema [76]. Smoke concentration measured in total particulate matter (TPM) per liter can also affect CS-induced emphysema development. Mice exposed to NHO CS inhalation, at concentrations of 75, 250, and 600 mg of TPM/L, show only a 13% increase in airspace enlargements (mean linear intercept) in animals exposed to 600 mg TPM/L after 28 weeks of exposure [77]. The TPM of mainstream CS is comprised of 4–9% of the total weight of cigarette smoke and is made up of many components, including polycyclic aromatic hydrocarbons, nitrosamines, phenol and nicotine. The TPM measurement excludes the gas and vapors in smoke. Therefore, regulated smoke concentration and the duration of CS exposure are critical steps in establishing a CS exposure model in animals. To prevent contrasting results when doing CS studies, Kentucky reference cigarettes (from the Center for Tobacco Reference Products) are used by many research groups throughout the world to aid in the standardization of the CS exposure model. Other species are very susceptible to CS-induced COPD, such as guinea pigs [78] and ferrets [79]. They develop COPD-like lesions and emphysema-like airspace enlargement within a few months of active CS exposure. By contrast, rat strains seem to be more resistant to the induction of emphysema-like lesions [80–83]. Equally, animal sex has been reported as a confounding factor in COPD susceptibility. One example are female A/J mice which develop emphysema 6 weeks earlier than their male counterparts [84].

Animal	Reference	Strain	Exposure	Outcomes
Mouse	[87, 89, 94]	C57BL/6	NHO	Enhanced lung hyperinflation, total lung capacity, compliance, alveolar enlargements, systolic blood pressure, circulating platelets and erythrocyte numbers, attenuate alveolar macrophage responses to inflammation, production of reactive oxygen species in heart and kidney and lipid peroxidation in heart, liver and kidneys. Decreased total nitric oxide plasma concentration
	[29, 109–113]	C57BL/6	Whole-body	Enhanced airspace enlargements, lung capacity, compliance, lung inflammation, serum TNF- α , vascular and myocyte dysfunction, TLR9 signaling, protease activity, lung cell apoptosis, and cytokine production. Reduced weight gain, muscle mass and type I and IIA oxidative fibers, lung tissue elastance, lung PTP1B activity, EPAS1 lung expression
	[90, 91]	BALB/c	NHO	Increase in oxidative stress in lung and heart, mitochondrial respiratory dysfunction, chronic inflammation, mucus hypersecretion, airway remodeling and emphysema. Reduced lung function
	[114, 115]	BALB/c	Whole-body	Induced lung neutrophilia, IL1 α , IL1 β and CXCL1
	[92, 93]	A/J	NHO	Enhanced susceptibility to tumorigenesis, hyperplasia, metaplasia, and inflammation of the nose and larynx and proliferative lesions of the lungs. Delayed cutaneous wound healing

Animal	Reference	Strain	Exposure	Outcomes
	[29, 116–118]	A/J	Whole-body	Enhanced airspace enlargement, right ventricle heart hypertrophy, lung cell apoptosis, infiltration of macrophages, neutrophils, lymphocytes, kinase activity, cytokine and protease expression. Reduced FEF50%/FVC, EPAS1 lung expression
	[115]	Swiss	Whole-body	Enhanced mast cell recruitment to cutaneous wound
	[119]	FVB/NJ	Whole-body	Enhanced airspace enlargements, immune cell recruitment to the lungs, RSV lung infection, lung cell apoptosis, BALF protein concentration, expression of S100A9 and MCP-1. Reduced PTP1B activity
	[120]	FVB	CSE IP injection	Enhanced airspace enlargements, prothymosin α expression, NF κ B activity, MMP2 and MMP9
	[76, 121]	AKR/J	Whole-body	Increased inflammation, Th1 responses, macrophages, neutrophils, and T cells, oxidative stress levels in diaphragm and gastrocnemius. Reduced weight gain
Rat	[80–83]	Wistar	Whole-body	Increased gastric ghrelin, plasma TNF- α . Reduced food intake, weight gain, abdominal fat, plasma levels of leptin, insulin-like growth factor-1
	[122, 123]	Sprague-Dawley	Whole-body	Areas of blebbing and microvillus-like projections from the luminal surface, and micro-thrombi proximal to intercostal branches. Enhanced lung parenchymal destruction, pulmonary hypertension and pulmonary inflammation
Hamster	[5, 99]	–	Whole-body	Induced elevation of right ventricular systolic pressures, medial hypertrophy of pulmonary arterioles, lung chymase activity, Ang II levels and enhanced TGF- β 1/Smad signaling. Reduces production of lysyl oxidase and the resynthesis of cross-linked elastin
Guinea pig	[97–99]	Hartley	Whole-body	Increased pulmonary artery pressure, right ventricle hypertrophy, raised respiratory resistance, airspace enlargement, intrapulmonary vessel remodeling, immune cell infiltration, CatK and CHOP expression, ERK and JNK phosphorylation. Decreased elastin and the loss of type III collagen in the alveolar walls
Ferret	[79]	<i>Mustela putorius furo</i>	NHO	Increased early-morning spontaneous coughs, sporadic infectious exacerbations, airway obstruction, goblet cell metaplasia/hyperplasia and mucus expression in small airways
Rabbit	[101–105]	New Zealand White	Whole-body	Decreased alveolar count, IRAK degradation. Increased ductal/destructive fraction, lung destruction, apoptosis, airspace enlargements, immune cell infiltration, intraparenchymal vascular congestion and thrombosis, intraparenchymal hemorrhage, respiratory epithelial proliferation, alveolar destruction, emphysematous changes and bronchoalveolar hemorrhage, lung and aorta expression of MMP1 and lung expression of TLR4

Animal	Reference	Strain	Exposure	Outcomes
Pig	[124]	–	Lungs only	Enhanced vasodilator response in the bronchial circulation, bronchodilatation and bronchial vasodilatation
Dog	[125]	Greyhounds	Whole-body	Increased parenchymal damage and inflammation
	[126, 127]	Beagle	Through a tracheostoma	Histological evidence of emphysema
Sheep	[128]	–	Unilateral inhalation	Increased gas exchange impairment and metabolic activity

Abbreviations: ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; TLR, toll-like receptor; EPAS1, endothelial PAS domain protein 1; CXCL, chemokine (C-X-C motif) ligand; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; FEF, forced expiratory flow; FVC, forced vital capacity; MCP1, monocyte chemoattractant protein-1; Th1, type 1 T helper; Smad, mothers against decapentaplegic homolog; Cat, cathepsin; CHOP, C/EBP homologous protein.

Table 2. Animal models of COPD induced by cigarette smoke.

The experimental details of CS smoke and tobacco usage in humans are so varied that, currently, no single experimental CS exposure system can replicate the diversity of human smoking patterns. Therefore, CS models probably reflects only facets of COPD but they still represent the closest model of early human disease. In the following sections, we will outline the COPD models that utilize CS exposure. There are advantages and disadvantages with both types of CS exposure approaches, such as animal number, exact quantifiable exposure, exposure duration and end points of study. Additionally, a detailed breakdown of every published CS exposure publication up to 2013 is summarized by Leberl and colleagues [85].

2.2.1. Nose or head only exposure

The nose or head only (NHO) exposure model (sometimes referred to as mainstream CS exposure) was developed for the induction of COPD in animals that allows quantifiable concentrations administered to animals. Several species, including mice, are obligate nose breathers, which allows direct delivery of CS or aerosolized solutions to be delivered to the lungs. The NHO system requires restraining the animal so that their nose is inserted into a cone where they inhale the cigarette smoke [86]. During this exposure time animals are deprived of food and water. This method generates a uniform exposure that produces the desired emphysematous changes. However, the prolonged periods of restraint are stressful for the mice and the machine can usually accommodate only a limited number of mice (e.g. the Jaeger system has eighteen ports which is depicted in **Figure 1B**). NHO exposures are usually for short exposure times (30–60 min) and CS exposures are repeated several times daily [87]. The level of exposure can range from 75 to 600 mg TPM/m³ [77, 87]. In animal research, TPM concentrations is typically a defining factor for characterizing whether a study is a passive or secondhand exposure model, in addition to a NHO or whole-body method. A recent study examining gene expression profiles in CS exposure models in mice compared to human cohorts demonstrated that low TPM induces genes mainly related to xenobiotic/detoxification responses,

while higher TPM activated immune/inflammatory and xenobiotic/detoxification responses [88]. In the same study, one human cohort clustered closer to low TPM but another cohort clustered closer to a high TPM [88]. Therefore, certain biological features of the CS exposure model are dose dependent and may represent a critical factor in establishing a model.

There are multiple companies that manufacture NHO exposure systems, e.g. Scireq, CH Technologies, Buxco Research Systems, In-Tox Products and Promech Lab AB. NHO exposure machines are typically automated for unattended operation (delivery, lighting, positioning, and ejection of cigarettes). They control the CS puff volumes and rates, are adjustable for direct smoke or indirect CS exposures, and contain a sealed chamber that prevents smoke leakage. The machines are relatively compact in size. Typically, an animal is placed in an individual exposure tube, with a rubber seal around the animal's neck to minimize exposure to the head. The tube is mounted onto the exposure tower (see **Figure 1B**) and CS is applied to the system. Some exposure models are equipped with a pneumotachograph, to measure respiratory flow from movement of the animal's chest wall. Alternatively, these models can be modified to test potential new aerosolized therapies.

Similar to PPE-induced emphysema models, mice are the primary species utilized in CS exposure models. C57BL/6 mice are frequently utilized in NHO exposures [87, 89], but others have utilized BALB/c [90, 91] and A/J mice [92, 93]. NHO exposure to CS has been documented to result in increased lung hyperinflation, total lung capacity, compliance, alveolar enlargements, systolic blood pressure, circulating platelets and erythrocyte numbers. This exposure model attenuates alveolar macrophage responses to inflammation, production of reactive oxygen species in heart and kidney and lipid peroxidation in heart, liver and kidneys and reduced total nitric oxide plasma concentrations in C57BL/6 mice [87, 89, 94].

Gaschler and colleagues [89] report that NHO and whole body CS exposure systems attenuate innate immune responses in a comparable manner. However, some outcomes appear to be different between both exposures, with NHO smoke exposures failing to produce soleus muscle weight reduction [87]. TPM concentrations [88] and exposure duration may also be confounding factors leading to differences in these models. Several larger animal species are more susceptible to CS exposure and NHO exposure resulting in phenotype changes similar to the human disease. Ferrets have several similarities to human airway physiology and submucosal gland distribution. In a recent NHO chronic CS exposure model, ferrets demonstrated clinical features close to human COPD, such as early-morning spontaneous coughs, sporadic infectious exacerbations, airway obstruction, goblet cell metaplasia/hyperplasia and increased mucus expression in small airways [79]. Despite larger animals displaying a lung morphology closer to human, the mouse model is the favored model of NHO CS exposure induced COPD. However, new models like the ferret may be a better model to evaluate and characterize further in future studies.

2.2.2. Whole-body exposure

Whole-body systems (sometimes referred to as side-stream CS exposure) can expose animals to a mixture of both passive and mainstream smoke, released from the burning cigarette and puffed through the cigarette [95]. The passive and mainstream smoke streams are mixed and

then propelled by a fan to a chamber containing the mice that are housed within their cages. The advantage of this system is that the mice freely move about and have access to food and water (**Figure 1B**). Thus, mice in this system can be exposed for longer periods of time daily. In addition, the whole-body exposure system allows for the exposure of large groups of mice. Some systems allow the exposure of greater than 120 mice simultaneously, enabling researchers to use large numbers of mice and to perform multiple experiments without stressing the animals beyond the CS exposure. There are several companies that manufacture whole-body exposure systems, e.g. Scireq and Teague Enterprises. These systems are also automated to help with ease of use.

Similar to the other COPD models already discussed, rodents are the most common species used in whole-body CS exposure models due to the wide variety of applicable gene expression manipulations. Several recent advances in pulmonary function testing in mice [96] will further enhance the usage of mice in CS exposure models. Other animal models include the guinea pig model, which has been shown to be susceptible to whole-body CS-exposures. CS exposure in guinea pigs induces several pulmonary and cardiovascular changes, such as pulmonary artery pressure, right ventricle hypertrophy, raised respiratory resistance, airspace enlargement, intrapulmonary vessel remodeling, loss of elastin and type III collagen in the alveolar walls, immune cell infiltration, protease and kinase activity [97–99]. Measurable emphysematous changes are also detected in rats following 2-months of whole body CS exposure [100]. Very few larger animals have been utilized in the whole-body exposure system, perhaps due to insufficient access to chambers large enough to contain these animals. Several studies have successfully exposed rabbits to whole body CS [101–105]. Mice do not produce MMP1, the major collagenase associated with COPD [106], but rabbits express MMP1 and its induction is CS triggered [103]. For the study of proteins not expressed in smaller animals or expressed at a different frequency to human, several larger animals may represent a better model option. Rabbits are relatively easy to handle and several transgenic animals are available [107], making them a good model for investigating lung-related diseases. Rabbits are also large enough to allow non-lethal monitoring of physiological changes during the course of the CS exposure but rabbits still have multiple differences in lung physiology to human [108]. Importantly, when deciding on a CS exposure model of emphysema, it must be noted that both NHO and whole-body exposure methods result in emphysema and other COPD symptoms in animals, and both represent good but not perfect models of COPD.

2.3. Genetic models of COPD

Prior to genome-wide association studies (GWAS) on COPD samples, several genes have been investigated in animals for susceptibility to COPD development and pathogenesis (**Table 3** is a summary from multiple mouse studies). GWAS and other genomic based studies have identified many further genes that are associated with COPD [26, 29] but functional studies are required to prove their importance in lung function and disease development. Currently, mice represent the most favored laboratory animal species to manipulate gene expression. The discovery of new gene manipulation approaches may alter this view, such as CRISPR-Cas9. CRISPR-Cas9 approaches in larger animals is an exciting new method for future COPD research. Several existing models will be briefly outlined here that alter susceptibility to environmental exposure

Reference	Mouse	Exposure	COPD susceptibility	Outcomes
[129]	<i>Itgb6</i> ^{-/-}	–	Increased	Spontaneous development of age-related lung emphysema due to lack of ITGB6-TGF-β1 regulation of the MMP12 expression
[130]	<i>Klotho</i> ^{-/-}	–	Increased	Increased aging, enlargement of the air spaces, destruction of the alveolar walls
[131]	Human <i>MMP1</i> expression	–	Increased	Increased disruption of the alveolar walls and coalescence of the alveolar spaces
[136]	<i>Mmp12</i> ^{-/-}	CS	Resistant	Reduced smoke induced alveolar macrophage infiltration and emphysema
[139]	<i>Tnfr</i> ^{-/-}	CS	Resistant	Reduced airspace enlargements and neutrophil infiltration
[138]	<i>iNOS</i> ^{-/-}	CS	Resistant	Mice protected against emphysema and pulmonary hypertension
[143]	<i>Cd8</i> ^{-/-}	CS	Resistant	Blunted inflammatory response and did not develop emphysema
[111]	<i>Tlr9</i> ^{-/-}	CS	Resistant	Protected from smoke-induced airspace enlargements, loss of lung function, inflammation, protease activity, apoptosis, and inflammation
[144]	SOD1 overexpression	CS or elastase	Resistant	SOD1 prevented smoke or elastase induced airspace enlargements, neutrophil infiltration and lipid peroxidation product accumulation
[137]	<i>Cav1</i> ^{-/-}	CS	Resistant	Reduced senescence of lung fibroblasts and pulmonary emphysema
[140]	<i>Mrp1</i> ^{-/-} <i>Mdr1a</i> ^{-/-} <i>Mdr1b</i> ^{-/-}	CS	Resistant	Reduced inflammatory and emphysema
[145]	<i>Il-18Ra</i> ^{-/-}	CS	Partially protected	Decreased inflammation and emphysema
[144]	<i>Il-1R</i> ^{-/-}	CS	Partially protected	No induction of inflammatory cell infiltration, small airway remodeling or matrix breakdown
[146]	Adiponectin ^{-/-}	CS	Partially protected	No induction of inflammatory cell infiltration, airspace enlargements, tissue elastance or TNFα
[147]	SOD3 overexpression	CS or elastase	Partially protected	SOD3 prevented smoke or elastase induced airspace enlargement, impairment of lung function and exercise capacity
[148, 149]	<i>Il-17a</i> ^{-/-}	CS	Partially protected	Exacerbated macrophage and γδ T cells frequency, which trigger emphysema
[119]	<i>Ptp1b</i> ^{-/-}	CS	Increased	Enhanced airspace enlargements, immune cell recruitment, apoptosis, and inflammation

Reference	Mouse	Exposure	COPD susceptibility	Outcomes
[132]	<i>Nrf2</i> ^{-/-}	CS	Increased	More inflammation, oxidative stress, apoptosis and reduced antioxidants
[133, 134]	<i>Gpx1</i> ^{-/-}	CS	Increased	Enhanced airspace enlargements, BALF neutrophils, macrophages, proteases, IL-17A, MIP1α, NFκB and AP1 activation. Reduced PP2A and PTP1B activity
[135]	<i>p53</i> ^{-/-}	Elastase	Increased	Increased emphysema severity, macrophages, neutrophils, BALF CCL2, BALF TNF-α and lung oxidative stress

Abbreviations: ITGB6, Integrin Subunit Beta 6; SOD, superoxide dismutase; Cav1, caveolin 1; Mrp1, multi-drug resistance associated protein 1; Mdr1, multi-drug resistance gene 1; AP1, activator protein 1, CCL, chemokine (C-C motif) ligand.

Table 3. Gene modulated models of COPD in mice.

induced COPD or genetic alterations that result in spontaneous COPD development. There are two major approaches, i.e. gain-of function or loss-of-function. These gene manipulations can be targeted within the whole body or within specific tissue or cell types. Gain-of-function is achieved by gene overexpression in transgenic mice or expressing a human gene or variant of that gene. Alternatively, loss of function is achieved by specifically targeting loss of expression of a gene, by direct or chemical mutagenesis. The majority of studies performed in COPD utilize whole body knockout animals, where the gene was genetically manipulated in the embryo prior to development. Several genes have been linked with COPD since several knockout animals develop spontaneous COPD as they age, such as *Itgb6*^{-/-} or *klotho*^{-/-} mice [129, 130]. Equally, introducing a human gene into a mouse can result in COPD development without environmental exposures, such as lung expression of human *MMP1* in mice [131]. Most genetic manipulations require a “second-hit” to observe lung changes causing disease. Inducing a COPD phenotype by CS inhalation or PPE instillation in animals deficient for *Nrf2* [132], *Ptp1b* [119], *Gpx1* [133, 134] or *p53* [135] exaggerates COPD-like changes. However, most genetic manipulations have been associated with protecting against CS or PPE induced COPD, e.g. *Mmp12*^{-/-} [136], *Cav1*^{-/-} [137], *iNOS*^{-/-} [138], *Tnfr*^{-/-} [139] and *Mrp1*^{-/-}*Mdr1a*^{-/-}*Mdr1b*^{-/-} [140] mice. Since COPD is an extremely complex heterogenous disease, many of these genes and others may all contribute to disease initiation and progression. The GWAS studies in humans have identified several other possible targets for future investigation, e.g. HHIP, CHRNA5, HTR4, FAM13A, RIN3, TGFB2, GSTCD-NPNT, CYP2A6, IL27-CCDC101, ADGRG6-GPR126, THSD4, ADAM19, TET2, CFDP1, AGER, ARMC2, RARB, EEFSEC, DSP, MTCL1, SFTPD, IREB2, HHIP, and FAM13A [141, 142]. The mouse model still remains the best species to manipulate gene expression in the lungs but new techniques will aid in larger animals being utilized in the coming years.

2.4. Models of bronchitis

As mentioned previously, the second phenotype in COPD is an airway model for chronic bronchitis. Several studies have focused on induction of bronchitis phenotypes in animals (see **Table 4** for a summary of several studies). Bronchitis models utilize noxious inhalants

Animal	Reference	Strain	Exposure	Outcomes
Mouse	[158]	BALB/c	SO ₂	Enhanced neutrophilic inflammation, epithelial sloughing, ET-1 and TGFβ expression
	[154]	C57BL/6	NO ₂	Increased airway (neutrophils and macrophages), goblet cell hyperplasia, collagen deposition in the lung parenchyma and airspace enlargements
Rat	[150, 151]	Sprague Dawley	SO ₂	Increased pulmonary resistance, airway responsiveness to methacholine, immune cell infiltration (notably neutrophils), immune dysregulation, oxidative stress, mucin, accumulation of surfactant in lamellar bodies of alveolar type II cells, BALF levels of lactate dehydrogenase and N-acetyl glucosaminidase activity. Reduced dynamic compliance
	[159, 160]	Sprague Dawley	NO ₂	Increased dopamine D(2) receptor expression
Ferret	[152]	<i>Mustela putorius furo</i>	SO ₂	Observation of lesions with changes in ciliated cells, edema and cell infiltration. Increased coughing, nasal discharge and dried mucus
	[161]	<i>Mustela putorius furo</i>	NO ₂	Reduced thoracic clearance function
Hamster	[160]	DSN	NO ₂	Moderate/severe bronchiolitis and alveolitis development. Increased mitosis in Club cells and BALF surfactant

Animal	Reference	Strain	Exposure	Outcomes
Guinea pig	[162]	–	SO ₂	Increased hyperresponsiveness to intravenously administered serotonin, degeneration, desquamation of epithelium, and edema of the lamina propria of the trachea and bronchi
	[163]	Hartley	NO ₂	Increased BALF eosinophils and neutrophils, microvascular leakage in the trachea and main bronchi.
Rabbit	[164]	–	SO ₂	Increase in sputum viscosity. Decreased respiratory rate and pO ₂ blood level
	[165]	–	NO ₂	Enhanced destructive of walls and abnormal enlargement of the distal air spaces
Dog	[166]	–	SO ₂	Significant ciliated cell damage
	[167]	–	NO ₂	Changes in edema, congestion, interstitial irritation, bronchiolitis, and interstitial fibrosis

Abbreviation: ET-1, endothelin-1.

Table 4. Animal models of bronchitis.

such as sulfur dioxide (SO₂), nitrogen dioxide (NO₂), or ozone. SO₂ is a gaseous irritant which can be used to induce COPD-like lesions in animal models. Exposure to high concentrations of SO₂ daily results in chronic injury and repair of epithelial cells in rats [150, 151]. The exposure to high-levels of this gas ranging from 200 to 700 ppm for 4 to 8 weeks has been demonstrated to lead to neutrophilic inflammation, morphological signs of mucus production and mucus cell metaplasia and damage of ciliated epithelial in rats [150, 151] and ferrets [152]. Exposure of SO₂ to capsaicin-treated rats results in increased airway smooth muscle mass and increased airway responsiveness observed in these animals [153]. NO₂ is another gas that induces COPD-like lesions in a concentration, duration of exposure, and species susceptibility dependent manner [154]. Exposure to NO₂ (50–150 ppm) can reduce animal survival

due to extensive pulmonary injury, including pulmonary edema, hemorrhage and pleural effusion. Sub lethal levels of NO₂ causes damage to cilia, hypertrophy of the bronchiolar epithelium and a type II pneumocyte hyperplasia in rats and hamsters [155, 156]. Mice exhibit similar lung responses to NO₂ exposure [154]. The administration of ozone [157] also causes significant lung injury with several features associated with human COPD. All three of these exposure models were utilized in several species of animals but are rarely utilized in current studies of COPD. A recent NHO chronic CS exposure model in ferrets demonstrated clinical features close to human bronchitis [79] and suggests that the animal species may be a deciding factor in bronchitis research. Overall, new approaches are needed to access bronchitis in animal models of COPD.

2.5. New models of COPD

Several novel models of COPD have been utilized recently, including investigation of biomass fuel and individual components of CS, i.e. nicotine. Several of these models will briefly be discussed in this section. One research group has developed a rapid COPD model in mice by daily injecting CS extract (CSE) into the abdominal peritoneal cavity [120, 168]. Airway enlargements and injury of cardiac and skeletal muscles are reported within 6 weeks of initiation [120, 168] but comparative studies to whole-body or NHO systems are required. Recent genetic analyses have identified $\alpha 3$ and $\alpha 5$ nicotine acetylcholine receptors (nAChR) as susceptibility loci for COPD [169]. The airway epithelium expresses $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\alpha 9$, $\beta 2$ and $\beta 4$ subunits for nAChRs [170], and their expression are highest on the apical cell surface, where exposure to inhaled nicotine occurs [171]. Several animal studies have already utilized nicotine in models of COPD, airway lung injury and tumorigenesis. CS from high-dose nicotine cigarettes induces more emphysematous changes than low-dose nicotine cigarettes in PPE pre-treated rats [172]. Nicotine enhances airway hyperreactivity in lipopolysaccharides (LPS)-challenged mice and inflammation in lung epithelial cells [173]. Nicotine also suppresses apoptosis in lung tumors [174].

These observations are important considering several new approaches for nicotine replacement therapy now being marketed, such as e-cigarettes. E-cigarettes are a relatively new product that has grown in popularity over recent years. E-cigarettes are devices that effectively deliver vaporized liquid nicotine to the lungs. The user can choose the nicotine concentration and devices can deliver a range of volumes. However, several additional studies have suggested that these products require further testing in animal models and human studies to evaluate short and long term effects. Recently, the US Surgeon General concluded that e-cigarette use among the younger population is now a significant public health concern [175]. The use of e-cigarettes enhances oxidative stress and inflammation in mice [176] and impairs immune defense against bacterial and viral infection [177]. Nicotine in e-cigarettes has also been implicated as the driving force of these changes in mice, with long-term inhalation of nicotine-containing e-cigarettes shown to increase airway hyperreactivity, distal airspace enlargement, mucin production, cytokine and protease expression [178]. There are some direct human disease correlations, with normal human bronchial epithelial (NHBE) cells grown at the air liquid interface exposed to nicotine-containing e-cigarette vapor showing impaired ciliary beat

frequency, airway surface liquid volume, conductance of ion channels, decreased expression of genes associated with ciliogenesis and increased cytokine production [178]. The animal models used for these studies use either the e-cigarette device connected to a whole-body CS exposure model, outlined in section b.ii, or a whole-body nebulizer. Many companies have modified their NHO or whole-body exposure systems for e-cigarette research, which will expand on new animal models for lung research. Therefore, these models and others should be utilized in future studies of COPD and nicotine replacement therapy.

Models of air pollution have been used to examine several phenotypes of COPD, with exposures to diesel exhaust particulates (DEP) and other biomass fuels under scrutiny. Animals exposed to DEP daily via intratracheal instillation for 3-months have decreased IFN- γ levels in bronchoalveolar lavage (BAL) but elevated CD3+ T and CD8+ T cells increased in the lung parenchyma and airway enlargement [179]. Equally, exposure of mice to smoke or particles generated from dung [180] and wood biomass [181] have been associated with several parameters of COPD. These models used modified CS whole-body exposure systems. Studies like these are important as it is estimated that air pollutants and biomass smoke could contribute to over 4 million deaths annually in the developing world [182] and contribute to the 10–15% of COPD cases not related to CS exposure. Therefore, new models or modification of the current group of COPD models are required to identify new biological targets of COPD and potential new therapies for treatment.

3. Exacerbation models

Exacerbations are one of the most important outcomes for COPD patients, as they contribute to decreasing quality of life, loss of lung function, and increasing mortality and health-care cost [183]. Modeling acute exacerbations of COPD (AECOPD) in animals is challenging due to the complex clinical manifestations. Most AECOPD are triggered by infection, usually viral [184] or bacterial [185]. This association of the presence of a microbial pathogen at the onset of AECOPD is the primary basis for the majority of AECOPD animal models. These models typically entail exposure of an animal, usually a mouse, to CS followed by infection with a virus or bacteria. Live pathogens can be difficult to work with and often require special containment facilities. Therefore, some groups have used purified components of the pathogen, such as lipopolysaccharides (LPS) [186, 187] or *Staphylococcus aureus* enterotoxin B (SEB) [188], or a stimulus that will mimic the immune response upon infection, such as polyinosinic:polycytidylic acid (poly(i:c)) [189, 190]. Several of these approaches and outcomes are summarized in **Table 5**. The most frequently used live pathogens utilized in AECOPD models are influenza [188, 191], rhinovirus [192], respiratory syncytial virus (RSV) [112, 119], *Streptococcus pneumoniae* [193] and non-typeable *Haemophilus influenzae* (NTHi) [193]. Most models utilize the CS exposure system, but several have favored the PPE method [192, 193]. The general consensus appears to be that the COPD status enhances the lungs susceptibility to infection, inflammation and worsens lung function in a similar manner to the human disease. A study by Foronjy and colleagues [112] infected mice multiple times with RSV during a chronic CS exposure to mimic repeat viral AECOPD observed in the human disease. Repeated

Animal	Reference	Pathogen	Exposure	Outcomes
Mouse	[188, 191]	Influenza	CS	Enhanced airway damage, inflammation and alters IL33 responses
	[192]	Rhinovirus	PPE	Increased airway inflammation, obstruction, goblet cell metaplasia, total lung volume and alveolar chord length. Reduced lung elastance
	[112, 119]	RSV	CS	Increased airspace enlargements, inflammation, cytokines, chemokines, protease activity, apoptosis and fibrotic areas. Reduced phosphatase activity
	[118]	Modified HIV	CS	Reduced FEF _{50%} /FVC and enhanced distal airspace enlargement, inflammation, apoptosis and protease activity
	[189, 190]	Poly(i:c)	CS	Enhanced pulmonary inflammation, airway hyper responsiveness, corticosteroid resistance, remodeling, apoptosis, fibrosis, TLR3 signaling, type I IFNs, IL-18, IL-12/IL-23 p40, IFN- γ , PKR activation and eIF2 α
	[193]	Streptococcus pneumoniae	PPE	Increased mortality, inflammatory cells in BALF, and MMP-12, as well as enhanced emphysema progression
	[199, 200]	NTHi	CS	Increased pulmonary inflammation (MCP-1, -3, and -5, IP-10, and MIP-1 γ)
	[186]	LPS	CS	Enhanced susceptibility to airway lung injury

Animal	Reference	Pathogen	Exposure	Outcomes
	[188]	SEB	CS	Increased BALF lymphocytes and neutrophils, CD8+ T lymphocytes and granulocytes in lung tissue, IL-13, CXCL13, CCL19 and goblet cell hyperplasia
Rat	[201]	NTHi	CS	Increased airway bacterial load, aggravated mucus hypersecretion and delayed mucociliary clearance
Guinea pig	[187]	LPS	CS	Enhanced airway resistance, lung volume, neutrophils, epithelial hyperplasia and emphysema

Abbreviations: eIF2 α , eukaryotic initiation factor-2alpha; PKR, protein kinase; SEB, *Staphylococcus aureus* enterotoxin B; NTHi, non-typeable *Haemophilus influenzae*.

Table 5. Animal models of COPD exacerbations.

infection heightened airspace enlargements, inflammation, cytokines, chemokines, protease activity, apoptosis and fibrotic areas, while reducing lung phosphatase activity [112]. The same group recently infected mice systemically with chimeric HIV-1 virus that is capable of establishing chronic infection in immunocompetent mice [194] and exposed these mice to CS [118]. This altered HIV-1 infection enhanced CS-induced COPD features in the lungs of these mice and this new murine model can be utilized to study HIV-related COPD, which results in a heightened form of the disease in the HIV+ population [195–198]. Therefore, several animal models exist that can aid in our understanding of AECOPD.

4. Modeling COPD comorbidities in animals

COPD is frequently observed with comorbidities that directly affect patient’s quality of life, increase the risk for exacerbations and increase rate of mortality [202]. There are many manifestations of COPD comorbidities, such as lung cancer, skeletal muscle wasting (cachexia), diaphragm muscle dysfunction, cardiovascular disease, osteoporosis and diabetes [202]. Several studies have suggested that a large proportion of COPD-associated deaths are attributed to comorbidities [203, 204]. Given that comorbidities have a significant impact on COPD patient survival and quality of life, researchers have utilized animal models to investigate systemic comorbidities associated with COPD. CS exposure models show extrapulmonary

manifestations, similar to those frequently observed in COPD. Mice and rats exposed to CS have reduced body weight, fat mass, hind-limb skeletal muscles mass, grip strength and aerobic endurance [82, 205]. CS exposure also enhances pulmonary artery wall thickening, increased contractility and endothelial dysfunction in guinea pigs [99]. Chronic CS exposure was recently shown to alter several cardiovascular parameters, such as enlarging ventricular end systolic and diastolic diameters, reducing myocardial and cardiomyocyte contractile function, and disrupting intracellular Ca^{2+} homoeostasis, regulating fibrosis, apoptosis and mitochondrial damage [206]. Mice and guinea pig exposed to CS, displaying lung inflammation and emphysema, have pulmonary hypertension and significant impairments to right ventricular diastolic and systolic function and contractility [99, 207]. CS in mice also causes hypertension, endothelial dysfunction and cardiac remodeling [208]. Chronic CS exposure have also been documented to increase systolic blood pressure, circulating platelets and erythrocyte numbers, attenuate alveolar macrophage responses to inflammation, production of reactive oxygen species in heart and kidney and lipid peroxidation in heart, liver and kidneys in mice [94]. Multiple elastase instillations in mice leads to the development of pulmonary arterial hypertension [209]. Diaphragm muscle dysfunction can contribute to COPD comorbidity. Diaphragm fatigue and ROS production are observed in guinea pig PPE-induced emphysema [65] and mouse smoke-induced emphysema [121], which could contribute to this comorbidity. Enhanced susceptibility to tumorigenesis is also demonstrated in mice exposed to smoke [93]. The hamster inhalation model is the only model in which tumor induction by CS has been reproducibly achieved [210]. These combined changes would be predicted to contribute to multiple comorbidities in COPD patients. Research focusing on models of COPD comorbidities will greatly improve our understanding of key factors in loss of quality of life and mortality in the COPD patient population, and additional novel animal models investigating the link between COPD and comorbidities are needed.

5. Animal model limitations and what is missing in these models

Models of disease try to mimic the disease as closely as possible. Despite no disease model being perfect, many models are helpful tools in deciphering the pathogenesis of the disease. Regarding COPD and the models outlined here, all models yield several phenotypes of the disease. Each researcher must evaluate the outcomes they desire to observe and choose the appropriate model that best suits their needs. For example, most COPD models cannot reproduce the features of severe emphysema as observed in humans, i.e. GOLD stages 3 or 4. Most models, especially the mouse smoke model, only represent early COPD (i.e. similar to human GOLD stages 1 of COPD) regardless of exposure time. It is estimated that obstruction of 75% of the small airways is required before changes can be detected by routine pulmonary function tests (e.g. forced expiratory volume in 1 s (FEV1)) in humans [211]. The majority of morbidity and mortality occurs in COPD patients with severe disease [3]. This is a major challenge to undertake when developing a reliable model of COPD as this requires a considerable amount of time and enhances the frequency of animal death prior to testing the conditions

of the model. If a severe stage of COPD is required for investigation, the PPE model may be the best model currently available to examine COPD. However, if researchers are examining disease initiation or progression the PPE model would not be ideal. Another challenge in animal research is in the measurement of lung function in very small mammals, such as mice. The use of the enhanced pause (Penh) in conscious mice as an indicator of airflow obstruction is not ideal and invasive methods remain more reliable. Several new technical advances have aided in enhancing the pulmonary function readouts in animals but further work is needed to correlate animal data to the human disease.

The many differences in lung morphology and physiology between humans and the animals utilized in research will definitely impact on data interpretation, such as mice being obligate nose breathers, having lower numbers of cilia, fewer Club cells, differences in airway mucus production and reduced submucosal glands in the trachea. However, the experimental approach must also undergo scrutiny. Studies do not define whether a new therapeutic treatment is started following disease initiation or in parallel with the disease triggering exposure (i.e. CS or PPE exposure). Many treatments may be useful in preventing disease initiation but will have little impact on the lungs following disease establishment. Several recent studies are now addressing this experimental problem but scientific reviewers need to assert this difference in studies. In mice, lung lesions and inflammation induced by CS inhalation do not progress after cessation of CS exposure but airway enlargements persist [212]. This may have a large impact on the methodological design of experiments in mice. Whether other species recover following CS exposure is unknown. Equally, new genetic targeted approaches should be employed after disease establishment. Genes associated with disease progression can be selectively knocked down or enhanced after disease initiation, rather than utilizing whole body knockout animals that may have lung disease phenotypes due to embryo development problems. Modifying current methodology in these ways will further aid in advancing current animal model data and interpretations. Future animal model COPD studies should assess the histopathological patterns of COPD and examine functional parameters of human COPD using imaging, airflow limitation, mucus hypersecretion, corticosteroid resistance, β -adrenergic bronchodilator responses, chronic cough and exacerbations.

Overall, several major factors limit the interpretation of data from animal models to the human disease. Many investigators expect the animal model to exactly mirror the human condition but COPD is a complex and heterogenous disease and the human disease requires further sub-characterization prior to designing animal models or interpreting current data from animal models. We must also remember that without disease models, the burden on patients would be vast to participate in clinical trials and donate samples. Equally, not utilizing animal models would only allow clinicians to undertake COPD research. Deciphering the key players of a disease requires multiple approaches from many research fields and animal models may have a significant impact on COPD treatment in a similar manner to oncology and cardiovascular research. There are many benefits in development and use of animal models of COPD, especially the understanding of the fundamentals of immune and inflammatory mechanisms. Clinicians will benefit from the input of animal researchers, immunologists, microbiologists, bioinformaticians and statisticians.

6. Conclusion

Current animal model technology allows researchers to investigate the mechanisms of airways dysfunction, the influence of genetics and the immunological factors that define physiological and signaling changes that drive COPD. There are numerous well-established exposure models of COPD that exhibit several characteristics of the human disease, but no model captures every phenotype of the human condition. Mice are the most common species utilized for COPD research as they are small in size, they have a fast gestation period, and cost of feeding and housing are far less than larger animals. Also, the mouse genome is extensively characterized and many genetically modifiable mice are available, as well as equipment and biological reagents designed specifically for mouse anatomy. It is far more cost effective to generate genetically manipulated mice than it is to do so in other species. Another advantage to the use of mouse models is that exposure to CS for 1 year represents approximately 50% of the animal's lifetime, thereby allowing a better representation of lifetime CS exposure. While there are many physiological differences between the mouse and human pulmonary systems, the mouse models of COPD represent good tools to further our understanding of the human disease.

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