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Animal Models of Chronic Obstructive Pulmonary Disease Exacerbations: A Review of the Current Status

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Abstract

Chronic obstructive pulmonary disease (COPD), characterized by airflow limitation and manifested as emphysema and chronic airway obstruction, is a major cause of morbidity and mortality worldwide, resulting in an economic and social burden that is both substantial and increasing. The natural history of COPD involves systemic manifestations, such as skeletal muscle wasting and cardiovascular impairment, and frequent exacerbations. The latter are caused by bacterial or viral infections and have major implications for patients and healthcare systems. There are no effective therapies to prevent or reverse these events. Smoking cessation remains the most effective intervention for reducing disease progression. Animal models of COPD and of COPD exacerbations have been developed to advance understanding of the pathogenesis of COPD and the role of infections in its severity. Cigarette smoke exposure, tracheal instillation of elastase, and genetic manipulation are commonly used to reproduce baseline COPD conditions, each with its advantages and disadvantages. Intratracheal instillation of lipopolysaccharide (LPS), bacteria, or viruses are the most common interventions used to exacerbate baseline COPD. This review highlights the three major animal models used for induction of emphysema and its exacerbations. Further exploration of these models should facilitate identification of new therapeutic approaches for COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality throughout the world [1]. It is associated with progressive disability and functional impairment, and represents a major economic and social burden worldwide [2]. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines COPD as a disease characterized by progressive airflow limitation that is not fully reversible, associated with an abnormal inflammatory response of the lungs to noxious particles or gases [3]. Although COPD refers to a broad group of lung diseases characterized by airflow limitation, parenchymal destruction (emphysema) and small-airway obstruction (chronic bronchitis) are the most important phenotypes [4]. Emphysema, which is characterized by permanent inflammation and irreversible destruction of alveolar walls, leading to airspace enlargement, loss of elastic recoil and hyperinflation [5], is the most studied of these conditions.

Pathophysiology of COPD

COPD may be considered a complex disease, as several different mechanisms seem to be involved in its pathophysiology. The most accepted hypothesis is an imbalance between elastase and antielastase activity that leads to enzymatic degradation of elastin [6,7]. Such an imbalance is observed after long-term cigarette smoke (CS) exposure [8].

CS or other inhaled irritants activate epithelial cells to release growth factors, such as transforming growth factor beta (TGF-β) and fibroblast growth factor (FGF), which induce fibroblast proliferation, resulting in small-airway inflammation and fibrosis [9,10]. Simultaneously, macrophages are activated and release proinflammatory cytokines, such as tumor necrosis factor- alpha (TNF-α), interleukin (IL)-1β, and IL-6, which amplify lung inflammation and contribute to disease progression. Macrophages also release several chemokines that attract circulating cells into the lungs, such as CCL 2 (an attractant of monocytes, which later differentiate into macrophages within lung tissue), CXCL8 (an attractant of neutrophils, which can

also be recruited by LTB-4), and CXCL-9, CXCL-10, and CXCL-11 (attractants of Th1 cells, which release interferon gamma [IFN-γ]) [8, 10]. Many of the inflammatory genes overexpressed in COPD smokers are governed by upregulation of the transcription factor nuclear NF-κB [11]. In non-stimulated cells, NF-κB is found in alveolar macrophages and airway cells in an inactive non-DNA binding form, associated with its inhibitory protein κBα (IκBα) [12]. However, cigarette smoke promotes IκBα degradation, which unmasks NF-κB, allowing it to migrate to the nucleus, bind to DNA and initiate gene transcription [13]. NF-κB is a heterodimer composed of two subunits, p65 and p50; the former is increased in bronchial biopsy specimens [14] and sputum [15] from smokers as compared to nonsmokers.

Macrophages and neutrophils contribute to persistent inflammation and to an oxidant/antioxidant imbalance by releasing reactive oxygen species (ROS) that induce epithelial and endothelial cell apoptosis and inactivate antiproteolytic defense mechanisms, such as tissue inhibitors of metalloproteases (TIMPs) and alpha-1 antitrypsin (α1-AT) [16,17]. Moreover, the oxidative stress can reduce levels of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) [18-20], which regulates the cellular antioxidant response by upregulating genes involved in augmenting cellular antioxidant capacity and promotes expression of genes involved in detoxification of ROS and electrophilic compounds [21,22]. Thus, low levels of Nrf2 are associated with increased susceptibility to neutrophilic inflammation [23] and histone deacetylase-2 (HDAC2) degradation [24]. HDAC2 is an epigenetic regulator and a critical component of the corticosteroid receptor complex that mediates repression of NF-κB transcriptional activity by deacetylating histones in proinflammatory gene promoters [25] and deacetylating the corticosteroid receptor [26]. Its levels are substantially reduced in the alveolar macrophages and distal structural cells of patients with COPD, which is associated with increased disease severity and airway inflammation [27] and promotion of corticosteroid resistance [25,28]. Thus, the disruption of epigenetic mechanisms has important implications to disease progression. Apoptosis is also related to the release of granzymes and perforins by CD8+ lymphocytes [29] and to low levels of vascular endothelial growth factor (VEGF) as a result of the endothelial cell destruction process [30].

Matrix metalloproteinases (such as MMP-8, MMP-9, and MMP-12) released by macrophages and neutrophils can degrade a variety of matrix components, including collagen and elastin, leading to alveolar wall destruction [31,32]. Subsequently, elastin fragments become chemoattractants of further inflammatory cell influx [17]. The abnormal collagen remodeling that follows plays a major role both in COPD progression [33,34] and in lung function [35]. **Figure 1** illustrates the above concepts related to COPD pathophysiology.

Although COPD affects primarily the lungs, it is known to be

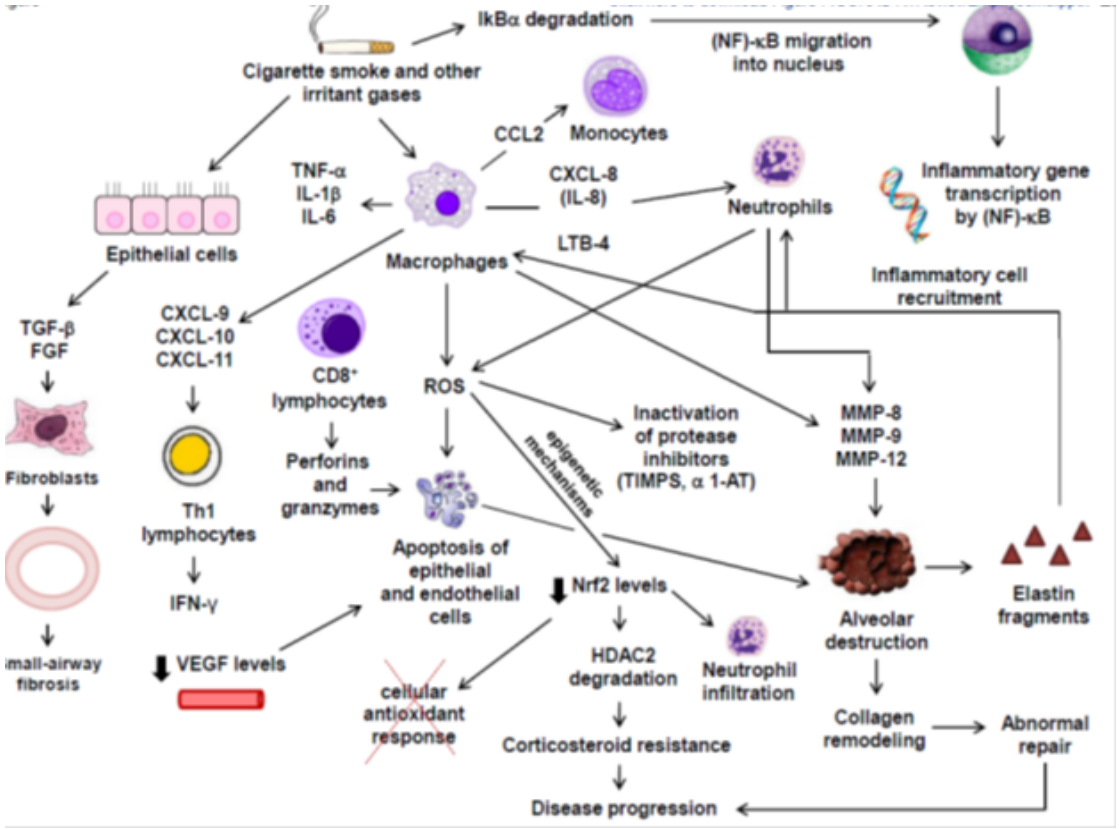

Figure 1 Pathophysiology of COPD. α1-AT, alpha-1 antitrypsin; CXCL and CCL, chemokines; FGF, fibroblast growth factor; HDAC2, histone deacetylase-2; IFN-γ, interferon gamma; IkBα, inhibitory protein κBα; IL-1β, interleukin 1β; IL-6, interleukin 6; LTB-4, leukotriene B4; MMP, matrix metalloproteinases; NF-κB, factor nuclear kappa B; Nrf2, nuclear factor (erythroid-derived 2)-like 2; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TIMPS, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor- alpha; VEGF, vascular endothelial growth factor.

associated with systemic effects [36]. The major comorbidities observed in COPD are: cardiovascular impairment, such as pulmonary hypertension and heart failure [37,38] diaphragmatic muscle dysfunction [39] skeletal muscle wasting [40] osteoporosis; diabetes [41] and malnutrition and weight loss [42,43]. These comorbidities reduce patient quality of life [43] and increase the risk of exacerbations [44] and mortality [45]. In addition, respiratory infections can worsen these comorbidities and compound their impact on the patient's life [43].

Major Experimental Models of COPD

There are three major experimental approaches for induction of emphysema: CS exposure, elastase instillation, and genetic manipulation.

Cigarette Smoke Exposure

The severity of emphysema induced by the CS exposure model can be influenced by some factors, such as differences in animal strains, smoke concentration, and sex. Vecchio et al. [46] showed that C57BL/6 mice exhibited higher levels of proinflammatory cytokines, ROS, and MMPs, with lower levels of glutathione peroxidase (GPX), than Institute of Cancer Research (ICR) mice. Smoke concentration also plays an important role. Hodge-Bell et al. exposed male C57 and ICR mice to nose-only CS inhalation from 2R4F reference cigarettes, at concentrations of 75, 250, and 600 micrograms of total particulate matter (TPM) per liter. They observed an approximately 13% increase in mean linear intercept (Lm) only in mice exposed to 600 mcg TPM/L [47]. Regarding sex, March et al. showed that female A/J mice develop emphysema earlier than male mice from the same strain [48]. CS studies have also been done in rats [49-54]. CS exposure produces not only pulmonary alterations, but also systemic manifestations, such as weight loss [55-59], oxidative modifications of muscle protein in respiratory and limb muscles [60], reduction of skeletal muscle strength and increase in catabolic factors [59], systemic inflammation [61], and pulmonary arterial hypertension [62] **(Table 1).** On the other hand, most models cannot reproduce the features of severe emphysema as observed in humans, which would translate into GOLD stages 3 or 4. Usually, only mild features (corresponding to GOLD stages 1 or 2) are observed, regardless of exposure time [63], whereas in humans, the majority of morbidity and mortality occurs in patients with severe disease [64]. Moreover, all changes induced by CS exposure take time to be observed [61,65,66]. Finally, unlike in human advanced COPD, the lesions induced by this model do not progress after cessation of CS exposure. Jobse et al. recently showed that, although CS exposure resulted in an increase in mononuclear cells and neutrophils, airspace enlargement, and V/Q mismatch, inflammatory cell levels returned to control values and V/Q parameters returned to normal after cessation of exposure [67].

Elastase Instillation

Porcine pancreatic elastase (PPE) offers the advantages of being inexpensive and able to induce features of panacinar emphysema [64,68,69] and more widespread lung damage [17]. In one study of C57BL/6 mice, intratracheal elastase administration produced a greater amount of low-density areas than CS exposure, as observed on quantitative micro-computed tomography [70]. A wide variety of studies have highlighted the proteolytic activity of elastase in causing structural changes, such as higher mean linear intercept and alveolar enlargement both in mice [71-75] and in rats [76-79] **(Table 2).** Furthermore, several studies reported changes in ECM composition after elastase administration, such as disorganized elastin [80, 81], degradation of proteoglycans [82], and abnormal collagen remodeling [83-88]. However, as in CS models, these effects are dependent on several factors, including strain; enzyme dose at each instillation; and number of elastase challenges **(Figure 2).** Limjunyawong et al. recently reported that BALB/C mice are more sensitive than C57BL/6J to elastase injury, as demonstrated by significantly greater mortality, weight loss, decline in lung function, and loss of alveolar tissue [89]. Regarding dose and number of elastase challenges, Lüthje et al. demonstrated that mice subjected to five elastase administrations with a 1-week interval between them developed not only a more severe alveolar destruction, but also systemic

Table 1 Models of emphysema induced by cigarette smoke (CS).

STUDY	STRAIN AND SPECIES	INTERVENTIONS	OUTCOMES
Gosker et al., 2009 [55]	C57BL/6 mice	Animals exposed to CS 5 days/week for 6 months	Pulmonary inflammation, weight loss, and reduction in type IIA oxidative fiber proportion in the soleus muscle
Tang et al., 2010 [56]	C57BL/6 mice	Animals exposed to CS daily for 8 or 16 weeks	Weight loss, reduction in citrate synthase and beta-hydroxyacyl CoA dehydrogenase in the soleus muscle
Tomoda et al., 2012 [57]	Wistar rats	Animals exposed to CS twice a day, 5 days/week for 4 weeks	Food intake, weight gain, and plasma levels of leptin reduced
Esquivel et al., 2014 [58]	Wistar rats	Animals exposed to CS 20 times/day, 5 days/week for 2 months	Body weight, abdominal fat, and plasma levels of leptin reduced
Kamiide et al., 2015 [59]	Wistar rats	Animals exposed to CS for 12 weeks	Body weight, food intake, and skeletal muscle strength reduced; mRNA expression of catabolic factors increased
Barreiro et al., 2012 [60]	AKR/J mice	Animals exposed to CS for 6 months	Weight gain reduced, oxidative stress levels in diaphragm and gastrocnemius increased. Proteins involved in glycolysis, ATP production and distribution, carbon dioxide hydration, and muscle contraction were carbonylated in respiratory and limb muscles
Krüger et al., 2015 [61]	C57BL/6J mice	Animals exposed to CS for 8, 16, 24, and 32 weeks	Reduced body mass, time-dependent decrease in muscle mass, type I oxidative fibers, and muscle cross-sectional area
Braber et al., 2010 [62]	A/J mice	Animals exposed to CS 5 days/week for 20 weeks	Right ventricle hypertrophy

manifestations, such as weight loss, diaphragmatic dysfunction, exercise intolerance, and pulmonary arterial hypertension. Unlike with the nonprogressive CS exposure model, these changes persisted for 6 months after injury induction [90]. Similar results have been demonstrated by other groups [74,81,82,91-94]. Furthermore, after multiple elastase instillations in mice, Cruz et al. and Antunes et al. reported the development of pulmonary arterial hypertension and cor pulmonale, one of the leading causes of death in human patients with emphysema [95,96].

Genetic Manipulation

With the advent of modern molecular biology techniques, transgenic mice can now be bred with artificially introduced

Table 2 Models of emphysema induced by elastase.

STUDY	STRAIN AND SPECIES	INTERVENTIONS	OUTCOMES
Takahashi et al., 2008 [72]	C57BL/6 mice	Single intratracheal injection of elastase	Higher mean linear intercept and alveolar epithelial damage
Harada et al., 2009 [73]	C57BL/6J mice	Single intratracheal injection of elastase	Alveolar enlargement, increased lung volume, upregulation of lung dendritic cells
Moreno et al., 2014 [74]	C57BL/6 mice	Single intratracheal injection of elastase	Alveolar enlargement, increased levels of matrix metalloproteinases, degradation of fibronectin
Santos et al., 2014 [75]	Swiss mice	Single intratracheal injection of elastase	Higher mean linear intercept, alveolar enlargement, reduced lung elastance
Onclinx et al., 2006 [76]	Sprague-Dawley rats	One or two intratracheal injections of elastase	Alveolar enlargement, increased dynamic compliance
Furuya et al., 2012 [77]	Wistar rats	Single intratracheal injection of elastase	Alveolar enlargement, reduced arterial oxygen tension
Bianchi et al., 2015 [78] Boiati et al., 2010 [79]	Wistar rats	Single intratracheal injection of elastase	Higher mean linear intercept

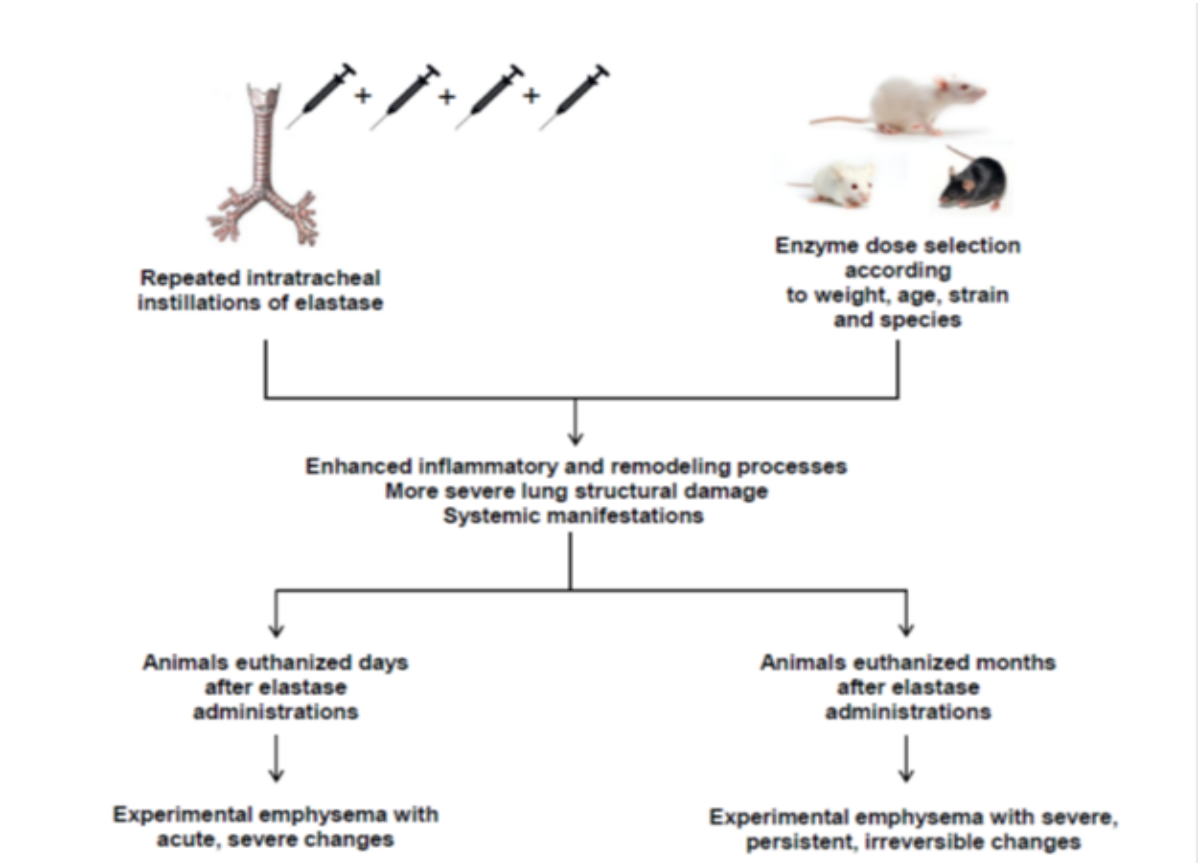

Figure 2 Pathophysiology of COPD. α1-AT, alpha-1 antitrypsin; CXCL and CCL, chemokines; FGF, fibroblast growth factor; HDAC2, histone deacetylase-2; IFN-γ, interferon gamma; IkBα, inhibitory protein κBα; IL-1β, interleukin 1β; IL-6, interleukin 6; LTB-4, leukotriene B4; MMP, matrix metalloproteinases; NF-κB, factor nuclear kappa B; Nrf2, nuclear factor (erythroid-derived 2)-like 2; ROS, reactive oxygen species; TGF-β, transforming growth factor beta; TIMPS, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor- alpha; VEGF, vascular endothelial growth factor.

alterations in their genome resulting in overexpression ("gain of function") or low expression ("loss of function") of the gene of interest. Gain-of-function models have added further proof to the protease-antiprotease imbalance hypothesis [97]. In this line, mice overexpressing human interstitial collagenase (MMP-1) showed airspace enlargement [98] and degradation of type III collagen [99,100]. Furthermore, in mice, Wang et al. have demonstrated that overexpression of interferon gamma (IFN-γ) caused prominent protease and antiprotease alterations, such as induction and activation of MMP-12, MMP-9 and cathepsins B, H, D, S, and L, and the selective inhibition of secretory leukocyte proteinase inhibitor [101].

Targeted mutagenesis also allows investigators to generate strains of COPD mice that lack individual proteins (loss-of-function mutations), such as elastin [102], fibulin-5 [103], platelet derived growth factor (PDGF) [104,105], fibroblast growth factor receptor (FGFR) [106], surfactant protein-D (SP-D) [107-109], tissue inhibitor of metalloproteinases-3 (TIMP-3) [110], and ATP binding cassette A3 (Abca3), a lipid transport protein required for synthesis and storage of pulmonary surfactant in type II cells in the alveoli [111]. Recently, Holm et al. developed a mouse model lacking microfibrillar-associated protein 4 (MFAP4), an important protein localized into elastic fibers in blood vessels and the interalveolar septa of the lungs. These mice exhibit increased total lung capacity and evidence of a decrease in mean density of the lung parenchyma on breath-hold gated microcomputed tomography (micro-TC) [112]. Rangasamy et al. showed that disruption of the Nrf2 gene led to earlier onset and more extensive features of emphysema in mice exposed to CS, suggesting that this gene determines susceptibility to lung inflammation, oxidative stress, and alveolar cell apoptosis [23]. Similarly, Adenuga et al., using Nrf2 and HDAC2 knockout mice, observed that HDAC2 deficiency led to an increase in CS-induced lung inflammation and steroid resistance [24] **(Table 3).**

Table 3 Loss-of-function knockout mouse models.

STUDY	STRAIN	INTERVENTIONS	OUTCOMES
Wendel et al., 2000 [102]	C57BL/6 mice	Animals lacking elastin (Eln -/-)	Dilated and few air sacs, impaired airway branching, attenuated tissue septae
Nakamura et al., 2002 [103]	C57BL/6 mice	Animals lacking fibulin-5 (fibulin-5-/-)	Disorganized elastic fiber system throughout the body, characterized by tortuous aorta with loss of compliance, severe emphysema, and cutis laxa
Boström et al., 1996 [104]	C57BL/6 mice	Animals lacking platelet-derived growth factor A (PDGF-A-/-)	Alveolar hyperinflation, abnormally large and air-filled cavities, failure of alveolar septation, loss of alveolar myofibroblasts, *cor pulmonale*
Lindahl et al., 1997 [105]	C57BL/6 mice	Animals lacking platelet-derived growth factor A (PDGF-A-/-)	Reduced deposition of elastin fibers in the lung parenchyma, development of early lung emphysema due to complete failure of alveogenesis
Weinstein et al., 1998 [106]	C57BL/6 mice	Animals lacking fibroblast growth factor receptors 3 and 4 (FGFR-3-/-, FGFR-4-/-)	Failure of alveogenesis, reduced elastin synthesis, bronchopulmonary dysplasia, growth retardation
Wert et al., 2000 [107] Yoshida et al., 2001 [108] Knudsen et al., 2009 [109]	C57BL/6 mice	Animals lacking surfactant protein-D (SP-D -/-)	Postnatal airspace enlargement, subpleural fibrosis, chronic inflammation with infiltration of foamy alveolar macrophages, increased MMP activity and oxidant production by alveolar macrophages
Leco et al., 2001 [110]	C57BL/6 mice	Animals lacking tissue inhibitor of metalloproteinases-3 (TIMP-3 -/-)	Spontaneous air space enlargement, impaired lung function, enhanced destruction of extracellular matrix molecules
Besnard et al., 2010 [111]	C57BL/6 mice	Animals lacking ATP-binding cassette A3 (Abca3 -/-)	Death shortly after birth from respiratory distress related to surfactant deficiency. Surviving mice exhibited decreased expression of mRNAs associated with lipid synthesis and fewer lamellar bodies
Holm et al., 2015 [112]	C57BL/6 mice	Animals lacking microfibrillar-associated protein 4 (Mfap-4 -/-)	Increased lung volumes and compliance, alveolar hyperinflation
Rangasamy et al., 2004 [23]	C57BL/6 mice	Animals lacking nuclear factor (erythroid-derived 2)-like 2 (Nrf-2 -/-) and exposed to CS for 6 months	Increased apoptotic alveolar septal cell (endothelial and type II epithelial cell) counts, oxidative stress, bronchoalveolar inflammation
Adenuga et al., 2010 [24]	C57BL/6J mice	Animals lacking nuclear factor (erythroid-derived 2)-like 2 (Nrf-2 -/-) and deacetylase 2 (HDAC-2 -/-) exposed to CS for 3 days and later treated with budesonide	Neutrophil influx in the lungs and BALF, increased levels of KC and MCP-1 in BALF, steroid insensitivity

BALF, bronchoalveolar lavage fluid; CS, cigarette smoke; KC, keratinocyte-derived cytokine; MCP-1, monocyte chemotactic protein 1; MMP, matrix metalloproteinase.

In some mouse strains, spontaneous development of emphysema has been observed in association with genetic abnormalities. Pallid [7,113,114] and tight skin [115,116] mice have α1-antitrypsin deficiency, which results in reduced elastase inhibitory capacity, leading to emphysematous lesions at the age of 2–4 weeks.

In short, gene-targeting techniques are very useful tools to identify the role of distinct genes in the regulation of pulmonary homeostasis and to examine potential mechanisms underlying human COPD [117]. However, a major disadvantage of these models is that the gene of interest is also expressed in other organs [97], which can cause systemic effects.

COPD Exacerbations

Frequent acute exacerbations are observed during the life course of COPD patients [118]. Most exacerbations are triggered by infection, usually viral [119] or bacterial [120]. Repeated exacerbations are associated with worse prognosis [121,122], airway inflammation [123], and lung function impairment [124] **(Figure 3).**

The susceptibility of smokers with COPD to respiratory infections is greater than that of nonsmokers, because CS exposure causes several disruptions to the innate lung defenses, such as impairment of mucociliary clearance [125], reductions in ciliary beat frequency and in the numbers of ciliated cells due to squamous metaplasia [126], reduction in the concentrations of surfactant proteins A and D [127], salivary lysozyme and sputum secretory leukocyte protease inhibitor deficiency [128,129], and impairment of phagocytosis by alveolar macrophages [130,131] **(Figure 4).** In addition, even a stable COPD condition is associated with respiratory pathogens in the airways, which worsens airflow conductance [132,133] and triggers an inflammatory response [15,123,134-136] and presence of inflammatory markers in sputum [133,137-139]. During exacerbations, these inflammatory cells and mediators increase [140,141], as does the H_2O_2 concentration in exhaled breath condensate [142,143].

Although many acute COPD exacerbations can be treated on an out¬patient basis, some cause a greater decline in lung function, requiring hospitalization [124]. Therefore, effective strategies to reduce the incidence of COPD exacerbations and their duration are needed. The administration of corticosteroids has long been a mainstay of therapy for acute COPD exacerbations; however, chronic corticosteroid use is associated with many adverse events [144] and with increased risk of pneumonia, possibly due to their immunosuppressive action [145-147]. Additionally, inhaled or systemic corticosteroids fail to attenuate chronic inflammation in some patients, due to increased oxidative stress [148,149]. Corticosteroids suppress inflammation by recruiting HDAC2 to NF-κB-driven pro-inflammatory gene promoters,

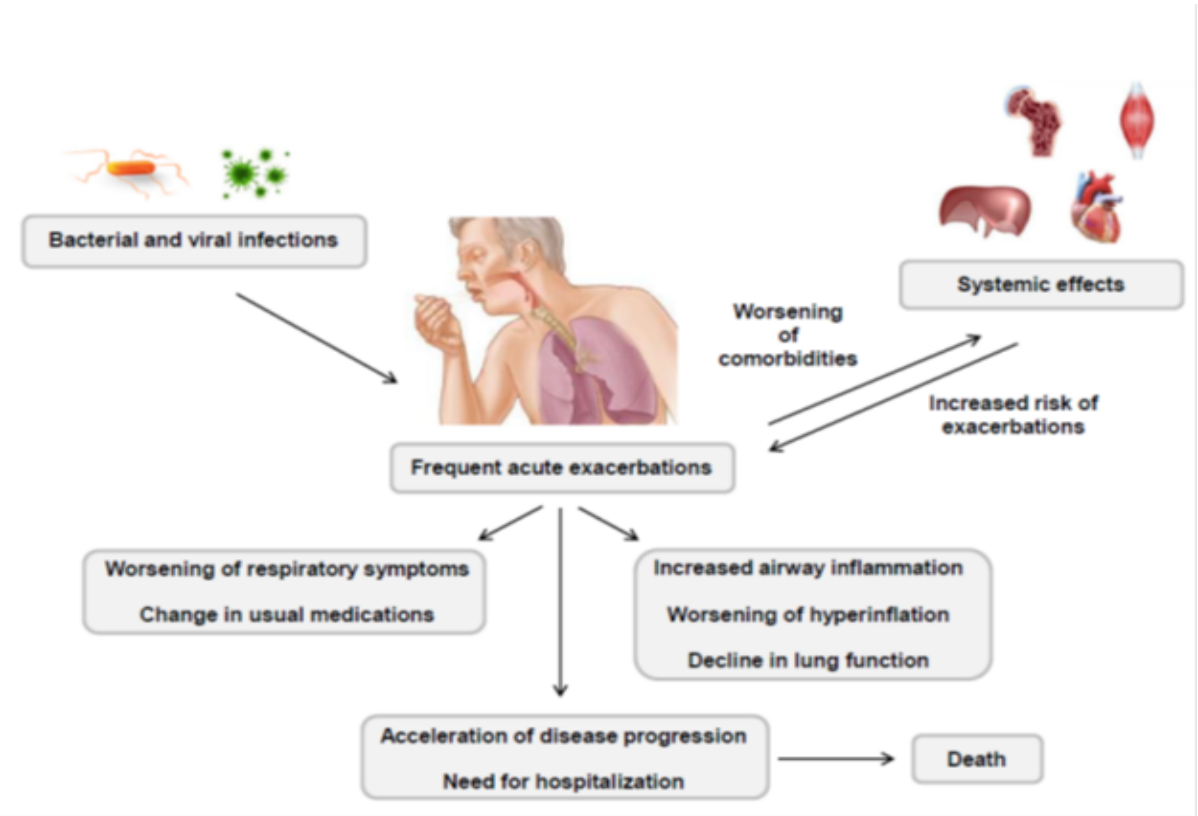

Figure 3 Causes and consequences of frequent acute exacerbations of COPD. Most exacerbations are caused by viral or bacterial infection. Air pollution and environmental conditions may increase airway inflammation or bronchomotor tone. Extrapulmonary effects can also increase the risk of exacerbations, as well as mortality. Exacerbations increase lung inflammation, worsen respiratory symptoms and lung function, and accelerate disease progression.

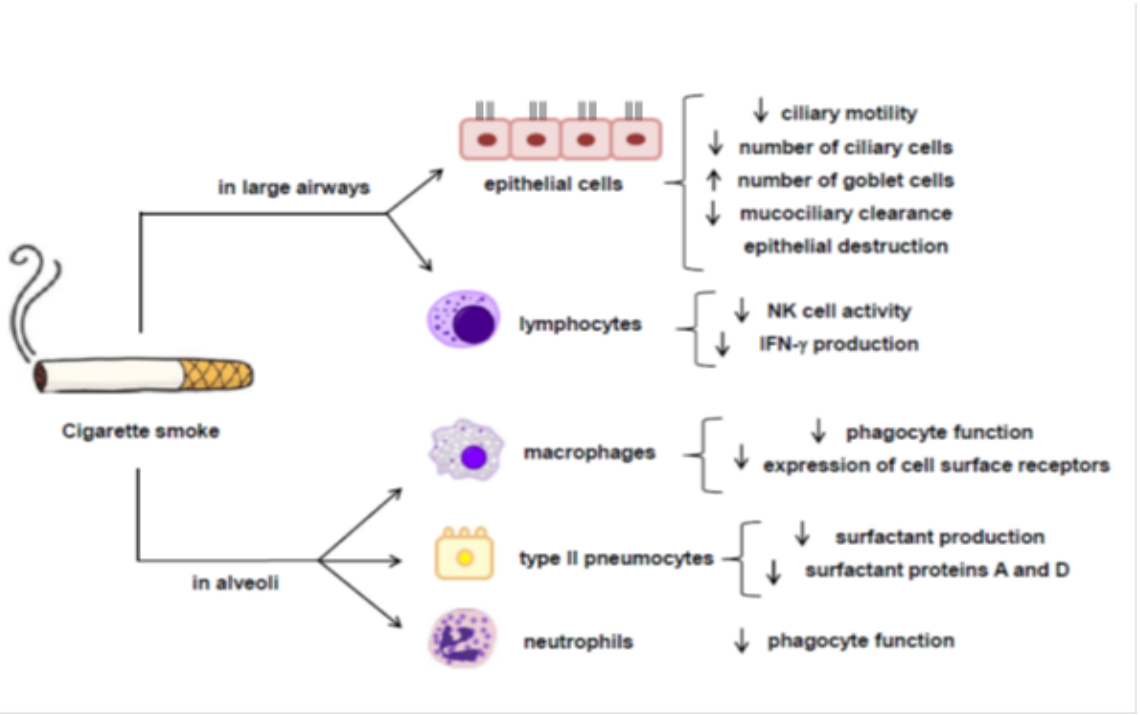

Figure 4 Influence of smoking on the function of immune cells throughout the respiratory system. IFN-γ: interferon gamma; NK: natural killer cell.

thereby inhibiting the transcription of these genes [150]. Thus, the inability of corticosteroids to suppress inflammation in some cases of COPD can be associated with loss of HDAC2 activity [151]. Several studies about the use of specific antibiotics in COPD exacerbations have been conducted [152-154], and it is known that antibiotic therapy can reduce sputum purulence [155]. On the other hand, such therapy may become ineffective against resistant bacterial strains, especially when treatment is provided in-hospital [156,157].

As previously reported, animal models with different purposes have been developed to elucidate the pathophysiology of COPD exacerbations. There are two major experimental approaches to induction of COPD exacerbations: 1) intratracheal instillation of bacterial lipopolysaccharides (LPS) and 2) challenge with specific bacterial or viral strains.

Experimental Models of COPD Exacerbation

LPS Infection

Using mice, Stolk et al. induced baseline emphysema through elastase administration and later induced exacerbations with intratracheal instillations of LPS (500 µg in 200 µL saline) twice a week for up to 5 weeks. Six months later, a severe bronchial mucus cell hyperplasia and persistent increase in mean linear intercept, indicating irreversible tissue destruction, were observed [158]. Recently, Kobayashi et al. induced exacerbations using only a single intratracheal administration of LPS (1 mg/kg) to mice with elastase-induced emphysema. Three days after LPS treatment, the authors observed neutrophil infiltration and CD8+ cells in bronchoalveolar lavage fluid (BALF), as well as increased levels of MMP-9 and TIMP-1, whereas after 12 weeks, they found severe alveolar destruction, using the parameter of low-attenuation area percentage (LAA%) on micro-computed X-ray tomography, suggesting intense acute inflammation and severe, irreversible alveolar destruction, respectively [159].

In rats, a single massive LPS insult (40 mg/kg, intratracheally) has been reported to cause an inflammatory response followed by mucus hypersecretion and bronchoconstriction, which reproduces symptoms of an exacerbation [160]. Hardaker et al. reported in a CS model that aerosolized LPS (0.3 mg/mL) was able to increase neutrophil infiltration, mucus, and edema in the lungs and in BALF and to impair lung function [161]. Accordingly, in a CS model, a single LPS instillation (200 µg/kg, intratracheally) promoted inflammatory cell infiltration and remarkable goblet-cell hyperplasia in tracheal, bronchial, and bronchiolar epithelium [162]. Recently, also in a CS model, Li et al. reported airspace enlargement, decreased expressions of surfactant protein (SP)-A and SP-C, and apoptosis of alveolar epithelial cells after two intratracheal LPS instillations (1 µg/µL) [163].

Bacterial Infection

Given that bacteria are the main cause of infections in COPD patients, the majority of studies use bacteria to induce exacerbations in emphysema models. In this line, Gashler et al. exposed C57BL/6 and BALB/c mice to CS for 8 weeks and subsequently challenged the animals with Haemophilus influenza (NTHI). In both strains, the authors observed an increase in

pulmonary inflammation and lung damage. Furthermore, NTHI challenge led to prominent upregulation of some inflammatory mediators, such as monocyte chemotactic protein 1 (MCP-1), MCP-3, and MCP-5 [164]. Huvenne et al. investigated the effects of Staphylococcus aureus enterotoxin B (SEB) in C57BL/6 mice exposed to CS for 4 weeks. CD8+ T lymphocytes and granulocytes increased in BALF, and goblet cell hyperplasia was observed in the airway wall [165]. In an investigation of the impact of CS on bacterial clearance and immune inflammatory processes in mice, Drannik et al. found higher levels of TNF-α, IL-1β, IL-6, MCP-1, and MIP-2 in lung homogenates after acute P. aeruginosa infection [166]. Recently, Voss et al. also showed enhanced inflammation in the upper airways and lung tissue of C57BL/6 mice exposed to CS after colonization with NTHI and Streptococcus pneumoniae [167]. Similar results can be obtained after elastase-induced emphysema. In this line, Pang et al. showed decreased intercellular adhesion molecule 1 (ICAM-1) in airway epithelium and low NTHI clearance, with pathologic findings consistent with pneumonia, supporting the hypothesis that ICAM-1 promotes clearance of NTHI [168]. Furthermore, Wang et al. induced exacerbation through NTHI challenge after elastase-induced emphysema, and observed lung consolidation, capillary congestion, atelectasis, hemorrhage, neutrophil infiltration, and higher levels of TNF-γ and IL-8 in BALF and plasma [169] typical clinical signs of severe pneumonia. Finally, Ganesan et al. developed a more complex animal model in which they combined a bacterial source and LPS. The authors administered LPS and elastase simultaneously for four consecutive weeks in C57BL/6 mice. One week after the last exposure to LPS, animals were challenged with NTHI. Elastase/LPS exposed mice exhibited delayed bacterial clearance with an increase in neutrophilic inflammation and prolonged mucus secretion as assessed by mucin gene expression and periodic acid–Schiff (PAS) staining of lung histology. Moreover, ex vivo macrophages showed deficient phagocytosis, possibly could be caused by decreased expression of scavenger receptor A [170]. Studies in rats are lacking.

Viral Infection

To date, very few studies have tried to induce viral infection to mimic COPD exacerbations. Bauer et al. exposed C57BL/6 mice to CS for 4 days and subsequently inoculated the animals with the influenza A(H1N1) virus. Mononuclear and neutrophil cells, MCP-1, MCP-3, and CXC (KC, MIP-2) chemokines increased in BALF [171]. Recently, using a single elastase treatment followed by rhinovirus (RV) infection in C57BL/6 mice, Singanayagam et al. reported increased airway neutrophilic and lymphocytic inflammation, increased expression of TNF-α and CXC motif chemokine 10 (CXCL10)/IP-10 (IFN-γ-induced protein 10), mucus hypersecretion, and preliminary evidence for increased airway hyperresponsiveness [172]. No major studies have been conducted in rats using viral infection.

Conclusions

Although no one animal model of COPD is able to mimic human disease, the different types of models available have provided valuable information not only about the complex and heterogeneous pathophysiology of this disease, but also about the mechanisms underlying its progression and exacerbations. A greater understanding of these mechanisms may help develop and test novel therapies to effectively prevent disease deterioration and minimize mortality.

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