Murine models of cardiovascular comorbidity in chronic obstructive pulmonary disease

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Submitted 8 January 2016; accepted in final form 15 March 2016

Khedoe PP, Rensen PC, Berbée JF, Hiemstra PS. Murine models of cardiovascular comorbidity in chronic obstructive pulmonary disease. Am J Physiol Lung Cell Mol Physiol 310: L1011–L1027, 2016. First published March 18, 2016; doi:10.1152/ajplung.00013.2016.—Patients with chronic obstructive pulmonary disease (COPD) have an increased risk for cardiovascular disease (CVD). Currently, COPD patients with atherosclerosis (i.e., the most important underlying cause of CVD) receive COPD therapy complemented with standard CVD therapy. This may, however, not be the most optimal treatment. To investigate the link between COPD and atherosclerosis and to develop specific therapeutic strategies for COPD patients with atherosclerosis, a substantial number of preclinical studies using murine models have been performed. In this review, we summarize the currently used murine models of COPD and atherosclerosis, both individually and combined, and discuss the relevance of these models for studying the pathogenesis and development of new treatments for COPD patients with atherosclerosis. Murine and clinical studies have provided complementary information showing a prominent role for systemic inflammation and oxidative stress in the link between COPD and atherosclerosis. These and other studies showed that murine models for COPD and atherosclerosis are useful tools and can provide important insights relevant to understanding the link between COPD and CVD. More importantly, murine studies provide good platforms for studying the potential of promising (new) therapeutic strategies for COPD patients with CVD.

chronic obstructive pulmonary disease; cardiovascular disease; mouse models

PATIENTS WITH CHRONIC OBSTRUCTIVE pulmonary disease (COPD) have an increased risk for developing cardiovascular disease (CVD) (29, 163), even after correction for common risk factors (145, 193, 194). Airflow limitation and emphysema are associated with arterial stiffness (29, 233), and impaired lung function is correlated with cardiovascular morbidity (4), indicating an association between the severity of COPD and CVD. Exposure to cigarette smoke (CS) is the most important risk factor for COPD. The major risk factors for the development of atherosclerosis, the main underlying cause of CVD, are an unhealthy lifestyle that includes inactivity, bad eating habits (high fat intake), and smoking, which cause dyslipidemia and systemic inflammation, the main contributors to atherosclerosis (163, 228).

The precise mechanism linking COPD and atherosclerosis development is unknown, and is likely a combination of several mechanisms (260) (Fig. 1). Chronic systemic inflammation is suggested to be the most important link between COPD and atherosclerosis (58, 59, 163, 228, 229). Proinflammatory mediators from the lung can be released into the circulation, cause systemic inflammation, and contribute to atherosclerosis development (59). This may especially occur during COPD exacerbations, following, for example, bacterial and/or viral infection, resulting in greater systemic inflammation (20, 38, 163, 244) and thus a higher proatherogenic status. Changes in airway and intestinal microbial colonization are also suggested to contribute to COPD and atherosclerosis (7, 71, 108). Colonization of the airways by specific pathogens is frequently associated with COPD exacerbations and increased systemic inflammation. However, even in a clinical setting in which COPD is stable, airway colonization increases the risk for atherosclerotic events due to increased systemic inflammation (71). Other important mechanisms comprise endothelial dysfunction (usually induced by CS), oxidative stress, and formation of reactive oxygen species (ROS) (59, 91, 151, 230), processes that aggravate both COPD and atherosclerosis. As mentioned above, CS exposure itself is also an important risk factor for several adverse events (64) including atherosclerosis initiation and progression, mainly because of its oxidative stress- and inflammation-inducing characteristics (5, 59). Hypoxia-induced pulmonary hypertension and changes in pulmonary blood flow (51) in COPD patients may further contribute to right ventricle hypertrophy and left ventricular diastolic dysfunction (138), processes that affect cardiac function.
COPD patients with atherosclerosis receive COPD treatment complemented with standard CVD treatment. However, it is unknown whether such treatment is the most optimal therapy. Because of the complexity of both diseases, mouse models are used to study the link between COPD and atherosclerosis and to develop novel treatments targeting both diseases. Several reviews provide overviews of the mouse models for COPD (21, 37, 39, 40, 69, 189, 190, 196, 197, 229–230, 240) and atherosclerosis (78, 96, 116, 236, 252) separately, and we will therefore discuss these only briefly in the present review. The aim of this review is to provide an overview of the available murine models in which COPD and atherosclerosis development are combined, and to discuss the relevance of these models in studying the pathogenesis of both diseases and the development of novel treatment options for COPD patients with atherosclerosis.

**CHRONIC OBSTRUCTIVE PULMONARY DISEASE**

COPD is a lung disease characterized by progressive, irreversible airflow limitation, chronic pulmonary inflammation and remodeling, and destruction of lung tissue (80). Repeated exposure of the lung to noxious substances resulting from active and passive smoking, as well as exposure to indoor (in-house cooking and heating) and outdoor air pollution contribute to the pathogenesis of COPD (36). In addition, CS enhances the risk for recurrent respiratory infections, which further contribute to the development and progression of COPD (71). Various factors contribute to the development and progression of COPD, including number of pack years smoked, age, and genetic predisposition. COPD patients clinically present with a variety of symptoms that include dyspnea, wheezing, chest tightness, cough and sputum production, and symptoms resulting from a range of comorbidities (80).

**COPD in Humans**

Development of COPD in humans occurs in response to repeated exposure to CS and recurrent respiratory infections (80). Upon exposure of immune and structural cells to CS, or bacterial or viral components, proinflammatory mediators such as monocyte chemoattractant protein-1 (MCP-1), TNF-α, IL-12, and CXCL8/IL-8 are secreted (22, 57, 260). These mediators induce recruitment of other (circulating) inflammatory cells such as monocytes, lymphocytes, and neutrophils, which contribute to the local inflammatory response (22, 230). Neutrophils and macrophages produce reactive oxygen intermediates, and a range of granule proteins including matrix metalloproteases (MMPs) and neutrophil elastase (NE), to combat pathogens. An imbalance between oxidants and antioxidants, and proteases and antiproteases is proposed to contribute to the pathogenesis of COPD (22). Although CS itself contains a large number of oxidants, CS exposure also increases ROS generation in the lung and the circulation, while antioxidant mechanisms such as those provided by glutathione are decreased (221). The array of cytokines, proteases, oxidants, and other inflammatory mediators produced as a result cause mucus hypersecretion by goblet cells, induce proliferation of smooth muscle cells, and activation of fibroblasts, resulting in airway remodeling and emphysema as observed in COPD patients. Importantly, prenatal and early life exposure to CS may also predispose to COPD development, and several studies have addressed this predisposition (243, 259).

**Mouse Models of COPD**

Modeling the variety of processes that contribute to COPD development and progression, and the interactions between these processes, is complicated (22, 36). A short summary of frequently used murine models to study COPD is provided below and in Table 1, and these are described in more detail elsewhere (21, 37, 39, 40, 42, 47, 56, 196, 197, 229, 230, 240).

CS exposure in mice is frequently used as model for COPD. Nose-only exposure to CS causes pulmonary inflammation and emphysema (45, 177). Whole body exposure is performed with mainstream or sidestream CS, or both (92). Sidestream CS induces high carbon monoxide levels, thus limiting inflammation, whereas mainstream CS induces strong inflammation (114). In addition, differences exist in duration, cigarette number, brand, and puffing and susceptibility to inflammation and emphysema of various mouse strains (88, 224, 247).

COPD-like features in mice can also be induced by other methods such as administration of an aqueous extract of CS...
Table 1. Mouse models of chronic obstructive pulmonary disease

<table>
<thead>
<tr>
<th>Exposure Model</th>
<th>Subtype</th>
<th>Emphysema</th>
<th>Inflammation Lung</th>
<th>Systemic Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term CS*</td>
<td>Mainstream or sidestream, nose-only or whole body</td>
<td>No</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>1, 26, 32–34, 63, 64, 103, 109, 111, 122, 123, 128, 160, 161, 169, 172, 173, 219, 225, 229, 230, 234, 239, 245, 247</td>
</tr>
<tr>
<td>Long-term CS†</td>
<td>Mainstream or sidestream, nose-only or whole body</td>
<td>Yes</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>2, 9, 10, 16, 27, 30, 31, 37, 39, 40, 43, 44, 46, 47, 52, 56, 61, 82, 88, 92, 93, 95, 109, 112, 113, 118, 121, 126, 129, 135, 153, 164, 167, 168, 174, 175, 177, 180, 181, 183, 187, 200–202, 206, 211, 220, 222a, 225, 229, 230, 234, 241, 249, 253, 254</td>
</tr>
<tr>
<td>CS extract</td>
<td>Intraperitoneal or intranasal</td>
<td>Yes, after chronic</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>35, 97, 98, 152, 245, 256</td>
</tr>
<tr>
<td>PPE</td>
<td>Intratracheal or intranasal</td>
<td>Yes</td>
<td>Transient</td>
<td>No</td>
<td>75, 120, 155, 191, 201, 203, 204, 209, 229, 230</td>
</tr>
<tr>
<td>LPS/PolyI:C</td>
<td>Intratracheal or intranasal acute or chronic</td>
<td>Yes, after chronic</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>18, 54, 62, 75, 132, 133, 134, 207, 226</td>
</tr>
<tr>
<td>VEGF blockade/antiendothelial antibody</td>
<td>Combined models: CS, PPE or LPS</td>
<td>Yes</td>
<td>No</td>
<td></td>
<td>117, 205, 217</td>
</tr>
<tr>
<td>+ infection</td>
<td>CS, PPE or LPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diet-induced</td>
<td>Bacterial or viral</td>
<td>Yes, after chronic</td>
<td>Yes</td>
<td>Increased inflammation, oxidative stress</td>
<td>74, 105, 108, 120, 144, 150, 179</td>
</tr>
<tr>
<td>IL-13 overexpression</td>
<td>Yes</td>
<td>Yes/No</td>
<td></td>
<td>Increased inflammation, oxidative stress</td>
<td>12, 66</td>
</tr>
</tbody>
</table>

CS, cigarette smoke; LPS, lipopolysaccharide; poly I:C, polyinosinic:polycytidylic acid; PPE, porcine pancreatic elastase; VEGF, vascular endothelial growth factor. *Several days to 6 wk; †4–6 mo.

(97, 242, 256), or intratracheal or intranasal administration of proteases such as porcine pancreatic elastase (PPE), NE, or papain (21), all of which induce emphysema by proteolytic degradation of the alveolar walls, accompanied by a transient acute inflammatory process (21, 120). Administration of antibodies to endothelial cells (49) and blockade of the receptor for vascular endothelial growth factor (VEGF) also results in alveolar septal cell apoptosis, oxidative stress, and emphysema development (50, 117, 205, 217). Exposure to microorganisms or microbial components such as lipopolysaccharide (LPS) (156) and polyinosinic:polycytidylic acid [poly(I:C)] are also frequently used to induce features of COPD in mice (18, 20, 69, 134). The combination of CS (144), LPS, or PPE (65, 74, 75) together with infection with respiratory syncytial virus, nontypeable Haemophilus influenzae (74), or Chlamydia pneumoniae has been used to mimic COPD exacerbations (179). Other COPD models include the use of air pollutants (79), ozone exposure (216), and genetic models using overexpression or deletion of genes such as α1-antitrypsin (8, 189).

Although these models do mimic the various aspects of COPD pathology, they have some limitations. These include differences in susceptibility between mouse strains (88), differences in anatomy of the lungs between humans and mice, and the absence of substantial airway involvement in most smoking models. Furthermore, differences in tissue analysis, fixation procedure, and other necropsy procedures may contribute to the differences observed between studies and groups (17). Despite such limitations, these models are considered suitable for studying COPD pathogenesis and novel COPD treatment strategies (69, 190).

ATHEROSCLEROSIS

Atherosclerosis is the main underlying cause of CVD and primarily affects the large arteries. Development of atheromatous plaques, a process called atherogenesis, starts with endothelial injury and activation, for example, by oxidative stress, which induces recruitment and migration of circulating monocytes into the intima (Fig. 2). Two important factors in the development of atherosclerosis are dyslipidemia and inflammation. Dyslipidemia arises primarily from unhealthy eating habits, causing increased circulating levels of proatherogenic lipoproteins. Triglyceride-derived fatty acids can be used as fuel for organs such as the heart and muscles, stored in white adipose tissue or burned in brown adipose tissue. In addition, the remaining triglycerides can be taken up by the liver again for repackaging into nascent VLDL. Cholesterol, an important component for membranes; vitamin D; bile fluids; and steroid hormones can be packaged into nascent VLDL and HDL or cleared into bile fluid (99). The liver recognizes triglyceride (TG)-rich lipoproteins mainly through apolipoprotein E (ApoE) on the surface of
lipoproteins, which is a ligand for receptors including the LDL-receptor (LDLr).

**Atherosclerosis in Humans**

During (CS-induced) oxidative stress, lipoproteins can be oxidized in the circulation or locally after entrapment in the vessel wall, resulting in the formation of oxidized LDL (ox-LDL). OxLDL is taken up by macrophages that are infiltrated in the vessel wall, which subsequently transform into foam cells, thereby enabling recruitment and infiltration of circulating monocytes into the intima. Oxidized lipoproteins can be ingested by the macrophages and cause formation of foam cells. A fatty streak is formed. When influx of lipoproteins is continued, the foam cells keep producing proinflammatory mediators, inducing further recruitment of immune cells from the circulation and proliferation of smooth muscle cells (SMC) from the media. The SMC migrate to the luminal side of the developing plaque and form a fibrous cap by producing collagen and elastin. Due to the growth of the plaque, the core is deprived of oxygen, causing apoptosis and necrosis of (foam) cells, eventually causing formation of cholesterol clefts in the necrotic core. At the same time, the lumens of the blood vessel becomes smaller.

Further induces plaque progression and growth. During growth of the plaque, the inner core of the plaque is deprived of oxygen, which causes apoptosis and necrosis, leading to formation of extracellular cholesterol crystals. The fibrous cap prevents rupture of the plaque and subsequent spilling of the inner core into the lumen. However, upon stress, the fibrous cap becomes thinner, causing the plaque to be more prone to rupture. Plaque rupture results in spillover of the plaque content into the lumen and formation of a blood clot, and is the most common cause of myocardial infarction and stroke.

**Mouse Models of Atherosclerosis**

Because wild-type (WT) mice do not spontaneously develop atherosclerosis, genetically modified mice are used to create atherosclerosis-prone models (Table 2). The classical murine atherosclerosis models are the Apoe<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice in which the hepatic ApoE/LDLr-mediated lipoprotein clearance pathway is disrupted, resulting in high circulating VLDL/LDL levels (116). These two frequently used models show differ-
Table 3. Combined mouse models of COPD and atherosclerosis

<table>
<thead>
<tr>
<th>COPD Model</th>
<th>CVD Model</th>
<th>Emphysema</th>
<th>Inflammation</th>
<th>Atherosclerosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term CS</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>No</td>
<td>Systemic Pulmonary</td>
<td>Yes/No</td>
<td>4, 72, 91</td>
</tr>
<tr>
<td>Long-term CS</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Ldlr&lt;sup&gt;−/−&lt;/sup&gt;, human transgenic ApoB100</td>
<td>Yes</td>
<td>systemic pulmonary</td>
<td>Yes</td>
<td>4, 6, 14, 15, 28, 52, 72, 73, 81, 90, 91, 124, 128, 131, 140, 148, 186, 232, 250</td>
</tr>
<tr>
<td>Caloric restriction/high cholesterol/high-fat diet</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Yes</td>
<td>Systemic Pulmonary</td>
<td>Yes/No</td>
<td>12, 30, 81, 146, 147, 159</td>
</tr>
<tr>
<td>Combined models: CS, PPE/LPS/infection</td>
<td>Apoe&lt;sup&gt;−/−&lt;/sup&gt;, Ldlr&lt;sup&gt;−/−&lt;/sup&gt;, APOE&lt;sup&gt;*3-Leiden&lt;/sup&gt;/cuff/collar</td>
<td>Yes</td>
<td>Systemic Pulmonary</td>
<td>Yes</td>
<td>3, 23, 89, 104, 110, 120, 125, 154, 157, 257</td>
</tr>
<tr>
<td>CS</td>
<td>Other effects: Metabolic organs</td>
<td>Yes/No</td>
<td>Systemic Pulmonary</td>
<td>Yes/No</td>
<td>9, 10, 27, 30–34, 52, 103, 135, 181, 182, 249, 250, 256</td>
</tr>
</tbody>
</table>

**Chronic Systemic Inflammation Linking COPD and Atherosclerosis**

Systemic inflammation is suggested to be one of the most important mechanisms to link COPD and atherosclerosis. Increased systemic levels of the acute phase C-reactive protein (CRP), TNF-α, and IL-6 in COPD patients are associated with an increased risk of atherosclerosis development (163, 228). In particular, COPD exacerbations increase circulating levels of proinflammatory mediators and are associated with atherosclerotic events and death. Several factors may contribute to systemic inflammation in COPD, including CS exposure and recurrent infections. Several murine studies on systemic inflammation linking COPD and atherosclerosis are described below.

The role of CS in systemic inflammation. Most murine studies aimed at the interaction between COPD and CVD are performed with subchronic or long-term CS exposure. CS exposure alone generally results in increased pulmonary leukocyte recruitment and pulmonary endothelial cell adhesion (178), inflammation, and oxidative stress in mice (21, 111). Long-term CS exposure in WT mice not only induced pulmonary inflammation and emphysema, but also increased systemic proinflammatory cytokines including TNF-α and IL-1β (82). Although that study did not combine COPD and CVD, the increased systemic cytokines were indicative of a proinflammatory systemic response after CS (82) in a normolipidemic WT model. In two other studies, exposure to sidestream CS in WT mice not only increased systemic proinflammatory cytokine levels, but also decreased heart stroke volume and antioxidant levels in the heart, and increased vascular resistance (253, 254), suggesting that CS exposure directly affects the cardiovascular system. Long-term CS exposure of transgenic mice with human APOB100, which develop moderate hyperlipidemia when fed an atherogenic diet, resulted in higher levels of MCP-1 in the circulation, heart, and aorta, and...
enhanced atherosclerotic lesion area and macrophage content in the atherosclerotic plaque (250). These data suggest that CS exposure leads to systemic inflammation, possibly in part through spillover of inflammatory mediators from the lung.

Leukocyte recruitment toward the lung during local pulmonary or systemic inflammation may also be different. Systemic intravenous LPS administration, for example, induced leukocyte trapping in pulmonary capillaries, whereas intratracheal LPS administration induced leukocyte accumulation in venules. Furthermore, local intratracheal LPS administration induced alveolar space leukocyte recruitment, which was not observed after intravenous administration of LPS. The difference in recruitment toward the lung after local or systemic LPS administration may be a result of leukocyte priming after systemic inflammation (235).

The role of respiratory infections in systemic inflammation. Smokers with normal lung function and COPD patients have a high risk for recurrent respiratory infections, which may contribute to systemic inflammation and atherosclerosis. In particular, COPD exacerbations associated with respiratory infections cause a rise in circulating inflammatory mediators and are associated with CVD morbidity and mortality (20, 58, 163). Respiratory infections alone can aggravate atherosclerosis through induction of systemic proinflammatory mediators (165, 215). Combining Chlamydia pneumoniae infection with sidestream CS exposure in Apoe−/− mice resulted in exacerbated atherosclerosis due to enhanced pulmonary inflammation, apoptosis, and defective pulmonary phagocytosis of pathogens, whereas systemic lipid levels and inflammation were not altered (257). Respiratory viral infections are also positively associated with atherosclerosis development (188). Upon exposure of Apoe−/−, Ldlr−/−, and WT mice to intranasal influenza virus, live virus was used in lungs, heart, and aorta, and Apoe−/− mice showed increased atherosclerotic plaque area and inflammatory cell content in the plaque compared with controls (89, 157).

CS exposure and respiratory infections may have direct effects on atherosclerosis development because of leakage of CS components or pathogens to the vessel wall. Therefore, several studies also examined the effect of emphysema alone on atherosclerosis development, in the absence of CS or infection. We showed, for example, that PPE-induced emphysema in WTD-fed APOE*3-Leiden mice did not affect pulmonary inflammation, or systemic inflammatory or lipid parameters, and did not affect atherosclerosis development, suggesting that emphysema alone does not aggravate atherosclerosis (120). Addition of biweekly chronic intranasal LPS administration to mimic chronic pulmonary inflammation did induce low-grade systemic and pulmonary inflammation and increased atherosclerosis development. This proatherogenic effect is likely caused by leakage of inflammatory mediators into the circulation, but this remains to be elucidated.

These studies collectively show that CS and respiratory infections induce systemic inflammation in mice, similar to COPD patients with atherosclerosis. Systemic inflammation most likely arises in part from leakage of proinflammatory mediators from the lung to the circulation (194, 207). It needs to be noted that development of features of atherosclerosis and COPD require long-term exposure, and therefore, aging of the mice during the experiment may affect outcomes. Although most studies have an age-matched control group, aging is associated with low-grade systemic inflammation (“inflammaging”) and may affect the lungs, immune responses, and response toward CS exposure and infections (238). Furthermore, there is evidence that in the absence of systemic inflammation, the extent of atherosclerosis development is limited. In addition, models of diet-induced atherosclerosis may be more lipid- and less inflammation-driven, suggesting that further research is necessary with more inflammation-driven atherosclerosis models such as the cuff or collar models. However, other CS-induced mechanisms linking COPD and CVD could also be involved and are described below.

Hyperlipidemia Linking COPD and Atherosclerosis

Another proposed mechanism that links COPD and atherosclerosis is hyperlipidemia, a common feature in COPD patients, which may arise from unhealthy eating habits and other lifestyle factors such as smoking. Smokers and COPD patients show increased circulating levels of VLDL, LDL, and triglycerides and low levels of HDL (5). Dyslipidemia not only affects circulating lipids and lipoproteins, but lipids are also important in normal physiology and homeostasis of organs and tissues, including the lung (84). Furthermore, diet-induced hyperlipidemia may contribute to systemic inflammation and pulmonary pathophysiology. Murine studies in which the role of hyperlipidemia was examined as a link between COPD and atherosclerosis are described below.

The role of hyperlipidemia in systemic inflammation. Apart from spillover of proinflammatory mediators from the lungs, systemic inflammation may also arise from hyperlipidemia. For example, intraperitoneal LPS injection resulted in higher plasma levels of IFN-γ and TNF-α in Apoe−/− and WT mice fed an atherogenic diet, and aggravated atherosclerosis development in Apoe−/− mice. Interestingly, it also induced leukocyte infiltration around pulmonary vessels in WT mice, and this was even more pronounced in hyperlipidemic Apoe−/− mice (162), suggesting that systemic inflammation, especially in combination with hyperlipidemia, increases pulmonary inflammation. Also, diets rich in cholesterol and fat increase pulmonary inflammation (214) and emphysema development (81). Feeding Apoe−/− mice the highly atherogenic Paigen diet, for example, resulted in higher plasma IL-6 and IL-1 levels, and induced pulmonary arterial hypertension and right ventricular hypertrophy (139). This is further supported by the observation that 12 wk of a high-fat diet in Apoe−/− mice also resulted in increased circulating inflammatory cytokines and pulmonary inflammation (159). Furthermore, pulmonary inflammation was associated with collagen deposition and MMP-9 activity, suggesting that a high-fat diet also induced remodeling in the lung. CS exposure resulted in greater pulmonary inflammation and emphysema associated with decreased lung function in chow-fed Apoe−/− mice, which still experienced mild hyperlipidemia compared with chow-fed WT mice (4). Apoe−/− mice fed a Western-type diet for 10 wk also developed emphysema, which was not observed in WT or Ldlr−/− mice (81). Because macrophages from these Apoe−/− mice have a reduced cholesterol efflux and increased expression of proinflammatory genes, a link between abnormal cholesterol efflux and lung inflammation was suggested.

These data show that diet-induced hyperlipidemia contributes to systemic inflammation in mice and may, vice versa, also
induce pulmonary inflammation, tissue destruction, and emphysema through MMP activation and other mechanisms. These findings may be relevant for COPD patients with dyslipidemia due to a Western lifestyle with high fat intake, high cholesterol levels, and insufficient exercise (222). These factors may thus, in addition to smoking, affect pulmonary inflammation, emphysema, and systemic inflammation, which enhance the risk for atherosclerosis development.

The effect of CS on metabolic organs. CS exposure is also known to affect metabolic organs including liver, muscle, and white and brown adipose tissue, organs that are important in lipid and lipoprotein metabolism. These organs are also implicated in the metabolic syndrome, which is more prevalent among smokers and contributes to CVD development (107).

The liver is a key organ in regulation of lipid metabolism and (systemic) inflammation. Hepatic steatosis (fatty liver disease) has been linked to atherosclerosis, although causation is unclear. CS exposure may increase the risk of hepatic steatosis through inflammation, lipid accumulation, and hypoxia, which worsens on a hypercholesterolemic background (6, 249). Low body weight and muscle wasting are commonly observed in patients with severe COPD and contribute to overall morbidity and mortality. Muscle wasting in patients with severe COPD (69) may contribute to atherosclerosis development, most likely by induction of systemic inflammation involving cytokines such as TNF-α (129, 136). Chronic CS exposure lowers body weight and white adipose tissue mass in WT mice (167), at least in part exerted via CS-mediated reduction of the neurotransmitter neuropeptide Y, which decreases appetite (30–34). There are also indications that caloric restriction increases pulmonary resistance and decreases lung volume (12). CS-induced reduction of body weight is driven by cytokines such as IL-6 and the adipokines leptin and adiponectin (93, 195). Adiponectin is considered protective in several diseases such as in metabolic syndrome and COPD (94). Adiponectin-deficient mice develop alveolar enlargement and other COPD-like characteristics in addition to systemic inflammation, wasting, and endothelial dysfunction similar to that in COPD patients (158). Also, mice with PPE-induced emphysema have low levels of adiponectin (153). Moreover, leptin expression in bronchial epithelial cells and alveolar macrophages was higher in WT mice exposed to CS compared with air-exposure (227). In spite of these findings, the physiological role of these adipokines in lung pathology and inflammation is not clear (195).

Several murine models have shown that exposure to CS activates brown adipose tissue, which in contrast to white adipose tissue, burns triglyceride-derived fatty acids and produces heat (101, 119). CS exposure induces expression of genes related to brown adipose tissue activation and thus may contribute to the lower body weight observed in smokers (31, 34). Moreover, brown adipose tissue activation reduces hypercholesterolemia and atherosclerosis development in ApoE/Leiden.CETP mice (11, 101). Because this cholesterol-reducing effect of brown adipose tissue activation is dependent on an intact ApoE-LDLr-mediated hepatic lipoprotein remnant clearance pathway, the cholesterol- and atherosclerosis-reducing effect was not observed in ApoE−/− and Ldlr−/− mice (11, 52). Although brown adipose tissue activation in ApoE/Leiden.CETP mice appears atheroprotective, the relationship between brown adipose tissue activation and the relatively unfavorable lipid profile in smokers and COPD patients is unclear. And in contrast to murine studies, the effect of CS exposure on brown adipose tissue in humans is not known yet, although resting energy expenditure in smokers was increased compared with individuals who do not smoke (13, 48), suggesting that brown adipose tissue may be activated. Furthermore, we have found no reports that have investigated the role of brown adipose tissue activation in the link between COPD and CVD.

As described above, murine models and human studies have shown that CS has various effects on metabolic organs such as the liver, brown and white adipose tissue, and muscle. Whether CS-induced metabolic changes in these organs contribute to atherosclerosis development remains to be elucidated.

The role of CS in hyperlipidemia. In addition to contributing to systemic inflammation and dysfunction of metabolic organs as described in the previous sections, CS may also contribute to atherosclerosis development by inducing hyperlipidemia. However, the effects of CS exposure on lipid levels in murine COPD and CVD models are inconclusive. Long-term exposure to both mainstream (232) and sidestream (72, 73) CS alone or in combination with feeding a Western-type diet to ApoE−/− mice aggravates atherosclerosis development, but this could not be explained by higher plasma cholesterol levels. However, in CS-exposed Ldlr−/− mice fed the same saturated-fat diet, both systemic cholesterol levels and atherosclerotic lesion area were increased, also without affecting pulmonary inflammation (91). Importantly, pulmonary inflammation was determined only in the latter study, and was not changed in either ApoE−/− or Ldlr−/− mice (91).

In other studies using ApoE−/− mice, CS exposure did result in higher circulating lipid levels (15, 140). Exposure to mainstream CS in ApoE−/− mice resulted in enhanced levels of cholesterol, triglycerides, and phospholipids in the circulation, liver, and aorta. Furthermore, pulmonary inflammation was determined only in the latter study, and was not changed in either ApoE−/− or Ldlr−/− mice (91).

Because most combined studies for COPD and atherosclerosis have been performed using ApoE−/− mice, one should keep in mind that lungs of these mice are congenitally different from lungs of WT mice. ApoE−/− mice have impaired developmental alveologenesis, increased airway resistance, and a rapid loss in lung elastic recoil compared with WT mice, predisposing ApoE−/− mice to pulmonary problems (146). In addition, ApoE itself has anti-inflammatory, antioxidative, antiproliferative (e.g., on smooth muscle cells) and antithrombotic properties, which may all contribute to its antiatherosclerotic effects. The immunomodulatory effects of ApoE may...
substantially contribute to immune activation and inflammation during atherosclerosis (252). Upon exposure to mainstream CS, chow-fed Apoe<sup>−/−</sup> mice showed an increased pulmonary inflammatory response, MMP activity, and emphysema development compared with chow-fed WT mice, suggesting that the lungs of Apoe<sup>−/−</sup> mice are more sensitive to CS (4). Deficiencies in other proteins such as scavenger receptors involved in intracellular and membrane cholesterol metabolism and trafficking in the lung, can result in altered immune responses in the lung (84). Furthermore, exposure to CS affects phagocytic activity of alveolar macrophages, which in combination with an abnormal cholesterol efflux in Apoe<sup>−/−</sup> mice, worsens pulmonary inflammation and contributes to atherosclerosis development (166). Mutations in ApoE thus not only affect atherosclerosis development, but can also affect both systemic and pulmonary inflammatory responses that are observed in models of COPD and airway hyperresponsiveness (248). Next to hyperlipidemia in Apoe<sup>−/−</sup> mice, obesity and diabetes may also affect lung function, structure, immune cell function (66), and pulmonary circulation and hypertension (67).

In conclusion, findings in murine studies with regard to the contribution of hyperlipidemia to the link between COPD and atherosclerosis are inconclusive so far. This difference in outcome is most likely explained by differences in duration of the studies, diet, outcome parameters, and smoking protocols.

Oxidative Stress Linking COPD and Atherosclerosis

Another proposed mechanism to link COPD and CVD is CS-induced oxidative stress and formation of ROS (26, 132). Oxidative stress causes dysregulation of atheroprotective mediators and induces endothelial injury and atherosclerosis development (151). Acute CS exposure causes oxidative (DNA damage in lungs, heart, and liver (103, 113, 161). Mitochondria are susceptible to oxidative stress, and CS-induced alterations in mitochondrial function can thereby affect energy production and metabolism (1), which may contribute to vessel wall damage and atherogenesis. This is supported by studies in hypercholesterolemic Apoe<sup>−/−</sup> mice exposed to sidestream CS, in which oxidative damage to mitochondrial DNA and protein damage in the heart was observed together with a lowering of antioxidants such as vitamins C and E (124). Increased systemic lipid peroxidation also caused depletion of vitamin E in liver and heart (254). CS-induced oxidative stress also causes lipid modification, such as formation of proatherogenic ox(V)LDL (208). In the lung, CS can oxidize lipids such as phospholipids, which impairs the phagocytic capacity of alveolar macrophage and thus may contribute to defective immune responses to invading pathogens (211).

Arginase is an enzyme that competes with endothelial nitric oxide synthase (eNOS) for the precursor of nitric oxide (NO), and is involved in many pathologies. Sidestream CS exposure in WT mice results in increased arginase activity and ROS levels, whereas NO levels were decreased, causing endothelial dysfunction and vascular stiffness (192). NO was not lower in arginase-2-deficient mice, indicating that CS resulted in lowered eNOS activity. Furthermore, CS extract also inhibits eNOS activity in pulmonary artery endothelial cells, which may contribute to CVD in cigarette smokers (198). Whereas eNOS is considered atheroprotective, inducible nitric oxide (iNOS) contributes to oxidative stress. CS exposure induces iNOS, causing ROS formation and cuff-induced intimal thickening in WT mice (3).

After removal or lowering of atherogenic components such as nicotine and tar from cigarettes, exposure to CS still resulted in higher biomarkers of systemic oxidative stress such as oxLDL. Other parameters, including plasma lipid levels (28), blood pressure, and blood leukocyte count, were not different, although lesion area was still higher compared with nonsmoking mice (28). Antioxidant therapy using vitamin E lowered these effects, indicating that atherosclerosis was accelerated in response to CS-induced systemic oxidative stress (131). Collectively, these data show that CS-induced oxidative stress in mice is an important contributor in the link between COPD and CVD.

Endothelial Dysfunction Linking COPD and Atherosclerosis

Injury to the endothelium induces endothelial dysfunction, an important step in atherosclerosis development (5, 76, 151). CS exposure causes endothelial damage and may thereby contribute to the link between COPD and atherosclerosis. CS is associated with endothelial apoptosis and reduced vascular barrier function, enabling leakage of inflammatory mediators to the circulation and vice versa, thereby contributing to inflammation (142, 180).

Chronic exposure of WT mice to CS causes high blood pressure, high blood cell counts, oxidative stress, systemic inflammation, low NO levels and lowered cardiac function (206), all of which may contribute to injury and activation of the endothelium. Also, recruitment of endothelial progenitor cells (EPC) from bone marrow and their function may be hampered by CS. Intraperitoneal injection of CS extract, used to induce emphysema in mice, interrupted EPC recruitment from bone marrow to the lung (97). Antioxidant therapy resulted in reduced CS-induced systemic oxidative stress, EPC dysfunction, and neovascularization development in WT mice (218), indicating that CS-induced EPC dysfunction is at least in part mediated via oxidative stress. CS exposure may also lead to enhanced platelet reactivity and thrombosis in Apoe<sup>−/−</sup> mice, further contributing to vascular injury (52). For example, circulating levels and procoagulant activity of tissue factor, which is involved in thrombus formation, were increased after CS exposure in Apoe<sup>−/−</sup> mice (148). In addition, thrombin generated upon CS exposure is involved in thrombus formation, but can also induce pulmonary endothelial hyperpermeability and decrease barrier protection (258). Interestingly, this process could be inhibited by bone marrow-derived EPC. Finally, CS affects vascular endothelial growth factor (VEGF), which is important in the maintenance of lung structure and endothelial function. CS exposure reduced both VEGF and VEGF receptor in the lung, which was also observed in COPD patients who smoke (202).

Although human studies on the effect of endothelial dysfunction in the link between COPD and CVD are scarce (19), data suggest that patients with mild COPD have higher levels of endothelial microparticles, suggesting endothelial activation and/or injury (212). Furthermore, patients with severe COPD show elevated markers of endothelial activation. Despite the limited data for humans, murine studies seem to show an...
important role for the endothelium and EPCs in the link between COPD and CVD.

**TREATMENT OF COPD PATIENTS AND ATHEROSCLEROSIS**

Although smoking cessation is the most effective measure to reduce COPD symptoms (5, 260), it is difficult in practice because of the addictive nature of tobacco. Smoking cessation does result in an increase in atheroprotective HDL and can be beneficial for reducing endothelial injury (5). However, even after smoking cessation, a considerable risk for both COPD and CVD remains, which was also shown in murine studies (140). COPD patients are treated with bronchodilators such as long-acting β2-agonists and anticholinergics, and anti-inflammatory agents such as inhaled corticosteroids, which are especially indicated for patients with frequent exacerbations (260) (Fig. 3). Treatment with inhaled steroids reduced all-cause mortality in COPD in a case-control study (25). However, in the TORCH study, no significant reduction was observed in all-cause mortality (including cardiovascular cause) after a combination therapy of a bronchodilator and an inhaled steroid. Nevertheless, treatment did reduce the frequency of exacerbations and improve overall health status (24). Most studies on the effect of bronchodilatory and anti-inflammatory agents on COPD and CVD parameters are performed in human trials and, unfortunately, murine COPD models are not often used.

COPD patients with CVD receive additional lipid-lowering drugs such as statins, which inhibit the rate-controlling enzyme of cholesterol synthesis, 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. Various murine studies have showed beneficial effects of statin therapy on the lungs, which is most likely mediated by its anti-inflammatory actions (237) in both acute and chronic disease states; for example, during sepsis after *Streptococcus*-induced acute lung injury (255), but also air pollution-induced pulmonary inflammation (210). Statins also result in lower acute CS-induced inflammation and oxidative stress (63), and elastase-induced emphysema is reduced upon statin treatment in WT mice (204). However, the importance of these findings for assessing the clinical benefit of statin treatment for COPD is a matter of ongoing debate.

There are several potential new drug targets (Fig. 3) for the treatment of COPD patients with CVD. Recent findings suggest that other lipid-lowering (i.e., liver × receptor agonists, ApoE-mimetic peptides) and HDL-raising strategies (i.e., apoAI-mimetic peptides) also may be beneficial for various lung disorders (237) including COPD (84), and are therefore attractive treatment targets and the subject of further research for both COPD and CVD. Injection of HDL, which is also a transporter for α-antitrypsin, injected together with α-antitrypsin, reduces CS-induced pulmonary inflammation and elastase-induced emphysema (155), and may be an attractive therapeutic option for both COPD and CVD.

Other treatment approaches include vitamin D therapy, microbiome alteration, and administration of EPC or mesenchymal stromal cells (MSC). Vitamin D deficiency is associated with COPD and CVD (100, 149, 184), and COPD patients with low vitamin D levels have a higher exacerbation risk (170, 171). Mice exposed to CS while being fed a vitamin D-deficient diet developed more severe emphysema compared with CS exposure alone (43, 171). Vitamin D also contributes to antimicrobial defense and control of inflammation, and thus may contribute to the control of the development of both COPD and CVD. Thus vitamin D may be an attractive target in combined therapy for COPD and CVD. Another potential new
The effects of COPD drug treatments on CVD outcomes and COPD treatments on COPD progression have been studied only in the past few years. In general, the effect of COPD treatment on CVD outcome and vice versa is currently largely unknown. Furthermore, because of the variety of drugs that COPD patients with CVD receive, it is difficult to determine the effect of a single drug, and of drug interactions. This issue limits the interpretation of study outcomes on the combined effects of COPD and CVD drugs in clinical trials and illustrates the necessity of new experimental models and studies. In such (murine) models, disease pathology and the effect of current and novel drug strategies for COPD with CVD can be assessed in a more controlled way.

In conclusion, combinations of models for COPD and atherosclerosis are very useful tools and can provide important insights relevant to understanding the link between COPD and CVD. More importantly, murine studies provide good platforms for studying the potential of promising (new) therapeutic strategies for COPD patients with CVD.

REFERENCES


