This thesis is based on studies of several aspects of airway inflammation in chronic obstructive pulmonary disease (COPD). Studies on inflammatory changes in induced sputum, bronchial wash (BW), bronchoalveolar lavage (BAL) fluid and bronchial biopsies, on hyperresponsiveness to adenosine 5'-monophosphate (AMP, an indirect stimulus in hyperresponsiveness testing, generally thought to better reflect airway wall inflammation than direct stimuli e.g. methacholine), on the role of airway inflammation in dyspnea, on the methodology of measurement of nitric oxide (NO) in exhaled air and on markers of NO metabolism in the airways are reported in the subsequent chapters. The overall aim was to further unravel the contribution of inflammation to the pathophysiology and clinical expression of COPD.

The results of these studies are summarized below.

Induced sputum is nowadays frequently used for studying airway inflammation. It is not known how findings in induced sputum relate to findings with other study tools. In chapter 2, we investigated how percentage and concentration of inflammatory cells and mediators in induced sputum relate to findings in BW, BAL fluid and bronchial biopsies in subjects with COPD. We studied 18 nonatopic subjects with COPD and 11 healthy controls. Sputum was induced by inhalation of hypertonic saline and the whole sample was processed. The airways were lavaged, using the first 50 ml for BW and the subsequent 150 ml for BAL, and biopsies were taken from subsegmental carinae. Neutrophils were the predominant cell type in sputum in COPD (median 77.3 %) but not in BW (5.5 %) and BAL fluid (1.7 %). Differential cell counts in sputum did not correlate with counts in BW, BAL fluid or biopsies, whereas sputum eosinophil cationic protein (ECP) correlated significantly with BW ECP (rho=0.66, p=0.007) and sputum IL-8 with BAL IL-8 (rho=0.52, p=0.026). Subjects with COPD had a higher percentage of sputum neutrophils and eosinophils and higher concentrations of ECP and IL-8 than healthy controls. The higher percentages of eosinophils and concentrations of ECP were also seen in BW and BAL. Finally, higher numbers of macrophages and activated eosinophils were found in biopsies. We concluded that induced sputum is derived from a different compartment than BW, BAL and biopsies. Induced sputum may be useful for studying the contribution of luminal neutrophils and eosinophils to tissue degradation in COPD, and studying soluble markers of inflammation in the large airways and bronchoalveolar compartment.

In chapter 3, we investigated which airway cell or mediator contributes to the differences between subjects with COPD and healthy subjects in percentage or concen-
tration of inflammatory cells or mediators in induced sputum, BAL fluid and bronchial biopsies. We studied 18 nonatopic subjects with COPD and 11 nonatopic healthy subjects. To avoid direct effects of smoking on the inflammatory measurements performed, we excluded current smokers. Sputum induction and bronchoscopy with BAL and biopsies were performed. COPD patients had more mucosal eosinophils and macrophages and a tendency towards more CD4+ but not CD8+ lymphocytes than healthy controls. Furthermore, COPD patients had higher percentages of sputum neutrophils and eosinophils and BAL fluid eosinophils and higher concentrations of sputum ECP. Concentrations of ECP expressed per eosinophil were not higher. COPD patients with high mucosal eosinophil numbers had also high mucosal CD4+ lymphocyte numbers. Sputum eosinophilia was associated with a decrease in FEV1/VC and BAL fluid eosinophilia with a decrease in mucosal neutrophils. We conclude that subjects with COPD who do not currently smoke have increased numbers of inflammatory cells. Eosinophils are increased in number in the airways in COPD but do not seem to be activated. The increased eosinophil numbers are probably due to recruitment as a result of ongoing inflammation. Macrophages and lymphocytes may play a role in this inflammation.

In chapter 4, we have investigated the role of airway inflammation in hyperresponsiveness to AMP in COPD. Inflammatory indices in induced sputum, BAL fluid and bronchial biopsies were measured in 12 nonatopic non/exsmokers with COPD with and in 6 without hyperresponsiveness to AMP and in 11 healthy controls. Subjects with COPD who presented with hyperresponsiveness to AMP had significantly higher numbers of mucosal CD8+ cells and higher percentages of sputum eosinophils than those without hyperresponsiveness. Otherwise, no differences in inflammatory markers were observed. We conclude that hyperresponsiveness to AMP in COPD is associated with airway inflammation, that is characterized by increased numbers of mucosal CD8+ cells and increased percentages of sputum eosinophils. Hyperresponsiveness to AMP may be used as a marker of airway inflammation in COPD, but its significance in the clinical course remains to be determined.

In chapter 5, we have investigated the role of histamine and cholinergic activity in hyperresponsiveness to AMP in COPD. These pathways had been found to be important in AMP induced bronchoconstriction in asthma. Histamine activity was investigated using the H1-receptor antagonist terfenadine and cholinergic activity was investigated using the anticholinergic drug ipratropium bromide. Forty-four hyperresponsive smokers with COPD were challenged with Mch and AMP. They used either 180 mg of oral terfenadine, 120 μg of inhaled ipratropium bromide, or placebo before the challenge in a randomized, double blind cross over trial. Geo-
metric mean PC_{20}AMP was 5.44 mg/ml after placebo and increased with 0.9 doubling concentration (p<0.0001) after terfenadine and decreased with 0.3 doubling concentration after ipratropium bromide (NS). We concluded that histamine is involved in AMP induced bronchoconstriction in smokers with COPD, whereas vagal nerve stimulation does not play a role.

In chapter 6, we investigated whether hyperresponsiveness to AMP decreases after a short-term treatment with inhaled corticosteroids in smokers with COPD. We hypothesized that AMP would be a more sensitive marker of airway inflammation than Mch in assessing effects of corticosteroids on hyperresponsiveness in COPD. Additionally, we studied the effects of inhaled corticosteroids on terfenadine and ipratropium bromide an on serum levels of interleukin-8 and histamine. Forty-four hyperresponsive smokers with COPD were challenged with Mch and AMP on 3 study days, after pretreatment with single doses of ipratropium bromide, terfenadine and placebo. Thereafter, subjects were randomized to 6 weeks of treatment with either 1,600 µg budesonide or placebo, and the same 3 study days were repeated. Budesonide, as compared to placebo, did not significantly change PC_{20}AMP, PC_{20}Mch or FEV_{1} after placebo pretreatment. Budesonide increased PC_{20}Mch after ipratropium bromide pretreatment, from 5.05 to 10.20 mg/ml (p=0.036). Budesonide decreased serum IL-8 from 9.2 ± 3.1 to 6.2 ± 2.1 pg/ml (p < 0.001). We conclude that PC_{20}AMP did not decrease after a short-term treatment with budesonide and that AMP did not elicit greater sensitivity than Mch in assessing short-term effects of budesonide on hyperresponsiveness in smokers with COPD. We suggest that long-term treatment with inhaled corticosteroids might be beneficial, by reducing neutrophil load in the airways and improving the action of anticholinergic drugs.

In chapter 7, we investigated the role of airway inflammation in the sensation of dyspnea in induced bronchoconstriction in COPD. Dyspnea was measured before and after bronchoconstriction induced with on the one hand an indirect stimulus, AMP, and on the other hand a direct stimulus, methacholine (Mch). We hypothesized that comparison of dyspnea after AMP and Mch challenges could give information about airway inflammation, because in asthma AMP has been shown to induce more dyspnea than Mch, probably via stimulation of sensory receptors and airway wall oedema. Forty-eight smoking subjects with COPD were challenged with AMP and Mch, and Borg score was assessed before and after each challenge. We found that AMP tended to induce more dyspnea than Mch challenge (p=0.18). One third of all subjects with COPD did not perceive bronchoconstriction during both challenges. We concluded that airway inflammation may play a role in the per-
ception of dyspnea in COPD. More direct markers of inflammation than measurement of hyperresponsiveness to indirect stimuli are needed to clarify the role of inflammation in the perception of dyspnea in COPD.

In chapter 8, we investigated whether levels of exhaled NO are different in subjects with COPD and asthma compared with healthy subjects. Moreover, we investigated whether differences, when present, were affected by the method of measurement. NO was measured in exhaled air with the single breath method and the tidal breathing method in 16 nonatopic exsmokers with COPD, 16 nonatopic exsmoking healthy controls, 16 atopic nonsmoking asthmatics, 16 atopic currently smoking asthmatics and 16 nonatopic nonsmoking healthy controls. NO values were not significantly different between exsmokers with COPD and healthy exsmokers with both single breath and tidal breathing method. NO values were higher in nonsmoking asthmatics than in nonsmoking healthy subjects with both methods. Nitric oxide concentrations differed substantially between both methods and were not interchangeable. We concluded that subjects with COPD, in contrast to asthmatics, do not have higher exhaled NO concentrations than healthy subjects.

In chapter 9, we investigated whether markers of NO metabolism in exhaled air and sputum are increased in subjects with COPD and whether they correlate with inflammatory cells in induced sputum. Moreover, we assessed the influence of smoking on these investigations. Sixteen subjects with COPD and 16 healthy controls participated. Both groups had 8 current smokers. NO was measured with the tidal breathing method. Sputum was induced by inhalation of hypertonic saline and the whole sample was processed. No differences were observed between subjects with COPD and healthy controls in exhaled NO excretion rate, sputum macrophage inducible NO synthase (iNOS) expression and sputum supernatant nitrite + nitrate. NO in exhaled air correlated with the % of sputum eosinophils in COPD (rho = 0.65, p = 0.009) but not in healthy individuals. Exhaled NO and supernatant nitrite + nitrate were lower in healthy smokers than in healthy non/exsmokers. We concluded that NO metabolism is not increased in stable COPD. However, the close association between exhaled NO and sputum eosinophils suggests a role for NO in airway inflammation in COPD.