Haemophilus influenzae and airway inflammation in chronic bronchitis

Bresser, P.

Citation for published version (APA):
Chapter 1

General introduction
**Chapter 1**

**Chronic bronchitis**

Chronic bronchitis refers to a condition of chronic or recurrent increase in the volume of bronchial secretions sufficient to cause expectoration. Already in ancient times chronic respiratory disorders characterized by cough, sputum production, shortness of breath and/or wheeze were recognized [1]. In modern times, chronic (obstructive) bronchitis is one of the most common respiratory diseases of the developed world, which represents a major health problem and a considerable socio-economic burden. In Great Britain in the late 1980s [2], the prevalence of chronic bronchitis in men and women was 15-20% and about 8%, respectively.

Chronic bronchitis is defined by the British Medical Research Council [3] as a productive cough on most days for at least three consecutive months of the year during two or more successive years. Chronic bronchitis may be associated with irreversible airflow limitation, i.e. chronic obstructive pulmonary disease (COPD) [3]. COPD is defined by the American Thoracic Society (ATS) as "characterized by airway obstruction that does not change markedly over periods of several months of observation" [4,5]. COPD is in fact a generic term covering several clinical syndromes with the common component of a degree of fixed airflow limitation. In addition to obstructive chronic bronchitis, COPD also comprises emphysema and peripheral airway disease [4,5].

**Natural history of chronic bronchitis and COPD**

Both chronic bronchitis and COPD are smoking-related disorders, however, less than half of heavy smokers develop mucus hypersecretion [6-8]. Mucus hypersecretion in smokers is associated with the degree of cigarette consumption [6,9], and cessation of smoking results in a marked reduction in sputum production in chronic bronchitis patients [6,8,9].

In most smokers, the annual decline in lung function, as estimated among others by measuring the forced expiratory volume in one second (FEV1), is similar to the decline expected from normal age regressions [7,8]. A subset (15-20%) of smokers, however, will develop chronic airway obstruction [6-8]. In these patients the annual decline in FEV1 is correlated with the degree of cigarette consumption [6,7,10]. Cessation of smoking is considered to slow-down the rate of progression of airway obstruction, since the annual decline of FEV1 in ex-smokers is similar to lifelong non-smokers [7].

The presence of chronic bronchitis in addition to irreversible airflow limitation in COPD patients was demonstrated by Vestbo and coworkers [11] to be associated with a more rapid decline in FEV1. Other studies, however, failed to show such a correlation [6,7,12], or demonstrated only a very limited additive effect of the presence of mucus hypersecretion.
to the decline in FEV1 in these patients [13]. It was suggested, therefore, that mucus hypersecretion and airway obstruction, although both considered the consequence of smoking, represent two distinct phenomena [7,12].

**Airway pathology in non-obstructive and obstructive chronic bronchitis**

The characteristic pathological findings in the airways of chronic bronchitis patients are goblet cell metaplasia, and hypertrophy of submucosal glands with an increased ratio of mucus to serous acini [14-16]. In addition, the surface epithelium may show squamous cell metaplasia with ulceration and epithelial shedding [14,17-19]. In obstructive chronic bronchitis also an increase in smooth muscle mass and in the deposition of collagen beneath the basement membrane can be demonstrated which may result in (reversible) airway constriction and fixed airway obstruction, respectively [14,17,20-23].

The inflammatory cells infiltrating the airway wall in both non-obstructive and obstructive chronic bronchitis consist of lymphocytes, plasma cells, macrophages, eosinophils, mast cells, and neutrophils [24-27].

**Neutrophils in non-obstructive and obstructive chronic bronchitis**

A role for neutrophils in the pathophysiology of non-obstructive and obstructive chronic bronchitis is particularly suggested by studies analysing bronchoalveolar lavage fluid (BALF) and sputum. Increased numbers of neutrophils and high levels of myeloperoxidase (MPO), a marker of neutrophil activation, can be detected in BALF of these patients [24,28-30]. The number of neutrophils was demonstrated to be associated with smoking history, sputum production, and airway obstruction [28,30]. High numbers of neutrophils and increased levels of MPO can also be detected in sputum samples of chronic bronchitis patients, especially during exacerbations [31-33].

Mediators involved in the recruitment of neutrophils are particularly interleukin-8 (IL-8) [34-37] and leukotriene B4 (LTB4) [32,38,39]. IL-8 can be produced locally in the airways by epithelial cells [40-42], macrophages [36,43] and neutrophils [44-46]. In BALF and sputum samples of non-obstructive and obstructive chronic bronchitis patients, high levels of IL-8 can be detected [29,34,47,48]. Like IL-8, LTB4 can be produced locally by epithelial cells [49], macrophages [50,51] and neutrophils [52]. LTB4 has been implicated as the major chemoattractant responsible for neutrophil recruitment in alpha-1-antitrypsin (α1AT) deficiency [33,51,53,54]. Alpha-1-Antitrypsin is the main inhibitor of most neutrophil proteases in the lung, and its deficiency is classically associated with the early onset of emphysema in smokers [53,55,56]. The potential role of LTB4 in the generation of airway
inflammation in subjects without α1AT deficiency is less well studied. Recent reports have indicated, however, that LTB4 may be of significant importance in (obstructive) chronic bronchitis too [32,39]. High levels of LTB4 were detected in sputum samples of obstructive chronic bronchitis patients, in particular during acute bacterial exacerbations [32].

The recruitment of neutrophils to the lung is considered to be an important factor in the pathophysiology of smoking-induced non-obstructive and obstructive chronic bronchitis [53,54]. The azurophilic granules of mature polymorphonuclear leucocytes contain the proteases neutrophil elastase, cathepsin G and proteinase 3. In animal models, these proteases were demonstrated to induce bronchial disease and/or emphysematous lesions [57,58]. The concentration within the azurophilic granules of neutrophil elastase is approximately 5 mM [59,60]. During activation of the cells, these granules are exocytosed and the granular protease is released in an active form. The local concentration of just released elastase is much higher than the concentration of its inhibitor α1AT in the plasma (30 μM) and the interstitium of the lung (approximately 80% of plasma concentration [61]). This major disparity in concentration close to the cells is considered to explain why neutrophils are able to digest connective tissue even in the presence of normal or even high levels of α1AT [59]. However, as the enzyme diffuses away from the cell, its concentration falls exponentially until it equals that of the surrounding inhibitors. This overall process is considered to enable normal neutrophil penetration through connective tissue matrix during migration from the circulation, while limiting the area of damage [54]. In case of an α1AT deficiency, a local inactivation of α1AT by oxidation [62-65], or an enhanced recruitment of neutrophils, this restriction of connective tissue degradation only close to the cells is lost. As a consequence more extensive connective tissue destruction is likely to occur [53,54,66]. Furthermore, free neutrophil elastase can induce mucus secretion [67-69], and the recruitment of even more neutrophils by the induction of the release of IL-8 and LTB4 in resident cells [40,51]. Therefore, neutrophil elastase is likely to contribute to the pathophysiology of non-obstructive and obstructive chronic bronchitis.

Effects of cigarette smoking

Cigarette smoking is not only associated with airway inflammation in patients with chronic bronchitis and/or COPD. Niewoehner and coworkers demonstrated in young healthy smokers, at autopsy, pathological changes in the peripheral airways of all subjects characterized by the accumulation of inflammatory (mononuclear) cells and denuded epithelium [70]. Long-term smoking even in healthy subjects was demonstrated to be associated with mucus hypersecretion, metaplasia of squamous and goblet cells, smooth
muscle hypertrophy, and bronchiolar wall inflammation and fibrosis [19,21-23,71,72].

Smoking of cigarettes is associated with the accumulation of neutrophils and macrophages in the lung [27,73-75]. In the BALF of healthy smokers, elastase activity derived from neutrophils and macrophages can be detected [76,77]. Alveolar macrophages isolated from BALF of smokers were demonstrated to release a chemotactic factor for neutrophils [73]. In addition, in a proportion of healthy smokers increased concentrations of IL-8 in BALF were detected, that were associated with increased chemotactic activity for neutrophils [78]. Furthermore, nicotine may contribute directly to neutrophil recruitment [79], and may prolong local neutrophil survival by suppressing apoptosis [80].

In vitro, cigarette smoke induced the release of a chemotactic activity for neutrophils by isolated human alveolar macrophages [73], and cultured bovine bronchial epithelial cells [81]. In human bronchial epithelial cells, cigarette smoke induced the release of IL-8 [82].

Furthermore, alveolar macrophages may also contribute to the pathophysiology of smoking-related emphysema by the release of proteases, particularly metalloproteases (MMPs) [83-85]. Increased levels of various MMPs were demonstrated in human lungs with emphysema compared with lungs without [85]. In addition, various studies on smoke-induced lung disease in animal models indicated that macrophage-derived proteolytic activity might be implicated in the development of emphysema [86-91].

The effects of smoking to the lung are attributed in part to the effects of oxygen radicals present in high concentrations in cigarette smoke [62,92,93]. Oxidants were demonstrated to increase epithelial permeability [94,95], to induce mucus secretion in guinea pig respiratory epithelial cells [96], and to impair ciliary function [97]. Oxidants may cause damage to DNA, lipids, and proteins [98,99]. In addition, oxygen radicals may inactivate α1AT [62-65], the main inhibitor of neutrophil elastase in the lung [55,100].

**Chronic respiratory tract infection with nonencapsulated Haemophilus influenzae**

Nonencapsulated H. influenzae is a Gram-negative bacterium that is a normal nasopharyngeal commensal only present in humans, that may cause mucosal infections of the upper and lower respiratory tract [101-105]. Carriage in the nasopharynx was demonstrated in 30-50% of healthy individuals [102-104]. In patients with (obstructive) chronic bronchitis, nonencapsulated H. influenzae may cause chronic or recurrent infections of the lower respiratory tract despite high levels of strain-specific antibodies and complement, and large numbers of neutrophils in the airways [106,107]. Clinical isolates from these patients were analysed phenotypically and genotypically. Phenotypical analysis was based on major outer membrane protein (MOMP) subtyping [108] and genotypical on
DNA fingerprinting after restriction endonuclease digestion [109] and randomly amplified polymorphic DNA analysis [110]. These analyses revealed that some *H. influenzae* strains persisted in the airways for longer periods of time, whereas others were isolated only once [107]. This indicates that chronic infections in these patients may be due to persistence of a specific (persisting) *H. influenzae* strain or to recurrent infections with different, unrelated (nonpersisting) strains.

Chronic bacterial infections in chronic bronchitis and COPD patients are considered to be the consequence of altered local conditions induced by chronic airway inflammation [111]. Airway inflammation in these patients is associated with epithelial damage [18,112,113], impaired mucociliary clearance [112,114], mucus hypersecretion [67-69], plasma protein exudation [115-117], and in COPD also with airway obstruction [17,118]. All of these factors are considered to promote bacterial adherence, infection and persistence [111].

In organ cultures from resected human adenoids [119] and nasal turbinates [120], *H. influenzae* did not adhere to undamaged ciliated epithelial cells, but did adhere very well to mucus, damaged epithelial cells and to exposed extracellular matrix components. Attachment of bacteria to mucosal surfaces is considered to be the first step in infection and bacterial persistence [119-121].

In addition to adherence to the ECM components [120,122], *H. influenzae* strains can bind plasminogen [123], that can be activated by tissue-type plasminogen activator (t-PA) to plasmin [122,124]. Deposition of plasminogen on the ECM and subsequent formation of plasmin is essential for normal repair processes of mucosal surfaces after injuries [125,126]. Plasmin generated on and bound to *H. influenzae* was demonstrated by Virkolak and coworkers [122] to degrade laminin, fibronectin and an ECM from human endothelial cells, and to potentiate bacterial penetration through a basement membrane preparation reconstituted on membrane filters. This suggests that in addition to adherence to exposed ECM components, *H. influenzae* is able to bind to locally present plasminogen and to penetrate into the subepithelial layers by local degradation of the ECM.

*In vitro*, *H. influenzae* also penetrated through epithelial cell layers of the human pulmonary mucoepidermoid carcinoma-derived cell line H292 [127]. Although strains that were adherent to H292 cells showed greater penetration (paracytosis), adherence was not an absolute prerequisite for bacterial penetration [127]. Passage was demonstrated, however, to be dependent on the synthesis of a specific bacterial protein (paracytin) [128]. Penetration of *H. influenzae* in epithelial cell layers of H292 cells resulted in shielding the bacteria from killing by antibody dependent defense mechanisms and by antibiotics [129].
In vivo, in sections of airways of chronic bronchitis patients infected with *H. influenzae* accumulation of bacteria between and underneath epithelial cells was demonstrated [130,131], indicating that this mechanism may be a factor of importance in local bacterial persistence.

During persistence, *H. influenzae* may show antigenic variation of outer membrane proteins [106,132,133]. Antigenic variation is considered to be the consequence of immunological pressure leading to selection of MOMP variants [134]. This antigenic variation allows escape from immune surveillance and is thus considered to be another essential aspect of bacterial persistence [134-137].

**Role of nonencapsulated *H. influenzae* in exacerbations**

Patients with non-obstructive and obstructive chronic bronchitis experience episodic exacerbations, characterized by increased cough and sputum production, increased sputum purulence, and shortness of breath [101]. The presence of bacteria, like *H. influenzae*, *Streptococcus pneumonia* and *Moraxella (Branhamella) catarrhalis* in sputum cultures was demonstrated to be associated with exacerbations and sputum purulence in these patients [101,138-141]. Stockley and coworkers observed that during exacerbations in obstructive chronic bronchitis, a positive bacterial culture was obtained in more than 80% of cases if the sputum was purulent, as compared to about 40% positive cultures in non-purulent, mucoid samples [142]. These microorganisms, however, can also be isolated from up to 40% of sputum samples of these patients during a clinically stable phase of their disease [101-103,106,107,139,142,143], and all bacteria involved can also be cultured from the throat of most (healthy) individuals [101,104,138,144]. Thus, the presence itself does not implicate a direct causal relationship between microorganisms and exacerbations.

In an attempt to assess the role of bacteria in the pathophysiology of exacerbations in these patients, antibody responses were studied [101]. In studies concerning *H. influenzae*, however, the antigenic heterogeneity of *H. influenzae* [145-147] was not taken into account. Exacerbations in patients with chronic bronchitis and COPD were demonstrated to be associated with endogenous (antigenic variant) and exogenous reinfections (new, unrelated strain) with *H. influenzae* [107]. Since, however, in most studies just one strain, not derived from the patients studied, was used as source of antigen, the results of these studies [106,148-153] remain inconclusive.

A role of acute bacterial infections in the pathogenesis of at least part of the exacerbations is suggested by several studies on the prophylactic use of antibiotics in patients with chronic bronchitis [154-157]. Prophylactic use of antibiotics in these studies
Chapter 1

reduced the rate of infectious exacerbations in these patients, especially in those suffering from frequent exacerbations. Additionally, in most studies also a reduction in days lost from work due to an exacerbation was demonstrated. Furthermore, in two out of five studies that failed to show an effect of antibiotic prophylaxis on the rate of exacerbations [158-162], a significant effect on the time lost from work in the antibiotic group was shown [158,160]. Also, the positive effect of antibiotic treatment of exacerbations points to at least a contribution of bacteria, like H. influenzae, in the pathogenesis of exacerbations in these patients, since patients suffering from exacerbations associated with purulent sputum were shown to benefit most from antibiotic treatment [101,163,164].

During acute bacterial infections in non-obstructive and obstructive chronic bronchitis patients there is an influx of neutrophils [31,32], associated with detectable activity of neutrophil elastase and enhanced plasma protein exudation [115,165,166]. Furthermore, an increase was demonstrated in the local secretion of secretory IgA (slgA) [137], that may prevent the adherence of H. influenzae to airway epithelial cells [167,168]. The inflammatory response is aimed at eradication of bacteria from the airways and should prevent bacterial adherence, invasiveness, and persistence. On the other hand, as discussed before, the increased local airway inflammation may also facilitate bacterial persistence.

**Interaction between viral and bacterial respiratory tract infections**

Viral respiratory tract infections were demonstrated to be associated with exacerbations in chronic bronchitis and COPD patients, and were suggested to be the cause of part of the exacerbations observed [139,143,169-171]. In addition, viral respiratory tract infections are also considered to predispose to a bacterial infection [172-175].

Influenza virus infection is well known for the occurrence of secondary bacterial pneumonia in humans [176-178], but also respiratory tract infection with other viruses were reported to be associated with bacterial infections [174,179]. Adherence of H. influenzae, Streptococcus pneumoniae and Staphylococcus aureus to human pharyngeal epithelial cells infected with influenza virus was demonstrated to be increased as compared to controls [180]. Furthermore, among others influenza, adeno, and parainfluenza viruses, were demonstrated to cause damage to airway epithelial cells in animal models [172,173,181,182]. Influenza virus infection in humans induces damage to ciliated epithelial cells of the trachea and bronchi [181]. In mice, an influenza virus infection was shown to cause damage to ciliated airway epithelial cells that was associated with an increased adherence of Pseudomonas aeruginosa to damaged cells [173]. Also H. influenzae was shown to adhere very well to damaged epithelial cells and exposed
extracellular matrix components [119,120,122]. Already about a century ago the association of H. influenzae with influenza was noticed. In 1892 Pfeiffer claimed that H. influenzae was the causative agent of influenza, since it was frequently cultured from sputum samples of patients suffering from influenza [183], an association that is now still remembered in its name.

Bacterial infections secondary to viral respiratory tract infection are known for their potential clinical severity [172,174,176-178]. This may be due to altered local conditions that predispose for bacterial infection and that may favour bacterial virulence since normal local defense is impaired. In mice, parainfluenza virus infection was demonstrated to decrease the capacity to clear bacteria from the lungs, and to facilitate the occurrence of pneumonia caused by a subsequent inoculation with H. influenzae type b [184].

**H. influenzae infections in the pathogenesis of (obstructive) chronic bronchitis**

Pulmonary function may worsen temporary during exacerbations in patients with (obstructive) chronic bronchitis and COPD, however, the role of acute and chronic bacterial infections in the pathogenesis of these disorders is far from clear [101,138].

Although acute respiratory tract infections in patients with non-obstructive and obstructive chronic bronchitis are associated with an enhanced influx of neutrophils and detectable elastase activity [31,32,165], most studies designed to assess the annual decline of the FEV1 in COPD [7-10] concluded that there was no correlation between recurrent respiratory tract infections and the decline in pulmonary function [7-9]. In one study only, frequent lower respiratory tract infections were associated with a more rapid decline in pulmonary function [10]. However, in this study a considerable number of patients with α1-antitrypsin deficiency were included. In this condition the proteinase-antiproteinase balance is considered crucial in the development of emphysema [53-55,100]. Therefore, the neutrophil elastase activity [165] that is associated with the recruitment of neutrophils during acute respiratory tract infections [31,32] may have a more severe impact on the progression of the disease in these patients, which might explain the more rapid decline in FEV1 observed in this study.

Whether chronic infection with H. influenzae contributes to the pathogenesis of (obstructive) chronic bronchitis is also still under debate [101,138]. Data from *in vitro* studies and animal models indicate that H. influenzae may aggravate the local inflammatory process by the induction of inflammatory mediators, like interleukin (IL)-6 and IL-8 in airway epithelial cells [42]. H. influenzae can cause damage to the airway epithelium [120,185], and can release components that induce mucus secretion [186] and impair
mucociliary clearance [187-189]. The potential interplay between microorganism and host is the basis of a vicious circle hypothesis, that has been proposed to explain the role of bacteria in the pathogenesis of bronchiectasis [190,191]. In this hypothesis, the microorganism is considered not just to reside locally in the airways of these patients, but to be crucial in the pathogenesis. It was suggested that similar mechanisms might be operative in chronic bronchitis and COPD [101]. Since airway inflammation in COPD was demonstrated to be associated with airway obstruction [28,30], this may implicate that persistent H. influenzae plays a role in causing irreversible airway obstruction in these patients by aggravating local airway inflammation [101].

**Therapeutic interventions**

Antibiotic treatment reduces sputum purulence and bacterial numbers. The effects, however, are just temporary as infection flares up shortly after cessation of treatment [192-194]. Strains of H. influenzae were demonstrated to persist in the airways of patients with non-obstructive and obstructive chronic bronchitis despite antibiotic treatment [107,139].

Since local airway inflammation is considered a crucial factor in bacterial persistence, it might be argued that reduction of local airway inflammation may reduce bacterial persistence.

Inhaled corticosteroids have proven to be effective in the treatment of asthma by reducing airway obstruction, bronchial hyperresponsiveness and local airway inflammation [117,195-197]. Inhaled corticosteroids are also widely used in the treatment chronic bronchitis and COPD. In various studies, treatment with (inhaled) corticosteroids was demonstrated to improve pulmonary function [198-202] and to slow down the annual decline in FEV1 [202,203]. In addition, corticosteroids were demonstrated to reduce the rate of severe exacerbations [202] and to reduce local airway inflammation [117,198,204,205] in non-infected patients with chronic bronchitis and COPD. However, others failed to show benefit from treatment [206-209], and it was suggested that corticosteroid reversibility in COPD is related to features of asthma being present in these patients [199,201,210,211]. Therefore, the role of inhaled corticosteroids in the treatment of chronic bronchitis and COPD is far from elucidated yet [211-213].

Corticosteroids modulate gene expression by various mechanisms [214]. Corticosteroids were shown to reduce among others the synthesis of pro-inflammatory cytokines, like tumor necrosis factor-α (TNF-α) [42], IL-6 [42,215] and IL-8 [42,216] in cultured airway epithelial cells. In cultured human bronchial epithelial cells, hydrocortisone inhibited the release of IL-6, IL-8 and TNF-α induced by lipopolysaccharide isolated from H. influenzae [42].
In several large trials, the mucolytic agent N-acetylcysteine (NAC) was demonstrated to reduce the rate of infectious exacerbations in patients with chronic bronchitis [217-221]. In addition, in some studies pulmonary function improved upon treatment with NAC [218,222]. Furthermore, the use of NAC in chronic bronchitis patients was associated with fewer positive bacterial cultures of BALF [223].

NAC has been shown to reduce cough frequency, and to facilitate sputum evacuation by the reduction of the viscosity of the sputum [217,222]. By the enhancement of the mucociliary clearance NAC may reduce infection and bacterial persistence. However, NAC may also act as an antioxidant by providing cysteine for the intracellular production of glutathione, an important physiological anti-oxidant [62,224]. This may have implications for bacterial persistence, since NAC was demonstrated to enhance the phagocytotic activity of neutrophils in vitro, by protection of the Fc-γ-receptor from oxidative damage [224,225].

Outline of this thesis

Recurrent and chronic infections of the lower respiratory tract with bacteria like *H. influenzae* are prominent in chronic bronchitis patients. Little is known about the course of infections with *H. influenzae*, possible differences between strains of *H. influenzae* and on the contribution of *H. influenzae* to airway inflammation. Therefore, in the studies presented in this thesis, we employed both ex vivo and in vitro approaches to study various aspects of *H. influenzae* in relation to airway inflammation.

Previously, we have demonstrated persistence of *H. influenzae* in an obstructive chronic bronchitis population, consisting mostly of non-smoking patients living in a home for the elderly [107]. Now (Chapter 2), we studied bacterial persistence in a well-defined chronic bronchitis population, most of whom were (ex-)smokers, all selected from the outpatient clinic of our hospital. We also studied whether exacerbations in these patients coincide with the occurrence of endogenous or exogenous reinfection with *H. influenzae*, as was demonstrated before in the previously mentioned population [107]. Since bacterial infections in chronic bronchitis patients are considered to be the consequence of the ongoing inflammatory process [111], we aimed to reduce local airway inflammation in chronic bronchitis patients. Therefore, chronic bronchitis patients with a documented history of recurrent infections with *H. influenzae* were treated with either an inhaled corticosteroid (budesonide), oral N-acetylcysteine or placebo for three months. The effect of treatment on bacterial persistence and local airway inflammation was studied.

Although infection of the airways with *H. influenzae* is considered to be the consequence of altered local conditions [111], the mechanism of bacterial persistence is
still far from clear. Since an inflammatory response is elicited against invading bacteria to eradicate bacteria from the airways, we hypothesized that strains that persisted for longer periods of time differ from non-persisting strains in the way they induce a local inflammatory response. Therefore, we studied the release of pro-inflammatory mediators (IL-6 and IL-8) in airway epithelial cells induced by persisting and nonpersisting clinical chronic bronchitis isolates (Chapter 3). In these studies also an initial analysis of the bacterial component(s) inducing the release of the pro-inflammatory mediators was performed.

Since adherence to ECM components and plasminogen receptor expression may promote bacterial persistence, strains causing persisting infections may differ from nonpersisting strains in their ability to adhere to ECM components. Therefore, we studied adherence to ECM proteins of persisting and nonpersisting clinical chronic bronchitis isolates (Chapter 4).

We also studied local airway inflammation in patients with non-obstructive and obstructive chronic bronchitis chronically infected with H. influenzae, and compared these data with those obtained in a non-infected, but otherwise similar COPD population [48,117] (Chapter 5).

As viral airway infections may contribute to exacerbations in chronic bronchitis and COPD patients with chronic H. influenzae airway infections, we also studied the impact of a viral infection on the regulation of IL-6 and IL-8 expression in airway epithelial cells (Chapter 6).

References


42. Khair, O.A., J.L. Devalia, M.M. Abdelaziz, R.J. Sapsford, H. Tarraf, and R.J. Davies. Effect of Haemophilus influenzae endotoxin on the synthesis of IL-6, IL-8, TNF-α and expression of


102. Murphy, T.F., and M.A. Apicella. Nontypable *Haemophilus influenzae*: a review of clinical


Chapter 7


Chapter 1


200. Weir, D.C., and P.S. Burge. Effects of high dose inhaled beclomethasone dipropionate, 750 µg and 1500 µg twice daily, and 40 mg per day oral prednisolone on lung function, symptoms, and bronchial hyperresponsiveness in patients with non-asthmatic chronic airflow


220. Grandjean, E.M., P. Berthet, R. Ruffman, and P. Leuenberger. Efficacy of oral long-term N-


