Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

ELSEVIER

Clinical and Applied Immunology Reviews 6 (2006) 21–36

Clinical and Applied Immunology Reviews

Exacerbations of chronic obstructive pulmonary disease and chronic mucus hypersecretion

Yohannes Tesfaigzi[a,*], Paula Meek[b], Suzanne Lareau[c]

[a]*Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive, SE, Albuquerque, NM 87108, USA*
[b]*College of Nursing, University of New Mexico, Albuquerque, NM 87131, USA*
[c]*Pulmonary Section, New Mexico Veterans Administration Medical Center, Albuquerque, NM 87108, USA*

Received 12 September 2005; received in revised form 9 February 2006; accepted 13 February 2006.

Abstract

Chronic obstructive pulmonary disease (COPD) exacerbations are an important cause of the considerable morbidity and mortality found in COPD. COPD exacerbations increase with increasing severity of COPD, and some patients are prone to frequent exacerbations leading to hospital admission and readmission. These frequent exacerbations may have considerable impact on quality of life and activities of daily living. Factors that increase the risk for COPD exacerbations are associated with increased airway inflammation caused by common pollutants and bacterial and/or viral infections. These inflammatory responses cause mucus hypersecretion and, thereby, airway obstruction and associated exacerbations. While chronic mucus hypersecretion is a significant risk factor for frequent and severe exacerbations, patients with chronic mucus hypersecretion have a lower rate of relapse after initial treatment for acute exacerbation. The benefit of antibiotics for treatment of COPD exacerbations is small but significant. While the mechanisms of actions are not clear, mucolytic agents reduce the number of days of disability in subjects with exacerbations. Reducing mucous cell numbers in small airways could be a useful way to reduce chronic mucus hypersecretion. Our studies suggest that programmed cell death is crucial in the resolution of metaplastic mucous cells, and understanding these mechanisms may provide novel therapies to reduce the risk of COPD exacerbations.

Keywords: Airway epithelium; Apoptosis; Mucous cell metaplasia; Small airways; Inflammation; Hospitalization

Abbreviations: CMH, chronic mucus hypersecretion; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; GCM, goblet cell metaplasia; IL, interleukin; LPS, lipopolysaccharide; NAC, *N*-acetylcysteine; PCR, polymerase chain reaction; RSV, respiratory syncytial virus; URI, upper respiratory infection.

* Corresponding author. Tel.: +1 505 348 9495; fax: +1 505 348 8567.
E-mail address: ytesfaig@lrri.org (Y. Tesfaigzi).

1529-1049/06/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi: 10.1016/j.cair.2006.02.001

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic condition whereby airflow is limited. This limitation can be caused by a combination of underlying conditions, most notably chronic bronchitis and emphysema. Chronic mucus hypersecretion (CMH) is a hallmark of chronic bronchitis. CMH is a common cause of morbidity and mortality, with an annual prevalence of diagnosed chronic bronchitis of 8.8 million in the United States [1]. In emphysema, alveolar destruction is associated with a loss of elastic recoil of the lung. Cigarette smoking is the major etiologic factor. The predominance of each condition varies among individuals. COPD causes significant morbidity, mortality, and economic consequences to individuals. COPD is the fourth leading cause of death and, of the five major diagnoses, only COPD is projected to have an increase in annual deaths by 2020 [2]. Once COPD has developed, there is no known cure for it. Despite the fact that COPD is often underdiagnosed, over 16 million people in the United States are estimated to have COPD, resulting in over 500 000 hospitalizations and 16 million office visits annually. COPD costs $24 billion annually [3], with 70% of medical expenditures occurring for hospitalization due to acute exacerbations [4].

Hospitalization is required when there is poor response to management such as unrelenting dyspnea and inability to perform activities of daily living. Of all COPD patients hospitalized, over 25% are admitted for acute exacerbations [4], and 25% of these are readmitted [5], often due to incomplete recovery [6]. About 30% of patients will have a recurrence within 6 months, and about 50% of these are readmitted within a year. The percentage of predicted forced expiratory volume in 1 second (FEV_1) is used to measure the severity of COPD and is associated with increasing occurrence of exacerbations as well as mortality. For example, Mannino et al. [3] found that patients with an FEV_1 of $<40\%$ have 2.3 exacerbations annually. Miravitlles et al. reported that those with less severe disease (FEV_1 $>60\%$) have an average of 1.6 exacerbations annually [7]. Frequent exacerbations in the past is the best predictor of exacerbations in the future.

Spencer and Jones demonstrated that recovery from exacerbations can take up to 26 weeks, with a pattern of rapid improvement over 4 weeks followed by prolonged recovery lasting up to 6 months [8]. In one of the most systematic studies of symptoms in exacerbations by Seemungal et al., 101 subjects were prospectively evaluated for 2.5 years [9]. Of this number, 91 had >1 exacerbation and 78 had ≥ 2, with 85% of exacerbations identified by symptom diary cards. Of a total 504 exacerbations, only 250 (49.6%) were actually reported to the investigators [9]. Mortality increases with exacerbations, with death rates during hospital admission reported to be as low as 8% but increasing to 23% within a year. In those with high-risk factors, the 6-month mortality is 43%. For those requiring mechanical ventilation, mortality is 24%, with half of those >65 years dying within 1 year [10].

2. Definition

There is no universal definition of exacerbations [11], likely because no single cause has been identified and often two patients can present on different occasions with different pathologic findings as well as a different constellation of symptoms. A frequently accepted

description of an exacerbation is a sudden, sustained worsening of COPD, ''beyond the normal day-to-day variations'' in breathing [12] or an event in the natural course of the disease characterized by a change in the patient's baseline dyspnea, cough, and/or sputum that dictates a change in management [13].

The majority of exacerbations have in common a combination of the following symptoms: dyspnea, wheezing, increased cough, and an increase in amount or purulence of sputum [14]. According to a commonly used definition, therefore, exacerbations are changes for 2 consecutive days in three ''major'' signs and symptoms (dyspnea, volume of sputum, and sputum purulence). Severity is determined by the presence of major or minor symptoms. ''Minor'' symptoms are increased cough or wheezing, upper respiratory infection (URI) (sore throat, nasal discharge) in the past 5 days, fever, and increased respiratory rate or heart rate 20% above baseline [15]. Severe exacerbation has been defined as an increase in all three major symptoms, moderate exacerbation as an increase in two major symptoms, and mild exacerbation as one major and one minor symptom. The importance of patient reports of symptoms in diagnosing exacerbations is underscored by the fact that objective measures (peak flow readings and spirometry) are not successful at detecting exacerbations early [9]. Early detection is key to early intervention and prevention of hospitalization. A greater understanding of the pathology of exacerbations most certainly should lead to better means of treating as well as preventing exacerbations.

Unfortunately, the cause of exacerbations is unclear and may vary within and between patients. COPD patients are at great risk for repeat exacerbations following the initial event. However, half of the patients do not seek care when symptoms occur. COPD patients tend to wait until experiencing extreme exacerbations either occasionally or frequently before reporting to the doctor.

Increased levels of interleukin (IL)-6 and IL-8 are found in the sputum of patients with frequent exacerbations at baseline when stable compared with those with infrequent exacerbations [16]. Because sputum cell counts for inflammatory cells were not increased at baseline in patients with more frequent exacerbations, the increased cytokine production may be originating from the bronchial epithelium in COPD. During an exacerbation, inflammatory markers include levels of plasma fibrinogen and serum cytokines; IL-6 rises [17] and is higher in viral than in nonviral exacerbations [18]. Plasma fibrinogen levels vary in response to virally induced exacerbations. In a study of 67 subjects, Seemungal et al. reported that mean plasma fibrinogen levels were 0.56 g/L during viral exacerbations, versus 0.27 g/L during nonviral exacerbations [18].

3. Viruses and bacteria in COPD exacerbations

Exacerbations can be caused by bacterial or viral infections and may lead to pneumonia. Other conditions associated with exacerbations are environmental pollution and cold weather. Seemungal et al. reported that in nearly 40% (66 of 168 events, n = 83) of COPD exacerbations, respiratory virus infections are found, rhinoviruses being the most common of the viruses [18]. Polymerase chain reaction (PCR) identified 67 organisms in nasal aspirates of subjects with COPD exacerbations. Rhinovirus was the predominant organism followed by respiratory syncytial virus (RSV), coronavirus, influenza B, parainfluenza, and *Chlamydia pneumonia*.

Viruses associated with change in sputum characteristics, such as increased volume or purulence, were not related to the viruses detected in nasal secretions. However, during COPD exacerbations, patients with viral infection had a higher symptom score at onset of exacerbation compared with nonviral exacerbations, and the time required for 50% of exacerbations to resolve symptomatically was significantly longer for viral versus nonviral exacerbations (13 vs. 6 days) [18]. A minimum of one respiratory virus was detected at exacerbation in 64% of patients, with these patients having a higher frequency of exacerbations ($P < 0.05$). However, RSV does not appear to be related to the severity of exacerbations.

Viruses have been identified by PCR in serum or nasal aspirate samples. Nasal cultures and serology, however, were negative in 83 subjects who reported at least one exacerbation [18]. Therefore, analysis of nasal cultures does not appear to be a good method for detecting exacerbations.

Airways of COPD patients often are colonized by bacteria, making the presence of bacteria insufficient to explain worsening of airway function. However, an increase in bacterial number, change in bacterial location in the airway, or acquisition of new, more virulent, or more pro-inflammatory bacterial species or strains could cause exacerbations [19]. Patients with frequent exacerbations have also an increased incidence of colonizations with bacteria (*Hemophilus influenza*, *Moraxella catarrhalis*, and *Streptococcus pneumonia*) when stable.

At exacerbation, there is an increased chance of detecting bacteria, especially if the exacerbation is associated with the presence of purulent sputum [20]. In work by Stockley et al., 84% of subjects with purulent sputum and 38% of those with mucoid sputum during an exacerbation tested positive for bacteria [20]. Patients who had *H. influenza* colonization when they were stable state experienced more symptoms and purulence during exacerbations [21]. Sputum purulence during an exacerbation is likely to be associated with a high bacterial load, while patients with symptoms of a cold or URI are likely to have exacerbations associated with a virus.

4. COPD exacerbations and chronic mucus hypersecretion

Chronic bronchitis is defined by the presence of chronic or recurrent increases in bronchial secretions that cannot be attributed to other pulmonary or cardiac causes and is sufficient to cause daily expectoration for a minimum of 3 months a year, for at least 2 successive years [22]. Population-based studies have reported an association of CMH with accelerated decline in FEV_1 [23].

Chronic bronchitis likely results from the interaction of both intrinsic and extrinsic factors [24]. To prevent further airway disease, it is crucial not only to limit exogenous causes of airway damage but also to identify and treat endogenous ones. Intrinsic factors may be based on genetic polymorphisms that make smokers susceptible to developing chronic bronchitis. Our studies in animal models of chronic bronchitis suggest that dysregulated expression of Bcl-2 may lead to prolonged presence of goblet cell metaplasia (GCM) [25]. Subjects with chronic bronchitis without airway obstruction show the same pathology in the airways as those with COPD.

The reasons for the natural cause of exacerbations in subjects with COPD are not clear; however, there is evidence that environmental pollution, such as the levels of SO_2 and NO_2

in the environmental air, as well as allergens and infections can increase the incidence of symptoms in some patients with chronic bronchitis or emphysema [26]. Some of these conditions are not known to induce a specific type of inflammation but are likely to stimulate innate (acute) response immunity. It is, therefore, possible that as a result of this innate immune response to environmental pollutants, allergens, and infections, sudden secretion of mucus can be triggered from the increased numbers of mucus-producing cells to cause airway obstruction and the associated exacerbations.

5. Increased risk of exacerbation in COPD patients with chronic mucus hypersecretion

Hospitalizations for exacerbations occur in over half of COPD patients, with patients averaging one to four events annually [27]. Given the lack of knowledge about the pathology of exacerbations, prevention or interventions to reduce hospitalizations are poorly understood.

Frequent exacerbations have been demonstrated to have a negative impact on the quality of life of patients with COPD [27]. Furthermore, they are the most frequent cause of hospital admission and death among patients with chronic lung disease. Since COPD exacerbations are an important event in the natural history of the disease, it is crucial to identify patients most at risk of suffering from them. The identification of risk factors for exacerbations may permit implementation of measures aimed at avoiding these complications. Patient recognition of exacerbation symptoms and early treatment improves the recovery process and reduces the risk of hospitalization because early therapy improves outcomes of exacerbations in COPD and reduces the cost of treatment [28].

CMH is a significant predictor of hospitalization due to COPD [29], and subjects with COPD and CMH are more likely to die from pulmonary infections than subjects without CMH [30]. CMH, which is the hallmark of chronic bronchitis, is particularly associated with mortality from an infectious cause [30]. These observations may be explained by the affinity that bacteria have for mucus and impairment of mucociliary clearance in chronic bronchitis, providing bacteria that are inhaled or aspirated into the bronchial tree an opportunity to colonize the mucosa. The impairment in respiratory function may be very important in governing the outcome of a COPD exacerbation caused by a bacterial infection [10].

When chronic bronchitis is exacerbated by these factors, bronchitis, in turn, may predispose to a secondary bacterial infection [31]. Bacterial products certainly have the potential to be an independent stimulus of inflammation, for example, lipopolysaccharide (LPS), a potent inducer of a variety of inflammatory mediators, including immunoregulatory cytokines from resident and inflammatory cells in the lung. We and others have shown that LPS initially induces mucous secretion followed by extensive mucous cell metaplasia in airway epithelia [32,38].

6. Factors affecting recovery from acute exacerbations

The speed of recovery from symptoms of exacerbations is important in patients with chronic bronchial disease because patients with milder symptoms may return to work or

to usual activities earlier, and patients with more severe symptoms may avoid respiratory failure if recovery of symptoms is achieved in a short period of time. Knowing the time course of symptoms and the risk factors for late recovery may help physicians to schedule follow-up visits and interpret the outcomes of therapy. Because failure to recover is associated with major economic implications, studies aimed at identifying risk factors for treatment failure in real-life conditions are required.

A study by Miravitlles et al. in 2004 [33] followed the rate of recovery from COPD exacerbation of 1656 patients. The primary outcome of this study was time to the resolution of symptoms. Fast recovery was defined as the resolution of symptoms under 5 days, the median duration of symptoms for the whole group, because 57% of the patients recovered before or at 5 days. Treatment failure was defined as failure to return to baseline before 10 days or persistence or aggravation of symptoms and the requirement of new treatments or medications during the first 10 days. Patients with two or more exacerbations or hospitalization for exacerbation during the previous year were likely to have a slow recovery. Interestingly, patients with chronic bronchitis had a reduced risk of treatment failure compared with patients with emphysema, suggesting that increased expectoration of sputum appears to help speedy recovery. These findings suggest that subjects unable to expectorate mucus may have breathing difficulties that limit activity—the hallmark of acute exacerbations—because of mucus obstructing the airways.

In a cross-sectional observational study on COPD patients, severity of FEV_1 impairment and the presence of CMH were found to be factors independently associated with increased risk of suffering two or more acute exacerbations of COPD per year [34]. While FEV_1 impairment was associated with increased risk of hospital admission, CMH was not significantly associated with the risk of admission, but the presence of significant comorbidity, such as diabetes mellitus, cardiac insufficiency, or ischemic heart disease. Miravitlles et al. [34] speculate that age and CMH are facilitating factors for exacerbations, but the severity and prognosis of exacerbation are best predicted by the presence or absence of significant comorbid conditions. Several studies have established that comorbid conditions are risk factors for being referred to the hospital after being treated for an acute exacerbation [35].

Another prospective study evaluated the influence of CMH on the risk of frequent exacerbations [36]. Exacerbation frequency was related to bronchitic symptoms, although Burrows and Earle mention that no significant association of daily sputum production was observed with frequent exacerbations even though patients with bronchitic symptoms are usually defined as presenting persistent cough with sputum production [36]. It is possible that patients reporting bronchitic symptoms were defined as having more severe symptoms than those only reporting sputum production. In this respect, one would consider CMH to be the daily emission of 30 mL of expectorated matter or more. Burrows and Earle did not show any information regarding the quantitation of expectoration. The study by Miravitlles et al. [34] suggests that CMH renders the patient more prone to suffering recurring exacerbations but does not determine the severity of the exacerbations, as it appeared not to be a factor associated with hospital admissions. Therefore, clinical assessment of COPD patients in general practice should include these important and easily measurable variables.

In summary, FEV_1 impairment explains part of the risk of hospital admissions caused by frequent exacerbations. CMH and increasing age are significantly associated with risk of

frequent exacerbations, and severity of exacerbations provoking hospital admissions is associated with the presence of significant comorbidity.

7. CMH and relapse after treatment

Relapse after initial treatment for acute exacerbation may lead to prolonged disability, a new course of antibiotics, an emergency room visit, or even hospital admission; therefore, it is crucial to identify patients most at risk for relapse. Identifying risk factors for ambulatory treatment failure may permit the implementation of more aggressive broad-spectrum treatment and closer follow-up. Therefore, risk factors associated with relapse should be incorporated into the management guidelines to aid general practitioners in identifying at-risk patients.

A study by Miravitlles et al. [37] estimated the probability of relapse after ambulatory treatment of an exacerbation of chronic bronchitis based on data collected in clinical records obtained after a visit to the general practitioner. In this study, diagnosis of acute exacerbation was based on the presence of any combination of the following symptoms: increased dyspnea and increased production and purulence of sputum. Relapse was defined as an unscheduled visit to the general practitioner before 1 month because of a persistence of or an increase in symptoms, which led to a change in drug prescription, an emergency visit, or a hospital admission. Treatment administered for acute exacerbations included antibiotics in 98% of cases and oral corticosteroids in 25% of cases. Only 38% of cases had mucolytic agents as treatment. Approximately 21% of individuals with exacerbations of chronic bronchitis suffered a relapse of exacerbation following treatment.

This study shows that after ambulatory treatment of acute exacerbations of chronic bronchitis, a patient with ischemic heart disease who visited the general practitioner three times in the last year for respiratory problems has a relapse probability of 32.4%, which is statistically significantly higher than the mean 21% probability of the general cohort.

Similarly, Ball et al. [35] found that the only factors significant in predicting failure to recover from an acute infective exacerbation of chronic bronchitis were historical. Neither clinical features of the presentation nor antibiotic treatment affected recovery. The best combination predicting return with a chest problem was history of cardiopulmonary disease and more than four acute exacerbations within the past 12 months.

8. Treatment for COPD exacerbations

Considering the high prevalence of chronic bronchitis in the general population and the high number of medical consultations generated by this population, it is crucial to identify patients at risk for failure of treatment of exacerbations or at risk for repeated consultations for respiratory problems. Patients exhibiting such factors should receive an energetic treatment with a broad-spectrum antibiotic and a short course of oral corticosteroids, and should be closely followed up in an attempt to avoid relapse, which in a significant number of cases may lead to hospital admission or even cause death [38].

In general, antibiotics are commonly prescribed during exacerbations, but evidence that they appreciatively alter the outcome is not accepted [39]. Anthonisen et al. [14] showed significant benefits for antibiotics compared with placebo in patients judged to have moderate to severe exacerbations on the basis of increased dyspnea, sputum production, and purulent sputum. Stockley et al. [20] further specified which of the patients who are likely to benefit from antibiotic treatment. The presence of green purulent sputum was specific for high bacterial load, and this subset of patients whose episodes were identified at presentation was likely to benefit from antibiotic treatment. The same study showed that patients who produced white mucoid sputum during acute exacerbations recovered without antibiotic treatment.

A recent meta-analysis of trials that included placebo-controlled studies involving small numbers of patients showed that, overall, there was a small but significant benefit from antibiotic treatment in acute exacerbations of COPD [40].

Most bacterial species that infect the bronchial tree also form part of the commensal of the nasal pharynx (e.g., *H. influenza*) or are opportunistic pathogens (e.g., *P. aeruginosa*). Mucosal infections are usually superficial and the majority of bacteria reside in the lumen, associated with secretions, whereas a proportion adhere in the epithelial surface, particularly in areas of epithelial damage, and some will infiltrate the mucosa [41]. Repeated injury from high-dose inhalation of atmospheric pollutants such as tobacco smoke or ozone leads to a chronic inflammatory cell infiltrate in the mucosa by lymphocytes, monocytes, and macrophages. Neutrophils and, to a lesser extent, eosinophils are attracted into the airway lumen, and their products may further impair the host defenses. Neutrophils release proteases such as neutrophil elastase and reactive oxygen species that damage epithelial cells and stimulate mucus production. Epithelial cells are also active participants in the inflammatory reactions because they release pro-inflammatory mediators, including cytokines, chemokines, and nitric oxide. This inflammatory response causes mucus hypersecretion, a change in the morphology of the airway involving a loss of ciliated cells, and an increase in the number of goblet cells in mucosal gland hypertrophy. The bronchitic airway is therefore vulnerable to infection, but there is also a well-primed immune system present that is ready to mount an inflammatory response to any further insult. Bacteria avoid clearance by producing factors that impair mucociliary clearance by paralyzing ciliary beat and stimulating mucus production; producing enzymes that break down local immunoglobulin; displaying antigenic heterogeneity to avoid immune surveillance; growing in biofilms; adhering to epithelium; surviving within epithelial cells; and forming microcolonies surrounded by polysaccharide gel [41].

A report by Poole and Black [42] summarized the therapeutic potential of mucolytic agents in preventing exacerbations of chronic bronchitis and COPD. In 21 studies, patients were defined as having chronic bronchitis that was most often defined as the presence of cough productive of sputum on most days for a minimum of 3 days per year for at least 2 consecutive years. In the remaining two studies, patients had COPD defined as a disease characterized by airflow limitation that is not fully reversible. It is likely that most of the patients with chronic bronchitis also had COPD, as most of these patients were smokers, and when lung function was measured, there was evidence of airway obstruction. In 12 of 23 studies, the mucolytic agent used were *N*-acetylcysteine (NAC), and mucolytic agents used were ambroxol, carbocysteine, sorbrerol, letosteine, cithiolone, iodinated glycerol,

N-isobutyrylcysteine, and myrtol. Ten of the studies were conducted in Italy, three each in Scandinavia and Germany, four in the United Kingdom, two in several European countries, and one in the United States. All 23 studies had similar scoring standards. This review found the annualized exacerbation rate of 2.7 per year in the control group being reduced by 29% with treatment of patients with mucolytic agents. A similar study showed a reduction by 25%. Another study [43] found that 25% of patients who received mucolytic agents were twice as likely not to have an exacerbation during the study period than if they had received placebo. Treatment with mucolytic agents resulted in 0.56 fewer days of disability per patient per month. This finding was based on six studies and was supported by the results of four other studies where mean days of disability but not standard deviation values were reported. In two systematic reviews of the effects of NAC in patients with chronic bronchitis, it was shown that patients treated with NAC were more likely to remain exacerbation free. These patients were more likely to report an improvement in symptoms with NAC than with placebo.

These mucolytic agents work in a variety of ways. Potential mechanisms of actions include thinning of produced sputum, resulting in promotion of expectoration, and antioxidant and antibiotic activity. It has also been postulated that mucolytic agents may work via an antioxidant mechanism, which is generally accepted to play a role in the pathogenesis of COPD. In acute exacerbations of COPD, this oxidant/antioxidant imbalance is very profound and further studies will be needed to determine whether mucolytic agents acutely reduce the morbidity of these events. A third possibility is that mucolytic agents may work through antibacterial or immunostimulatory mechanisms because pretreatment with NAC resulted in significantly fewer patients having positive cultures from intrabronchial samples [44]. In addition, NAC has been shown to reduce the adhesion of *S. pneumonia* to oropharyngeal epithelial cells [45].

Mucolytic therapy appears safe and well tolerated, with no suggestion from the studies of any increase in the incidence of adverse effects relative to placebo. NAC is the mucolytic agent that has been most extensively studied; however, other mucolytic agents, overall, had similar effects on exacerbation rates. Patients who have frequent or prolonged exacerbations or those who had repeatedly been admitted to a hospital would appear to have the most to gain.

The BRONCUS study [46] compared the effect of NAC and placebo in 523 COPD patients on the number of exacerbations per year and found that NAC failed to be effective at preventing exacerbations in these patients but appeared to reduce the risk of exacerbation in patients not taking inhaled steroids. The difference in findings between this study and previous studies may be because of the fact that previous studies assessed chronic bronchitis rather than COPD. Furthermore, the authors of the BRONCUS study acknowledge that greater doses of NAC, that is, increasing the dose from 600 mg daily, could have resulted in an improved effect. Another study showed that 1800 mg daily improved lung function in idiopathic pulmonary fibrosis and was well tolerated.

The main difficulties in interpreting the results or studies of treating chronic bronchitis exacerbations include the heterogeneity of the condition. The cause of an exacerbation can include acute viral bronchitis, environmental pollutants, and allergic responses as well as bacterial infections. Furthermore, heterogeneity within the definition of the severity of an exacerbation exists. Severity classifications based on the symptomatology of chronic

bronchitis exacerbations have, thus far, been largely empirical and have not been validated by clinical trials [14]. A classification of severity based on clinical parameters that can be easily evaluated would allow the detection of differences in efficacy of new therapies compared to placebo and improve conventional management of exacerbations.

9. GCM and airflow limitation

The site of most mucus hypersecretion is the large airway. Histological studies show enlargement of tracheobronchial submucosal glands and hyperplasia [47]. The presence of an increased number of goblet cells distinguishes patients with CMH and airway obstruction from patients with CMH but without obstruction [48]. Hyperplasia of mucous goblet cells and mucus plugging were noted in patients with chronic bronchitis but without emphysema [49]. Therefore, understanding the causes for the increased numbers of mucous cells is crucial to reduce the severity of COPD exacerbations.

Detailed morphometric analysis of the amount of sustainable stored secretory product in the tracheobronchial airways of the Rhesus monkey reveals at least twice as much mucus in the surface epithelium as in the submucosal glands [50]. In the lower airways of the Rhesus monkey, as in the majority of other animal species, glands are absent and surface epithelial mucous cells are the sole source of mucus. Because surface epithelial mucous cells represent a great potential for secretion of mucosubstances [51], it is important to investigate how their numbers are regulated in airway epithelia.

The normal tracheobronchial epithelium contains ciliated, basal, and secretory cells, which are maintained at fixed ratios by homeostatic mechanisms. The proportion of these cell types is perturbed following various inflammatory responses or mechanical injury to the epithelium due to proliferation [52]. Various studies have shown that nonciliated columnar cells are the main cell type that is recruited to the cell cycle in larger numbers [53]. GCM following acute injury or inflammatory responses results from differentiation of pre-existing epithelial cells into mucous cells and differentiation of proliferating cells into mucous cells [51]. Following cessation of allergen exposure, airway epithelia return to the original proportion of cell types [54]. Various mechanisms may be responsible for the reduction of GCM, including the fact that inflammatory mediators responsible for mucin synthesis are no longer present. However, full recovery of the epithelium necessitates the reduction of epithelial cell numbers to the original state [55]. Our studies suggest that abrogation of these mechanisms may be, at least in part, responsible for the persistent change in airway epithelia as is observed in airways of subjects with CMH.

The small airways of subjects with COPD are detached from the alveolar connection, which results in a loss of support and closure of small airways during expiration [56]. In addition, the airways are narrowed as a result of inflammation and scarring and, more importantly, due to the blocking of the lumen with mucous secretions [57]. Exacerbations can occur because a number of mucus-secreting cells in small airways are present that secrete their contents as a result of increased inflammation caused by exposures to new bacterial [21] or viral infections [58], environmental pollutants such as ozone or other particles, or allergens. It is widely believed that these factors cause COPD exacerbations, and they are

believed to do so largely by increasing inflammation. Increased inflammation may be primarily causing increased secretion of mucus from metaplastic goblet cells, which will immediately obstruct small airways and reduce or completely block airflow.

Increased numbers of mucous cells are present because the epithelium is not capable of regulating the numbers. While the presence of mucous cells is not detrimental under symptom-free conditions, increased inflammation can cause exacerbations, that is, reduced pulmonary function and increased cough or sputum secretion, due to the sudden secretion of large amounts of mucus into the lumen. The blockage of the airway is enhanced by the sticky nature of mucus that traps inflammatory cells [57] that release DNA and further increase mucus viscosity.

10. Apoptotic regulators in GCM

We have developed an animal model in which epithelial cell number and GCM are increased in airway epithelia, and we have studied the role of Bcl-2 in sustaining GCM. Instillation of bacterial LPS in rats causes the number of epithelial cells to increase by approximately 25%, and in addition to these proliferating cells, existing serous cells differentiate into mucous cells. Following a recovery of 16 days, the number of epithelial cells is reduced to that found in noninstilled control rats. In an attempt to understand the mechanisms that reduce numbers of metaplastic mucous cells after inflammatory responses subside, we analyzed the expression of regulators of programmed cell death [59].

The Bcl-2 family of cytoplasmic proteins can register diverse forms of intracellular damage, gauge whether other cells have provided a positive or negative death stimulus, and determine the progression or inhibition of the suicide program [60]. Bcl-2, an inhibitor of apoptosis, belongs to a member of a family of proteins with anti- and pro-apoptotic properties that when overexpressed prevent or induce apoptosis, respectively. Pro- and anti-apoptotic family members can heterodimerize, and their relative concentration determines whether a cell lives or dies [61]. These proteins function by regulating the release of cytochrome *c* from mitochondria, initiating a cascade of events leading to activation of caspases and DNases that are responsible for the appearance of apoptotic morphology including DNA fragmentation, chromatin condensation, membrane blebbing, cell shrinkage, and disassembly of the cell [62]. Bcl-2 enhances cell survival by inhibiting apoptosis induced under a wide variety of circumstances. Bcl-2 extends cell survival by preventing cell death in different cell types and in response to different stimuli; this suggests that Bcl-2 acts at a central control point in the pathway to apoptotic cell death [63]. Bcl-2 expression is regulated by cytokines and other death–survival signals transcriptionally or posttranscriptionally by phosphorylation [60].

We determined that the Bcl-2-positive mucous cells are derived not only from proliferating cells but also from pre-existing cells [25]. These results showed that Bcl-2 is not involved in cell cycle progression in these cells (Fig. 1). The following observations suggest that Bcl-2 expression sustains GCM: (1) We have demonstrated in Brown Norway and Fischer 344/N rats that following a single intratracheal instillation of LPS, Bcl-2, an inhibitor of apoptosis, must be downregulated before mucous cell numbers can be reduced

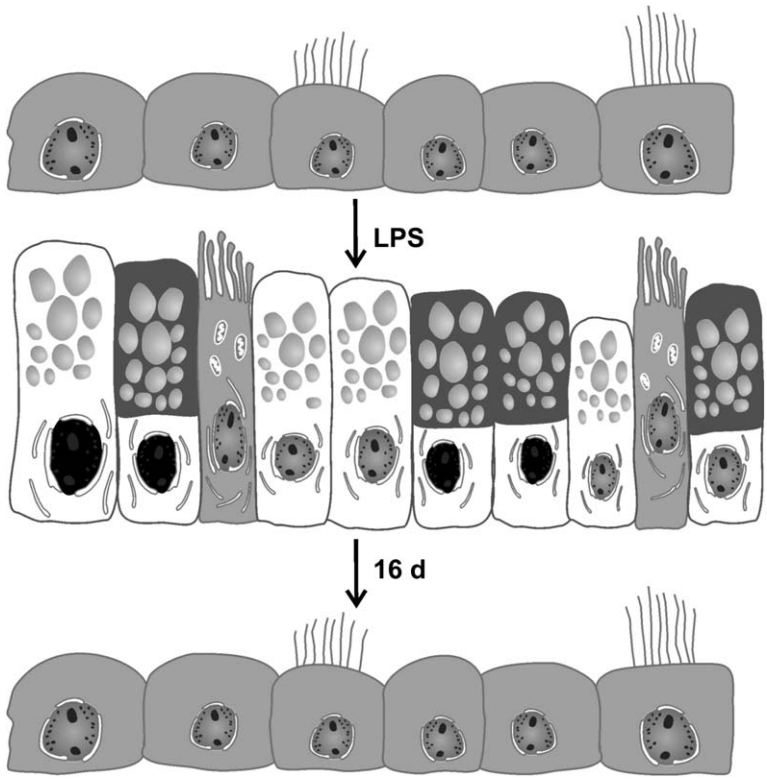

Fig. 1. Injury of the airway epithelium by LPS causes airway epithelial hyperplasia and mucous cell metaplasia. Proliferating (denoted by the black nuclei) and pre-existing cells can produce and store mucus (denoted by the vesicles in the columnar cells). Bcl-2-expressing cells are shown as mucous cells with black staining around the vesicles. Following 12–16 days, the epithelial cell numbers are reduced to essentially the numbers observed before injury to the epithelium.

[64] and (2) Bcl-2 mRNA is downregulated with antisense oligodeoxynucleotides and GCM is subsequently reduced in organ cultures and in *in vivo* rats [55].

In naïve rats, endogenous mucous cells are found in epithelia lining the proximal mid septa. In these cells, approximately 10% are Bcl-2 positive, and this percentage does not change significantly following exposure to ozone. However, exposure to ozone induces GCM in transitional epithelia lining the lateral wall, and the nasal and maxillary turbinates, and the percentage of Bcl-2 positivity in these metaplastic mucous cells exceeds 50% [59]. These results show that Bcl-2 is low in endogenous mucous cells but is expressed in a high percentage of metaplastic mucous cells in nasal epithelia. This observation suggests that Bcl-2 could be a useful target for eliminating metaplastic mucous cells without affecting endogenous mucous cells. Another group of researchers used microarray approaches to identify Bcl-2 as one of the genes expressed following a smoke-induced injury to a human bronchial epithelial cell line, HBE1. They confirmed their observation by Northern blot assays [65]. Collectively, these studies show that Bcl-2 expression in mucous cells is found in animals and humans.

11. Restoration of epithelial homeostasis may reduce GCM and COPD exacerbations

GCM may persist in subjects with chronic bronchitis because of repeated injury of the epithelium due to repeated exposure to allergen, cigarette smoke, or environmental and/or occupational pollutants, chronic infections, or interactions of these various factors. It is also possible that there is a deficiency in the ability of the epithelium to reverse these changes during the wound-healing process. While the inflammatory response may subside, the genetic makeup of the epithelium leads to the inability of the epithelium to heal itself, causing GCM to persist. The following observations support this hypothesis: (1) Bcl-2 transgenic mice do not show resolution of LPS-induced GCM at 8 days after LPS exposure, while their wild-type littermates do [55] and (2) Horses with recurrent airway obstruction, also called COPD, have increased airway secretions of mucus that obstructs airflow [66] and have a significantly higher percentage of Bcl-2-positive mucous cells compared with control horses (personal observation).

While increased GCM in COPD between symptom-free periods is not detrimental, sudden mucus secretion from increased numbers of mucus-producing cells triggered by infections, environmental pollutants, or allergens may block the nonelastic airways of emphysema patients and cause exacerbations. This process can lead to dyspnea and increased sputum production, the hallmarks of acute exacerbation. Identification of molecular mechanisms related to the development and persistence of COPD and CMH may help develop novel strategies to reduce the debilitating symptoms associated with COPD exacerbation.

12. Summary

While the precise cause of COPD exacerbations is not known, evidence suggests that an infectious process is taking place. This evidence is seen at the cellular level with biomarkers, infectious agents from cytology, and clinically observed changes in sputum. CMH is a risk factor for COPD exacerbation, and treatment with mucolytic agents has been shown to reduce the days of disability in subjects with COPD exacerbations. Therefore, the effect of reducing the number of metaplastic goblet cells in small airways may be useful to reduce the risk for exacerbations and the rate of relapse after treatment for COPD exacerbations.

Acknowledgments

This research was supported by grants from the National Heart, Lung, and Blood Institute (HL068111) and the National Institutes of Environmental Health Sciences (ES09237).

References

[1] DHHS, U.S.; Lethbridge-Cejku M, Vickerie J. Summary Health Statistics for U.S. Adults: National Health Interview Survey. 2005 Vital and Health Statistics, Series 10, Number 225.

[2] Mannino DM. Chronic obstructive pulmonary disease: definition and epidemiology. Respir Care 2003; 48(12):1185–91; discussion 91–3.

[3] Mannino DM, Brown C, Giovino GA. Obstructive lung disease deaths in the United States from 1979 through 1993. An analysis using multiple-cause mortality data. Am J Respir Crit Care Med 1997;156(3 Pt 1):814–8.
[4] Strassels SA, Smith DH, Sullivan SD, Mahajan PS. The costs of treating COPD in the United States. Chest 2001;119(2):344–52.
[5] Niewoehner DE, Collins D, Erbland ML. Relation of FEV(1) to clinical outcomes during exacerbations of chronic obstructive pulmonary disease. Department of Veterans Affairs Cooperative Study Group. Am J Respir Crit Care Med 2000;161(4 Pt 1):1201–5.
[6] Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2000;161(5): 1608–13.
[7] Miravitlles M, Mayordomo C, Artes M, Sanchez-Agudo L, Nicolau F, Segu JL. Treatment of chronic obstructive pulmonary disease and its exacerbations in general practice. EOLO Group. Estudio Observacional de la Limitacion Obstructiva al Flujo aEreo. Respir Med 1999;93(3):173–9.
[8] Spencer S, Jones PW. Time course of recovery of health status following an infective exacerbation of chronic bronchitis. Thorax 2003;58(7):589–93.
[9] Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. Eur Respir J 2000;16(4): 677–83.
[10] Seneff MG, Wagner DP, Wagner RP, Zimmerman JE, Knaus WA. Hospital and 1-year survival of patients admitted to intensive care units with acute exacerbation of chronic obstructive pulmonary disease. JAMA 1995;274(23):1852–7.
[11] Pauwels R, Calverley P, Buist AS, Rennard S, Fukuchi Y, Stahl E, et al. COPD exacerbations: the importance of a standard definition. Respir Med 2004;98(2):99–107.
[12] Burge S, Wedzicha JA. COPD exacerbations: definitions and classifications. Eur Respir J Suppl 2003;41: 46s–53s.
[13] Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 2004;23(6):932–46.
[14] Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med 1987;106(2):196–204.
[15] Wedzicha JA, Donaldson GC. Exacerbations of chronic obstructive pulmonary disease. Respir Care 2003; 48(12):1204–13; discussion 13–5.
[16] Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. Thorax 2000;55(2):114–20.
[17] Wedzicha JA, Seemungal TA, MacCallum PK, Paul EA, Donaldson GC, Bhowmik A, et al. Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. Thromb Haemost 2000;84(2):210–5.
[18] Seemungal T, Harper-Owen R, Bhowmik A, Moric I, Sanderson G, Message S, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001;164(9):1618–23.
[19] Chin CL, Manzel LJ, Lehman EE, Humlicek AL, Shi L, Starner TD, et al. Haemophilus influenzae from patients with chronic obstructive pulmonary disease exacerbation induce more inflammation than colonizers. Am J Respir Crit Care Med 2005;172(1):85–91.
[20] Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD. Chest 2000;117(6):1638–45.
[21] Patel IS, Seemungal TA, Wilks M, Lloyd-Owen SJ, Donaldson GC, Wedzicha JA. Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. Thorax 2002; 57(9):759–64.
[22] Stuart-Harris CH, Crofton J, Gison JC, Gough J, Holland W, Knowelden J, et al. Definition and classification of chronic bronchitis for clinical and epidemiologic purposes. Lancet 1965;7389:775–9.

[23] Vestbo J, Prescott E, Lange P. Association of chronic mucus hypersecretion with FEV1 decline and chronic obstructive pulmonary disease morbidity. Copenhagen City Heart Study Group. Am J Respir Crit Care Med 1996;153(5):1530–5.
[24] Morgan WJ, Taussig LM. The chronic bronchitis complex in children. Pediatr Clin North Am 1984;31(4): 851–64.
[25] Tesfaigzi Y, Harris JF, Hotchkiss JA, Harkema JR. DNA synthesis and Bcl-2 expression during the development of mucous cell metaplasia in airway epithelium of rats exposed to LPS. Am J Physiol Lung Cell Mol Physiol 2004;286(2):L268–74.
[26] Anderson HR, Spix C, Medina S, Schouten JP, Castellsague J, Rossi G, et al. Air pollution and daily admissions for chronic obstructive pulmonary disease in 6 European cities: results from the APHEA project. Eur Respir J 1997;10(5):1064–71.
[27] Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998; 157(5 Pt 1):1418–22.
[28] Wilkinson TM, Donaldson GC, Hurst JR, Seemungal TA, Wedzicha JA. Early therapy improves outcomes of exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004;169(12):1298–303.
[29] Vestbo J, Knudsen KM, Rasmussen FV. The value of mucus hypersecretion as a predictor of mortality and hospitalization. An 11-year register based follow-up study of a random population sample of 876 men. Respir Med 1989;83(3):207–11.
[30] Prescott E, Lange P, Vestbo J. Chronic mucus hypersecretion in COPD and death from pulmonary infection. Eur Respir J 1995;8(8):1333–8.
[31] Sethi S, Evans N, Grant BJ, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med 2002;347(7):465–71.
[32] Steiger D, Hotchkiss J, Bajaj L, Harkema J, Basbaum C. Concurrent increases in the storage and release of mucin-like molecules by rat airway epithelial cells in response to bacterial endotoxin. Am J Respir Cell Mol Biol 1995;12(3):307–14.
[33] Miravitlles M, Llor C, Naberan K, Cots JM, Molina J. Variables associated with recovery from acute exacerbations of chronic bronchitis and chronic obstructive pulmonary disease. Respir Med 2005;99(8):955–65.
[34] Miravitlles M, Guerrero T, Mayordomo C, Sanchez-Agudo L, Nicolau F, Segu JL. Factors associated with increased risk of exacerbation and hospital admission in a cohort of ambulatory COPD patients: a multiple logistic regression analysis. The EOLO Study Group. Respiration 2000;67(5):495–501.
[35] Ball P, Harris JM, Lowson D, Tillotson G, Wilson R. Acute infective exacerbations of chronic bronchitis. QJM 1995;88(1):61–8.
[36] Burrows B, Earle RH. Course and prognosis of chronic obstructive lung disease. A prospective study of 200 patients. N Engl J Med 1969;280(8):397–404.
[37] Miravitlles M, Murio C, Guerrero T. Factors associated with relapse after ambulatory treatment of acute exacerbations of chronic bronchitis. DAFNE Study Group. Eur Respir J 2001;17(5):928–33.
[38] Balter MS, La Forge J, Low DE, Mandell L, Grossman RF. Canadian guidelines for the management of acute exacerbations of chronic bronchitis. Can Respir J 2003;10(Suppl B):3B–32B.
[39] Wilson R, Tillotson G, Ball P. Clinical studies in chronic bronchitis: a need for better definition and classification of severity. J Antimicrob Chemother 1996;37(2):205–8.
[40] Saint S, Bent S, Vittinghoff E, Grady D. Antibiotics in chronic obstructive pulmonary disease exacerbations. A meta-analysis. JAMA 1995;273(12):957–60.
[41] Wilson R. Evidence of bacterial infection in acute exacerbations of chronic bronchitis. Semin Respir Infect 2000;15(3):208–15.
[42] Poole PJ, Black PN. Preventing exacerbations of chronic bronchitis and COPD: therapeutic potential of mucolytic agents. Am J Respir Med 2003;2(5):367–70.
[43] Burge PS, Calverley PM, Jones PW, Spencer S, Anderson JA, Maslen TK. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. BMJ 2000;320(7245):1297–303.

[44] Riise GC, Larsson S, Larsson P, Jeansson S, Andersson BA. The intrabronchial microbial flora in chronic bronchitis patients: a target for N-acetylcysteine therapy? Eur Respir J 1994;7(1):94–101.
[45] Riise GC, Qvarfordt I, Larsson S, Eliasson V, Andersson BA. Inhibitory effect of N-acetylcysteine on adherence of Streptococcus pneumoniae and Haemophilus influenzae to human oropharyngeal epithelial cells in vitro. Respiration 2000;67(5):552–8.
[46] Decramer M, Rutten-van Molken M, Dekhuijzen PN, Troosters T, van Herwaarden C, Pellegrion R, et al. Effects of N-acetylcysteine on outcomes in chronic obstructive pulmonary disease (Bronchitis Randomized on NAC Cost-Utility Study, BRONCUS): a randomised placebo-controlled trial. Lancet 2005;365(9470): 1552–60.
[47] Mullen JB, Wright JL, Wiggs BR, Pare PD, Hogg JC. Reassessment of inflammation of airways in chronic bronchitis. Br Med J (Clin Res Ed) 1985;291(6504):1235–9.
[48] Aikawa T, Shimura S, Sasaki H, Takishima T, Yaegashi H, Takahashi T. Morphometric analysis of intraluminal mucus in airways in chronic obstructive pulmonary disease. Am Rev Respir Dis 1989;140(2): 477–82.
[49] Hogg JC. Pathology. In: Weiss EB, Stein M, editors. Bronchial Asthma, Mechanisms and Therapies. Boston, MA: Little Brown and Co.; 1993. pp. 352–5.
[50] Plopper CG, Heidsiek JG, Weir AJ, George JA, Hyde DM. Tracheobronchial epithelium in the adult rhesus monkey: a quantitative histochemical and ultrastructural study. Am J Anat 1989;184(1):31–40.
[51] Rogers DF. Airway goblet cells: responsive and adaptable front-line defenders. Eur Respir J 1994;7(9): 1690–706.
[52] Basbaum C, Jany B. Plasticity in the airway epithelium. Am J Physiol 1990;259(2 Pt 1):L38–46.
[53] Wells AB. The kinetics of cell proliferation in the tracheobronchial epithelia of rats with and without chronic respiratory disease. Cell Tissue Kinet 1970;3(2):185–206.
[54] Blyth DI, Pedrick MS, Savage TJ, Bright H, Beesley JE, Sanjar S. Induction, duration, and resolution of airway goblet cell hyperplasia in a murine model of atopic asthma: effect of concurrent infection with respiratory syncytial virus and response to dexamethasone. Am J Respir Cell Mol Biol 1998;19(1):38–54.
[55] Harris JF, Fischer MJ, Hotchkiss JA, Monia BP, Randell SH, Harkema JR, et al. Bcl-2 sustains increased mucous and epithelial cell numbers in metaplastic airway epithelium. Am J Respir Crit Care Med 2005; 171(7):764–72.
[56] Barnes PJ. Small airways in COPD. N Engl J Med 2004;350(26):2635–7.
[57] Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004;350(26):2645–53.
[58] Seemungal TA, Wedzicha JA. Viral infections in obstructive airway diseases. Curr Opin Pulm Med 2003; 9(2):111–6.
[59] Tesfaigzi J, Hotchkiss JA, Harkema JR. Expression of Bcl-2 during mucous cell metaplasia and remodeling in F344/N rats. Am J Resp Cell Mol Biol 1998;18(6):794–9.
[60] Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science 1998;281(5381):1322–6.
[61] Reed JC. Bcl-2 family proteins. Oncogene 1998;17(25):3225–36.
[62] Cryns V, Yuan J. Proteases to die for. Genes Dev 1998;12(11):1551–70.
[63] Tesfaigzi Y. Roles of apoptosis in airway epithelia. Am J Respir Cell Mol Biol 2006;34:1–11.
[64] Tesfaigzi Y, Fischer MJ, Martin AJ, Seagrave J. Bcl-2 in LPS- and allergen-induced hyperplastic mucous cells in airway epithelia of Brown Norway rats. Am J Physiol Lung Cell Mol Physiol 2000;279:L1210–7.
[65] Yoneda K, Peck K, Chang MM, Chmiel K, Sher YP, Chen J, et al. Development of high-density DNA microarray membrane for profiling smoke- and hydrogen peroxide-induced genes in a human bronchial epithelial cell line. Am J Respir Crit Care Med 2001;164(10 Pt 2):S85–9.
[66] Jefcoat AM, Hotchkiss JA, Gerber V, Harkema JR, Basbaum CB, Robinson NE. Persistent mucin glycoprotein alterations in equine recurrent airway obstruction. Am J Physiol Lung Cell Mol Physiol 2001;281(3): L704–12.