Antiinflammatory Effects of the Phosphodiesterase-4 Inhibitor Cilomilast (Ariflo) in Chronic Obstructive Pulmonary Disease

Elizabeth Gamble, Diana C. Grootendorst, Christopher E. Brightling, Susannah Troy, Yusheng Qiu, Jie Zhu, Debbie Parker, Dean Matin, Swati Majumdar, Antonio M. Vignola, Claus Kroegel, Ferran Morell, Trevor T. Hansel, Stephen I. Rennard, Christopher Compton, Ohad Amit, Tri Tat, Jeffrey Edelson, Ian D. Pavord, Klaus F. Rabe, Neil C. Barnes, and Peter K. Jeffery

Department of Pulmonology, Leiden University Medical Centre, Leiden, The Netherlands; Instituto di Fisiopatologia Respiratoria, Palermo, Italy; Department of Pneumology and Allergy, University Medical Clinic, Jena, Germany; Servei de Pneumologia, Clinica Tres Torres, Hospital Vall d'Hebron, Barcelona, Spain; University of Nebraska Medical Center, Omaha, Nebraska; GlaxoSmithKline Pharmaceuticals, Collegeville, Pennsylvania; Department of Respiratory Medicine, Glenfield Hospital, Leicester; GlaxoSmithKline Pharmaceuticals, Harlow; Lung Pathology, Department of Gene Therapy, Faculty of Medicine, Imperial College, Royal Brompton Hospital; Royal Brompton Hospital; Imperial College School of Medicine; and Department of Respiratory Medicine, London Chest Hospital, London, United Kingdom

Cilomilast (Ariflo), a new oral phosphodiesterase-4 selective inhibitor, improves lung function in chronic obstructive pulmonary disease (COPD). We have evaluated its antiinflammatory effects in 59 patients with COPD randomized to receive cilomilast, 15 mg two times a day, or placebo for 12 weeks. Induced sputum differential cell counts were obtained at baseline and at five further visits. Interleukin-8 and neutrophil elastase were measured in sputum supernatant. Bronchial biopsies obtained at baseline and at Week 10 were immunostained and counted for neutrophils, CD8+ and CD4+ T-lymphocyte subsets, and CD68+ macrophages. Cells expressing the genes for interleukin-8 and tumor necrosis factor–α were identified by *in situ* hybridization and quantified. Compared with placebo, analysis of variance (ANOVA) of the change from baseline showed that cilomilast did not alter any sputum endpoint or FEV_1. However, bronchial biopsies demonstrated that cilomilast treatment was associated with reductions in CD8+ ($p = 0.001$; ANOVA) and CD68+ cells ($p < 0.05$; ANOVA). In addition, by Poisson analysis, comparison of cell counts analyzed as a ratio of active to placebo demonstrated reductions of CD8+ (48% $p < 0.01$) and CD68+ (47% $p = 0.001$) cells. This is the first demonstration of reduction by any agent of airway tissue inflammatory cells characteristic of COPD. Phosphodiesterase-4 inhibitors represent a promising new class of substances for use in antiinflammatory treatment of this disease.

Keywords: inflammation; bronchial biopsy; induced sputum; emphysema; chronic bronchitis

It is predicted that chronic obstructive pulmonary disease (COPD) that was in the sixth place in 2000 in the global ranking of causes of death will move to the third place by 2020 (1). COPD is an inflammatory condition (2–5) characterized clinically by poorly reversible airflow limitation and accelerated rate of decline in lung function (6). The inflammation underlying COPD differs from that of asthma in the predominance of CD8+ cells and macrophages in airway and alveolar tissues of smokers with COPD (2, 3), and CD8+ cell numbers in all lung compartments correlate inversely with FEV_1% of predicted (3, 7, 8). Increased numbers of neutrophils have been reported in bronchoalveolar lavage fluid and sputum (9, 10) and correlate with accelerated decline in FEV_1 over 15 years (11). The cytokines interleukin (IL)-8 and tumor necrosis factor–α (TNF-α) have been shown in one study to be increased in induced sputum (12), and acting on their respective receptors, they may play a role in the selective recruitment of the inflammatory cells (6, 13, 14).

Current treatment modalities for COPD are limited. These include symptom relief by bronchodilators (15). Also, the appreciation that COPD is an inflammatory condition has led to the frequent prescription of inhaled corticosteroids (ICS). However, ICS do not alter the long-term decline of FEV_1 (16), and there are conflicting data as to the effects of ICS on markers of inflammation in the sputum of patients with COPD (17, 18). A trial of ICS in COPD has failed to show changes in tissue CD8+, CD68+ cells, or neutrophils, albeit there is a reduction of subepithelial mast cells (19). The recently published Global Initiative for Chronic Obstructive Lung Disease guidelines identify a pressing need to develop agents that suppress the inflammation associated with COPD and prevent disease progression (6).

Cilomilast is a selective second-generation phosphodiesterase-4 (PDE4) inhibitor. Phosphodiesterases inactivate cAMP, and PDE4 is the predominant isoenzyme in inflammatory cells. Thus, it was proposed that selective PDE4 inhibitors might be effective in treating inflammatory lung conditions such as COPD (20). Indeed, experimental evidence suggests that cilomilast has antiinflammatory activity of relevance to COPD (21). In addition, data obtained from a recent relatively large-scale clinical trial demonstrate significant improvement in lung function after 6 weeks of treatment with cilomilast (22).

This study was designed specifically to evaluate the effects of cilomilast on inflammation in COPD and was not statistically powered to show differences in clinical endpoints such as FEV_1. We hypothesized that cilomilast would reduce the characteristic airway inflammation in patients with COPD treated for 12 weeks in a parallel-group randomized, placebo-controlled study. Induced sputum and endobronchial biopsies were obtained from patients before and after treatment. Neutrophil numbers and concentrations of IL-8 and neutrophil elastase were examined in induced sputum and neutrophils, CD8+ and CD4+ T lymphocytes, and CD68+ macrophages, and gene expression of the proinflammatory mediators IL-8 and TNF-α were evaluated in bronchial biopsies.

(*Received in original form December 17, 2002; accepted in final form June 16, 2003*)

Supported by GlaxoSmithKline Pharmaceuticals (formerly SmithKline Beecham), employers of O.A. and former employers of J.E., S.T., and C.C.

Correspondence and requests for reprints should be addressed to Peter K. Jeffery, Lung Pathology Unit, Imperial College, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK. E-mail: p.jeffery@ic.ac.uk

This article has an online supplement, which is accessible from this issue's table of contents online at www.atsjournals.org

Am J Respir Crit Care Med Vol 168. pp 976–982, 2003
Originally Published in Press as DOI: 10.1164/rccm.200212-1490OC on June 19, 2003
Internet address: www.atsjournals.org

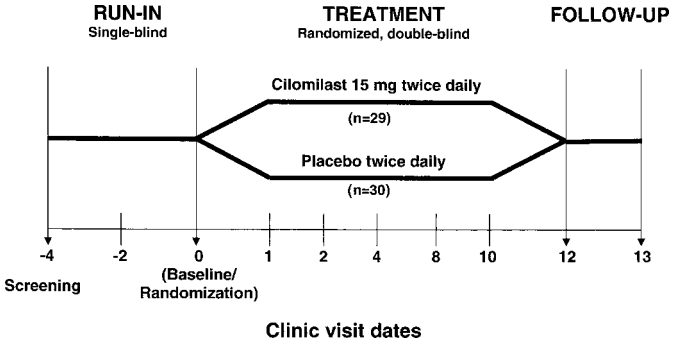

Figure 1. Study design.

METHODS

Subjects

Patients were aged 40 to 80 years with stable COPD, a smoking history of at least 10 pack-years, and fixed airflow obstruction. Only inhaled short-acting β2-agonists and anticholinergic treatments were used during the study. Subjects were volunteers who gave informed written consent, and approval was given by each local research ethics committee.

Study design

This was a 12-week, randomized, placebo-controlled, double-blind, parallel-group study. After screening, there was a 4-week single-blind placebo run-in period before randomization. Bronchoscopy was performed 2 weeks into the placebo run-in period and after 10 weeks of treatment. Any patient taking ICS had these withdrawn at least 4 weeks before screening, i.e., at least 6 weeks before the first bronchoscopy. Patients were randomized if study medication compliance was 80 to 120% during the run-in phase and FEV_1 was stable. Eligible subjects were randomly assigned to receive double-blind cilomilast, 15 mg, or placebo twice daily. Evaluations were performed after 1, 2, 4, 8, 10, and 12 weeks of treatment and 7 to 10 days after completion. Figure 1 shows the study design, and Figure 2 shows the numbers of patients in the study at each stage. Pulmonary function was measured at trough levels of cilomilast.

Sputum Induction and Processing

This was performed according to a previously published method (23). Differential cell counts were performed at the central laboratory (DP at Glenfield Hospital, Leicester, UK). An ELISA technique was used to measure IL-8 (R&D Systems, Minneapolis, MN) and TNF-α (Amersham Pharmacia, Piscataway, NJ) concentrations in the sputum supernatant. Free neutrophil elastase was measured using a spectrofluorimetric assay as previously described(24). Although TNF-α was recoverable from standards, it was not recovered from the study sputum samples; thus, this assay was unsuccessful.

Bronchoscopy Procedure

Bronchoscopy was performed according to the American Thoracic Society guidelines, as previously described (3). Six endobronchial biopsies were obtained from the carinae of the right middle/lower lobar bronchus and subsegmental airways. Cup forceps (Olympus FB 20-C; Olympus, Tokyo, Japan) were replaced after every five bronchoscopies to maintain specimen quality.

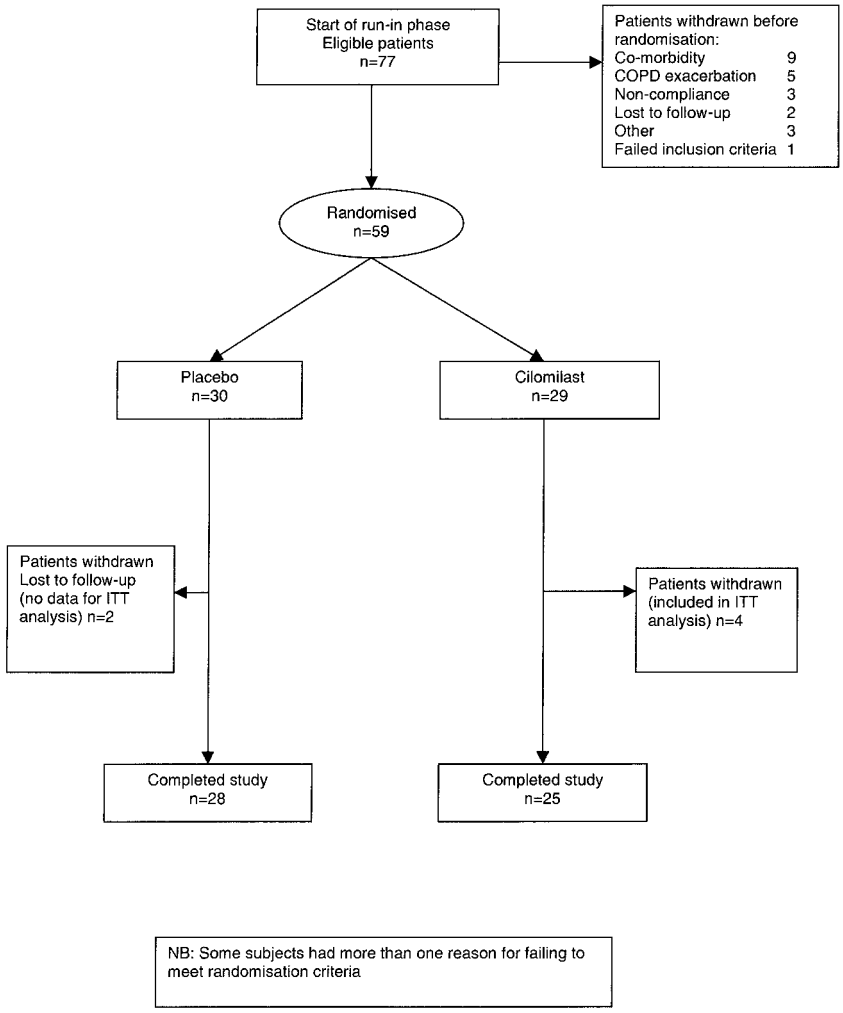

Figure 2. Number of patients remaining at each stage of the study.

TABLE 1. DEMOGRAPHIC CHARACTERISTICS (INTENTION-TO-TREAT POPULATION)

Characteristics	Placebo	Cilomilast
Sex		
Male	23	25
Female	5	4
Age, yr*	61 (7)	62 (11)
Smoking		
Current	16	15
Ex	12	14
Pack-years*	46 (18.2)	46 (20.4)
Chronic bronchitis, MRC criteria	23	24
FEV_1/FVC*	0.53 (0.10)	0.56 (0.09)
FEV_1 prebronchodilator, L*	1.62 (0.43)	1.81 (0.41)
FEV_1, % predicted*	53.9 (12.4)	58.2 (8.2)
FVC prebronchodilator, L*	3.08 (0.62)	3.30 (0.82)
Albuterol reversibility, %*	7.9 (5.9)	7.0 (6.2)

Definition of abbreviation: MRC = Medical Research Council.
Albuterol reversibility as percentage of change from prebronchodilator FEV_1.
* Mean (SD), otherwise actual numbers are given.

Processing of Bronchial Biopsies

Biopsies were fixed immediately in fresh 4% paraformaldehyde at room temperature for 4 hours before transfer to phosphate-buffered saline at 4°C overnight. After dehydration and clearing, biopsies were embedded in paraffin wax. All further processing and quantification were performed in the central laboratory (Lung Pathology, Royal Brompton Hospital, London, UK). Five-micrometer–thick sections were stained with hematoxylin and eosin and the following monoclonal antibodies: neutrophil elastase (neutrophils), CD8 (cytotoxic/suppressor T lymphocytes), CD68 (monocytes/macrophages), OPD4 (CD4+ T-helper lymphocytes). *In situ* hybridization was performed to identify cells' messenger RNA^+ for IL-8 and TNF-α. Positive cells were counted using light microscopy and tissue areas measured using computerized image analysis.

Statistical Analysis

The study was designed to randomize 60 patients in a ratio of 1:1. This provided at least 90% likelihood of detecting a treatment-related 20% change in sputum neutrophil count, with a SD of 15% and a significance level of 0.05. The primary efficacy variable was change in neutrophil percentage in induced sputum. Secondary efficacy variables were biopsy numbers of subepithelial CD8+ cells, CD68+ cells, epithelial and subepithelial neutrophils, and FEV_1. All endpoints were analyzed using an analysis of variance model. Biopsy cell counts were also analyzed using a Poisson model that was most suited to the distribution of the biopsy data and allowed for comparison of relative cell counts in the active compared with placebo groups. Additional details are provided in the online supplement.

RESULTS

Patient Characteristics

Fifty-nine patients met the eligibility criteria for randomization. One patient was lost to follow-up 3 days after randomization, and another was withdrawn for noncompliance 32 days after randomization, leaving 57 patients with available data in the intention-to-treat population. Four patients were withdrawn after adverse events (*see below*), leaving 53 patients who completed the study. The groups were comparable with respect to baseline demographic characteristics, lung function, and smoking history (Table 1). All patients were given albuterol for use as required, and 14 of 59 used ipratropium bromide at a constant dosage (eight in the placebo group, six in the cilomilast group). Before screening, six patients in each group had used ICS. According to the tablet count data, 93.1% of patients taking cilomilast achieved 80 to 120% compliance with the study medication compared with 96.7% of patients receiving placebo.

Sputum

Sputum neutrophil percentage did not change significantly from baseline in the cilomilast group or in the placebo group. Furthermore, the changes in neutrophil percentage were not different between the active and placebo groups (Table 2). Similarly, there were no significant differences in the changes of sputum macrophage percentage, total cell counts, IL-8, or neutrophil elastase concentrations (*see* Table E1 in the online supplement).

Bronchial Biopsies

Figures 3 and 4 show examples of biopsy tissue sections immunostained for CD8+ cells and neutrophils, respectively. The mean area per biopsy section was 0.69 mm^2. The mean length of reticular basement membrane per section was 3.63 mm. The data obtained for all cell counts in the cilomilast or placebo groups at baseline and at Week 10 are shown in Table 2. Compared with placebo, analysis of variance of the change from baseline demonstrated significant reductions in counts of bronchial biopsy CD8+ ($p = 0.001$) and CD68+ ($p = 0.04$) cells by cilomilast. Subepithelial cell counts for CD8+ and CD68+ cells for each patient at baseline and at Week 10 by treatment group are shown in Figures 5 and 6. *Post hoc* Poisson regression analysis confirmed the reductions in the cilomilast group compared with the placebo group in bronchial biopsy counts for CD8+ (48%, $p = 0.004$) and CD68+ (55%, $p < 0.001$) cells. In addition, by the *post hoc* analysis there were reductions of subepithelial CD4+ cells (42%, $p = 0.025$) and neutrophils (37%, $p = 0.049$) (Figure 7). There were no significant treatment-related effects seen in the numbers of epithelial neutrophils, IL-8 messenger RNA^+, or TNF-α messenger RNA^+ cells.

Lung Function

At baseline, the mean FEV_1 in the cilomilast group was 210 ml higher than in the placebo group (no significant difference). At 8 weeks, there was a 340-ml difference in FEV_1 in favor of the cilomilast group ($p = 0.02$), with a 40-ml rise in FEV_1 in the cilomilast group and a 90-ml fall in the placebo group. By the end of the study, the mean FEV_1 in the placebo group had fallen, from its baseline at the start of the study, by 60 ml (SEM, 0.04), whereas it had risen by a mean of 10 ml (SEM, 0.03) in the cilomilast group. This difference did not achieve statistical significance ($p = 0.16$). In those subjects who showed a cilomilast-associated reduction in CD8+ cell count, there was a positive albeit weak correlation with the improvement in FEV_1 ($r = 0.36$), which did not reach statistical significance ($p = 0.18$). There was no treatment difference in Borg breathlessness score.

Adverse Events

There were no side effects of cilomilast on hematology, biochemistry, vital signs, and ECG findings. Diarrhea occurred in six (20.7%) patients in the cilomilast group and in four (13.3%) patients in the placebo group (no significant difference). The diarrhea was mild to moderate in severity and did not result in treatment withdrawal. Three patients in the cilomilast group and two in the placebo group complained of nausea. Four patients in the cilomilast group were withdrawn from the study due to myocardial infarction, COPD exacerbation, dyspepsia, abdominal pain, and/or nausea ($p < 0.05$). One patient in the placebo group developed pancreatitis.

DISCUSSION

In COPD, the extent of inflammation has been linked to severity, and CD8+ T-cell numbers have been shown to correlate inversely with lung function (3, 25). In this study, we have demon-

TABLE 2. RESULTS OF SPUTUM AND BIOPSY CELL COUNTS (INTENTION-TO-TREAT POPULATION)

Cell Type	Placebo	Cilomilast	p Value
Sputum			
Total cell count, 10^6/ml			
Baseline	1.62 (0.60)	2.96 (0.59)	
Endpoint	3.57 (1.9685)	5.86 (1.92)	
Change	1.95 (1.750)	2.90 (1.72)	0.700
Neutrophils, %			
Baseline	76.0 (4.0)	70.5 (3.8)	
Endpoint	74.9 (3.6)	73.7 (3.5)	
Change	−1.1 (3.6)	3.3 (3.5)	0.372
Bronchial biopsy			
Epithelial neutrophils/0.1 mm^2			
Baseline	5.3 (0–26.7)	3.1 (0.7–174)	
Week 10	2.8 (0–28.5)	2.6 (0–30.3)	
Mean change	−0.8 (2.2)	0.9 (2.4)	0.597
Subepithelial neutrophils/mm^2			
Baseline	37.5 (0–188)	47.3 (0.7–174)	
Week 10	30.9 (0–287.4)	35.8 (0–171.8)	
Mean change	18.3 (15.8)	3.2 (17.1)	0.496
Subepithelial CD8+/mm^2			
Baseline	202.8 (8.4–1289.1)	240.9 (9.6–1351.1)	
Week 10	302.4 (4.4–851.6)	134.9 (20.0–860.6)	
Mean change	27.7 (45.2)	−185.0 (49.7)	0.001*
Subepithelial CD68+/mm^2			
Baseline	55.7 (4.0–190.4)	62.3 (6.8–235.9)	
Week 10	73.5 (7.1–370.7)	49.1 (7.8–166.7)	
Mean change	25.3 (15.0)	−21.1 (13.2)	0.039*
Subepithelial CD4+/mm^2			
Baseline	126.0 (26–651)	61.2 (20–618)	
Week 10	90.1 (12.3–428.0)	52.5 (7.3–575.5)	
Mean change	2.2 (33.3)	−29.4 (36.0)	0.496
Subepithelial IL-8+/mm^2			
Baseline	24.3 (0–284.6)	16.7 (0–117.5)	
Week 10	8.6 (0–186.9)	17.5 (0–69.1)	
Mean change	−16.0 (10.7)	0.7 (11.6)	0.270
Subepithelial TNF-α+/mm^2			
Baseline	215.7 (20–1,440)	232.0 (17–944)	
Week 10	237.5 (31–891)	190.8 (17–1,478)	
Mean change	42.1 (72.3)	−2.4 (78.1)	0.659

Definition of abbreviations: IL = interleukin; TNF-α = tumor necrosis factor–α.
Mean (SEM) sputum values before and after treatment.
Median (range) biopsy values before and after treatment.
Mean change (SEM) for biopsy (at Week 10) and sputum (at endpoint) values.
* Denotes a statistically significant difference by analysis of variance for the change from baseline between cilomilast and placebo groups.

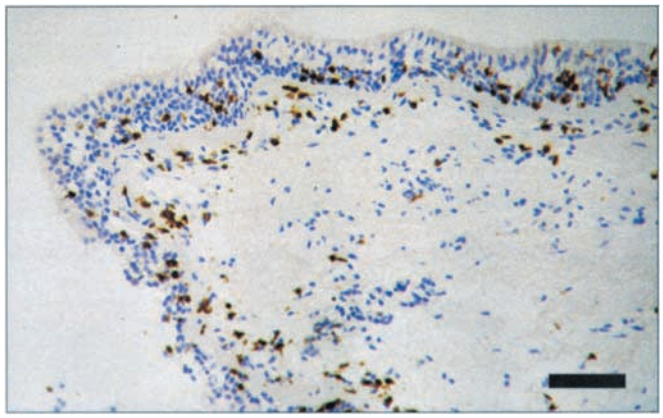

Figure 3. Postcilomilast treatment biopsy immunostained for CD8+ cells using the Envision technique that stains positive cells brown. The cut profiles of immunopositive cells were counted in three-step sections from each biopsy, each section separated by a step of 35 μm (*scale bar* = 80 μm).

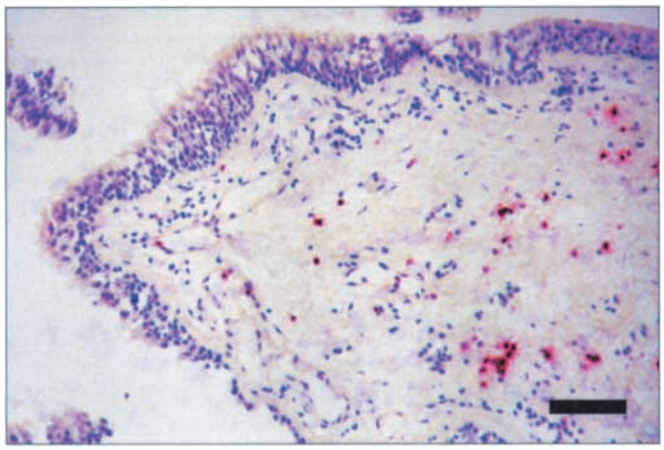

Figure 4. Postcilomilast treatment showing neutrophil elastase+ cells (*red staining*). Three-step sections were counted for each biopsy (*scale bar* = 80 μm).

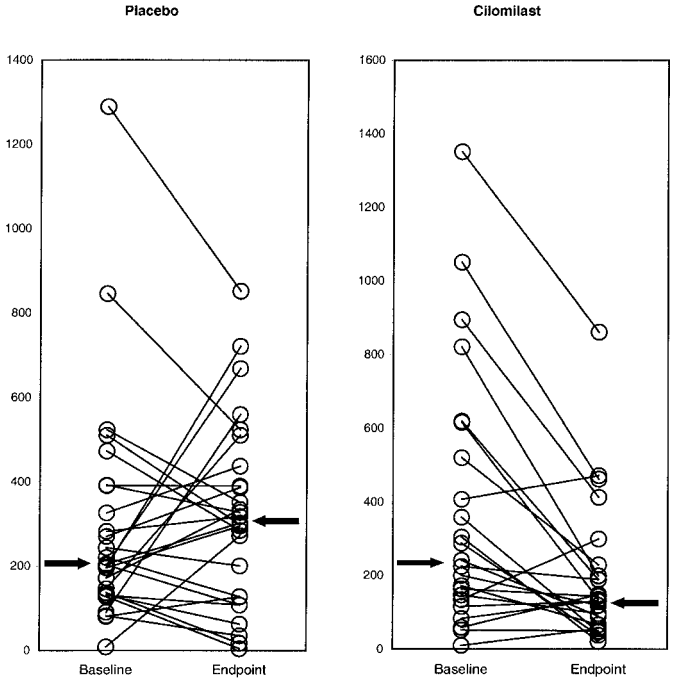

Figure 5. Cell counts for biopsy CD8+ cells for each patient before and after treatment for the cilomilast and placebo groups. *Arrows* indicate median.

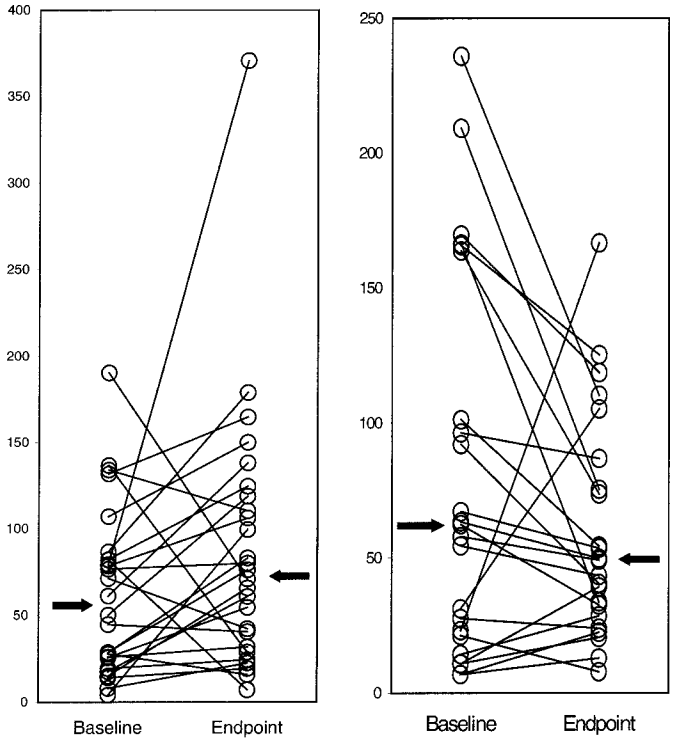

Figure 6. Biopsy CD68+ cell counts for each patient before and after placebo (*left*) or cilomilast (*right*) treatment. *Arrows* indicate median.

strated treatment-related reductions of inflammatory cells in bronchial biopsy tissue in COPD. Although glycophosphopeptical has been recently reported to be associated with alterations in natural immunity in COPD (26), no agent has been shown to reduce tissue CD8+ and CD68+ cell numbers in this disease. Cilomilast has previously been shown to improve lung function over 6 weeks at trough levels of the drug (22). Phosphodiesterase inhibitors promote the accumulation of intracellular cAMP, a second messenger that suppresses activity of immune cells and induces airway smooth muscle relaxation. PDE4 is the predominant phosphodiesterase isoenzyme in immune and inflammatory cells and cilomilast, an agent shown to have antiinflammatory effects *in vitro* (21), and is selective for the inhibition of this isozyme.

One key function of the CD8+ cell is the lysis of virally infected or altered host cells. However, it has been shown experimentally that excessive or inappropriate CD8+ activation can cause extensive tissue damage (27). In patients with similar smoking histories, the severity of COPD and of emphysematous lung destruction has been linked to the amplification of inflammation due to the persistence of (latent) viral infection (28). Cilomilast may attenuate such CD8+ cell–mediated damage by reducing the numbers of these cells.

Increased numbers of tissue macrophages are an early change reported in young smokers and in COPD (3, 29, 30). It is now realized that alveolar macrophages have the capacity to produce proteinases capable of the tissue destruction seen in emphysema (31). In experimental infection, CD8+ T-cell recognition of epithelial cell surface–expressed antigen has been shown to induce alveolar epithelial cell release of macrophage chemoattractants resulting in tissue infiltration and tissue damage by macrophages (32). We have shown in this study that cilomilast also reduces the numbers of tissue macrophages, which might attenuate such damage.

Neutrophilia is often reported in induced sputum of patients with COPD (33, 34). In contrast, tissue neutrophilia has been observed in some but not all studies (3, 30). Two studies of patients with COPD have reported a reduction in sputum neutrophils after ICS treatment (18, 35), but these results were at variance with two further reports (17, 36). A reduction in sputum neutrophils has been demonstrated after treatment with theophylline, a nonselective phosphodiesterase inhibitor (37). Although 3 months of treatment with ICS reduces biopsy mast cell numbers in COPD, there are no significant effects on the numbers of CD8+ T cells, CD68+ macrophages, or neutrophils (19). PDE4 is the predominant cAMP-metabolizing enzyme in human neutrophils (38). Although we found no effect on sputum neutrophils, our *post hoc* analyses demonstrated a reduction of tissue neutrophils and CD4+ cells and confirmed the reductions in CD8+ and CD68+ cells seen with analysis of variance. Inflammatory cell numbers in sputum and bronchial biopsies are poorly correlated (39) but provide complementary information. Moreover, the differences in treatment effect found between sputum and biopsy in our study would indicate that analyses of airway tissue should be included in any future interventional studies of COPD.

Our study was designed to investigate the mechanism of action of cilomilast in terms of its antiinflammatory activity. As a biopsy study, it was necessarily small. Yet, the number of patients in our study was larger than many previous bronchial biopsy studies. This study was not statistically powered to show changes in pulmonary function—this might explain the weak associations and lack of statistical significance between the fall in CD8+ cells and improvements in FEV_1. The placebo group showed a large (mean 60 ml) fall in FEV_1 during the 12 weeks of the study. All the patients met the European Respiratory Society criteria for the diagnosis of COPD (43). The change in FEV_1 was not related to smoking status, reported exacerbations, or earlier steroid use. Most of the patients were recruited and followed up over winter

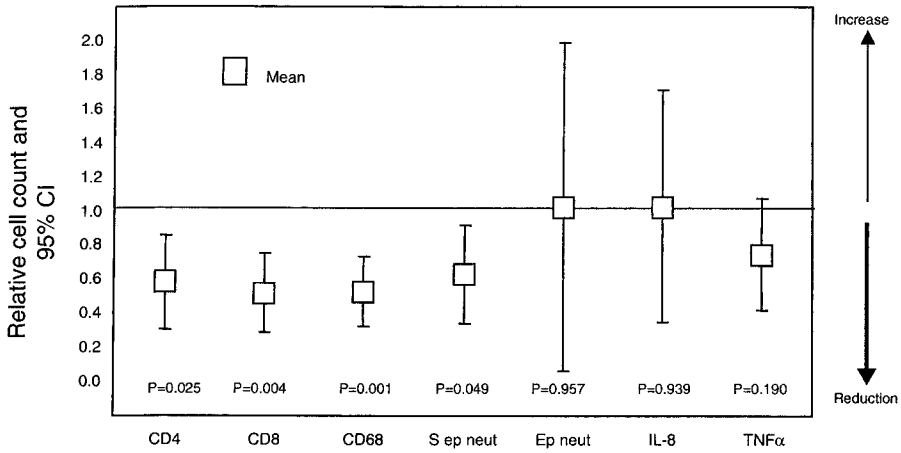

Figure 7. Comparison of biopsy cell counts by Poisson analysis. The results for the cilomilast group are expressed as a ratio of the placebo together with their 95% confidence intervals. The graph demonstrates the magnitude and statistical significance of the relative reductions of inflammatory cells in the subepithelial zone of the cilomilast-treated patients with chronic obstructive pulmonary disease. Epithelial neutrophils and subepithelial cells' messenger RNA^+ for interleukin-8 and tumor necrosis factor–α were unchanged.

months, and it is possible that some subjects had unreported (subclinical) exacerbations resulting in a fall in FEV_1. This may also explain why we failed to show a treatment-associated effect on FEV_1. Another study of 424 patients with COPD who were treated with cilomilast has shown a significant maximum difference in trough FEV_1 of 160 ml compared with placebo after 6 weeks of therapy (22). Four patients in the cilomilast group and none in the placebo group were withdrawn from this study due to serious adverse events. However, the larger study of cilomilast in COPD by Compton and coworkers (22) found that the withdrawal rate for serious adverse events in the cilomilast 15 mg two times a day group (107 patients) was not significantly greater than that in the placebo group.

In summary, this is the first study to evaluate the antiinflammatory effect of a selective PDE4 inhibitor in this increasingly common disease. We have, for the first time, shown a significant reduction by oral cilomilast treatment of tissue CD8+ T lymphocytes and CD68+ monocytes/macrophages in COPD. There is growing support for the hypothesis that these cells are the key effector cells responsible for the airway and lung damage of COPD and that this distinct pattern of inflammation requires alternative antiinflammatory treatment to that used in asthma. Finally, in contrast to many existing COPD treatments, oral cilomilast is available systemically. Such delivery may have added value in influencing the systemic aspects of COPD (40–42) and also may be more effective in targeting the inflammatory process in small airways and lung parenchyma, the predominant anatomic sites responsible for the airflow obstruction.

Conflict of Interest Statement: E.G. has been reimbursed by GSK for attending several conferences and her institution received a research grant of $200,000 from GSK for participating in this multicenter clinical trial; D.C.G. has no declared conflict of interest; C.E.B. has been reimbursed by GSK for attending conferences and has been a speaker at scientific meetings financed by various pharmaceutical companies (GSK, Astra-Zeneca, and MSD) and has also had an unconditional research grant from Schering-Plough for £78,000; S.T. was an employee of the sponsor company during the conduct of the study but is no longer employed by the sponsor; Y.Q. has no declared conflict of interest; J.Z. has no declared conflict of interest; D.P. has received support from GlaxoSmithKline and Merck to attend international conferences; D.M. has no declared conflict of interest; S.M. has no declared conflict of interest; A.M.V. has participated as a speaker in scientific meetings or courses organized and financed by various pharmaceutical companies (GlaxoSmithKline, Astra-Zeneca) receiving 5,000 Euros; C.K. has no declared conflict of interest; F.M. has no declared conflict of interest; T.T.H. has no declared conflict of interest; S.I.R. has consulted for GSK and provided service on advisory boards (approximately $8,000 in 2002), has been paid honoraria/lecture fees (approximately $30,000 in 2002), has received GSK-sponsored grants (approximately $350,000 in 2002), is co-inventor of a patent owned by the University of Nebraska Medical Center in use of the PDE4 inhibitor cilomilast to mitigate fibrotic scarring, provided ad hoc advice to GSK as a consultant on at least three occasions over the last three years totaling $6,000 annually, serves on three GSK advisory boards, one meets twice annually and the other two annually, with compensation for this $6,000 annually, directly sponsored by GSK or sponsored by a non-profit institution with funding from GSK numerous times over the last three years with compensation approximately $20,000 annually, currently funded by GSK to evaluate salmeterol, fluticasone, and the combination on survival in COPD (Torch study) for approximately $100,000, a laboratory study evaluating cilomilast, salmeterol, and fluticasone on fibroblast-repair responses for $120,000 and planning a study to evaluate smoke cessation effects on airway inflammation in COPD ($750,000) and recently completed a study to evaluate the genetics of COPD ($800,000), and a patent for the use of the PDE4 inhibitor cilomilast as a means to mitigate fibrotic scarring has been submitted; C.C. was an employee of GlaxoSmithKline from 1991 to 2002 and holds $40,000 of stock in the company; O.A. has been employed at GSK as a full-time statistician from May 1997 through the present; T.T. has no declared conflict of interest; J.E. was the global clinical project director for Ariflo from July 1998–July 2001; was global head of respiratory clinical research, development, and medical affairs during July 1998–January 2001, and is also Associate Professor of Medicine (Adjunct) at the University of Pennsylvania since November 2001; I.D.P. has received support for research, attending international meetings and advisory board from GSK, Merck, and Astra-Zeneca; K.F.R. has participated as a speaker in scientific meetings and has been a member of advisory boards for Astra-Zeneca, AltanaPharma, Boehringer, Pfizer, GSK, MSD, Novartis, and Schering-Plough, the Department of Pulmonology received research grants from AltanaPharma ($202,616), Novartis ($90,640), Bayer ($61,762), Astra-Zeneca ($103,155), and GSK ($299,495) in the years 2000 until 2002; N.C.B. has been reimbursed by GSK, Merck Sharpe & Dohme, and Schering-Plough for attending scientific conferences, has received consultancy fees of less than $12,000 from GSK and has served on advisory boards for ICOS, GSK, and Aventis, received lecture fees for $16,000 in total in 2002/2003 from GSK and lecture fees of less than $10,000 from Astra-Zeneca, Boehringer Ingelheim, and Pharmacia and received research grants of $200,000 from GSK for participation in research studies; P.K.J. has been reimbursed by GlaxoSmithKline (GKS), Astra-Zeneca (A-Z), and Merck, Sharpe & Dohme (Merck) for attending many conferences and has participated as a paid speaker in scientific meetings or courses organized and financed by various pharmaceutical companies (such as GSK, A-Z, Merck, and Boehringer Ingelheim); served as a consultant to GSK & Novartis; received research grants from several pharmaceutical companies over many years and currently holds research grants from GSK (approximately $750,000), Merck ($120,000), and A-Z ($140,000), the first of which includes a grant for a multicenter clinical trial; P.K.J.'s institution has received unrestricted grants from a wide variety of pharmaceutical companies.

Acknowledgment: The authors thank Drs. Daniella Haefner, P. Reinhard Grahmann, Franco Mirabella, Xavier Munoz, Sergi Marti, and M. Jesus Cruz, and Phil Lake for their valuable contributions to the study and Mr. Andrew Rogers for assistance with the illustrations.

References

1. Murray CJL, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: global burden of disease study. *Lancet* 1997; 349:1498–1504.
2. Jeffery PK. Comparison of the structural and inflammatory features of COPD and asthma (Giles F. Filley Lecture). *Chest* 2000;117:251s–260s.
3. O'Shaughnessy T, Ansari TW, Barnes NC, Jeffery PK. Inflammation in bronchial biopsies of subjects with chronic bronchitis: inverse relationship of $CD8^+$ T lymphocytes with FEV_1. *Am J Respir Crit Care Med* 1997;155:852–857.
4. Zhu J, Majumdar S, Qiu Y, Ansari T, Oliva A, Kips JC, Pauwels RA, De Rose V, Jeffery PK. Interleukin-4 and interleukin-5 gene expression and inflammation in the mucus-secreting glands and subepithelial

tissue of smokers with chronic bronchitis. *Am J Respir Crit Care Med* 2001;164:2220–2228.
5. Zhu J, Qiu YS, Majumdar S, Gamble E, Matin D, Turato G, Fabbri LM, Barnes N, Saetta M, Jeffery PK. Exacerbations of bronchitis: bronchial eosinophilia and gene expression for IL4, IL5 and eosinophil chemoattractants. *Am J Respir Crit Care Med* 2001;164:109–116.
6. Pauwels RA, Buist AS, Calverley PMA, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: NHLBI/WHO global initiative for chronic obstructive pulmonary disease (GOLD) workshop summary. *Am J Respir Crit Care Med* 2001;163:1256–1276.
7. Saetta M. CD8+ T-lymphocytes in peripheral airways of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998; 157:822–826.
8. Saetta M, Baraldo S, Corbino L, Turato G, Braccioni F, Rea F, Cavallesco G, Tropeano G, Mapp CE, Maestrelli P, *et al.* CD8+ cells in the lungs of smokers with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:711–717.
9. Thompson AB, Daughton D, Robbins RA, Ghafouri MA, Oehlerking M, Rennard SI. Intraluminal airway inflammation in chronic bronchitis: characterization and correlation with clinical parameters. *Am Rev Respir Dis* 1989;140:1527–1537.
10. Lacoste J-Y, Bousquet J, Chanez P, Vyve TV, Simony-Lafontaine J, Lequeu N, Vic P, Enander I, Godard P, Michel F-B. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 1993;92:537–548.
11. Stanescu D, Sanna A, Veriter C, Kostianev S, Calcagni PG, Fabbri LM, Maestrelli P. Airways obstruction, chronic expectoration and rapid decline in FEV_1 in smokers are associated with increased levels of sputum neutrophils. *Thorax* 1996;51:267–271.
12. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumour necrosis factor alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med* 1996;153:530–534.
13. Vernooy JH, Kucukaycan M, Jacobs JA, Chavannes NH, Buurman WA, Dentener MA, Wouters EF. Local and systemic inflammation in patients with COPD: soluble tumour necrosis factor receptors are increased in sputum. *Am J Respir Crit Care Med* 2002;166:1218–1224.
14. Churg A, Dai J, Tai H, Xie C, Wright JL. Tumour necrosis factor-alpha is central to acute cigarette smoke-induced inflammation and connective tissue breakdown. *Am J Respir Crit Care Med* 2002;166: 849–854.
15. Anthonisen NR, Connett JE, Kiley JP, Altose MD, Bailey WC, Buist AS, Conway WA, Enright PL, Kanner RE, O'Hara P, *et al.* Effects of smoking intervention and the use of inhaled anticholinergic bronchodilator on the rate of decline of FEV_1: The Lung Health Study. *JAMA* 1994;272:1497–1505.
16. 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;320:1297–1303.
17. Keatings VM, Jatakanon A, Worsdell YM, Barnes PJ. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. *Am J Respir Crit Care Med* 1997;155:542–548.
18. Yildiz F, Kaur AC, Ilgazli A, Celikoglu M, Kacar Ozkara S, Paksoy P, Ozkarakas O. Inhaled corticosteroids may reduce neutrophilic inflammation in patients with stable chronic obstructive pulmonary disease. *Respiration (Herrlisheim)* 2000;67:71–76.
19. Hattotuwa KL, Gizycki M, Ansari TW, Jeffery PK, Barnes NC. The effects of inhaled fluticasone on airway inflammation in chronic obstructive pulmonary disease: a double-blind, placebo-controlled biopsy study. *Am J Respir Crit Care Med* 2002;165:1592–1596.
20. Torphy TJ, Barnette MS, Underwood DC, Griswold DE, Christensen SB, Murdoch RD, Nieman RB, Compton CH. Ariflo (SB 207499), a second generation phosphodiesterase 4 inhibitor for the treatment of asthma and COPD: from concept to clinic. *Pulm Pharmacol Ther* 1999; 12:131–135.
21. Barnette MS, Christensen SB, Essayan DM, Grous M, Prabhakar U, Rush JA, Kagey-Sobotka A, Torphy TJ. SB 207499 (Ariflo), a potent and selective phophodiesterase 4 inhibitor: *in vitro* anti-inflammatory actions. *J Pharmacol Exp Ther* 1998;284:420–426.
22. Compton CH, Gubb J, Nieman R, Edelson J, Amit O, Bakst A, Ayres JG, Creemers JPHM, Schultze-Werninghaus G, Brambilla C, *et al.* Cilomilast, a selective phosphodiesterase-4 inhibitor for treatment of patients with chronic obstructive pulmonary disease: a randomised, dose-ranging study. *Lancet* 2001;358:265–270.
23. Pavord ID, Pizzichini MMM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997;52: 498–501.
24. Brightling CE, Monteiro W, Ward R, Parker D, Morgan MD, Wardlaw AJ, Pavord ID. Sputum eosinophilia and short-term response to prednisolone in chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* 2000;356:1480–1485.
25. Saetta M, Turato G, Maesrelli P, Mapp CE, Fabbri LM. Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;163:1304–1309.
26. Prieto A, Reyes E, Bernstein ED, Martinez B, Monserrat J, Izquierdo JL, Callol L, de Lucas P, Alvarez-Sala JL, Villarrubia VG, *et al.* Defective natural killer and phagocytic activities in chronic obstructive pulmonary disease are restored by glycophosphopeptical (Inmunoferon). *Am J Respir Crit Care Med* 2001;163:1578–1583.
27. Cannon MJ, Openshaw PJ, Askonas BA. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. *J Exp Med* 1988;168:1163–1168.
28. Retamales I, Elliott WM, Meshi B, Coxson HO, Pare PD, Sciurba FC, Rogers RM, Hayashi S, Hogg JC. Amplification of inflammation in emphysema and its association with latent adenoviral infection. *Am J Respir Crit Care Med* 2001;164:469–473.
29. Niewoehner DE, Kleinerman J, Rice DB. Pathologic changes in the peripheral airways of young cigarette smokers. *N Engl J Med* 1974; 291:755–758.
30. Di Stefano A, Capelli A, Lusuardi M, Balbo P, Vecchio C, Maestrelli P, Mapp CE, Fabbri LM, Donner CF, Saetta M. Severity of airflow limitation is associated with severity of airway inflammation in smokers. *Am J Respir Crit Care Med* 1998;158:1277–1285.
31. Shapiro SD. The macrophage in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:S29–S32.
32. Zhao MQ, Stoler MH, Liu AN, Wei B, Soguero C, Hahn YS, Enelow RI. Alveolar epithelial cell chemokine expression triggered by antigen-specific cytolytic CD8+ T cell recognition [abstract]. *J Clin Invest* 2000;106:R49–R58.
33. Peleman RA, Rytila PH, Kips JC, Joos GF, Pauwels RA. The cellular composition of induced sputum in chronic obstructive pulmonary disease. *Eur Respir J* 1999;13:839–843.
34. Keatings VM, Barnes PJ. Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. *Am J Respir Crit Care Med* 1997;155:449–453.
35. Confalonieri M, Mainardi E, Della Porta R, Bernorio S, Gandola L, Beghe B, Spanevello A. Inhaled corticosteroids reduce neutrophilic bronchial inflammation in patients with chronic obstructive pulmonary disease. *Thorax* 1998;53:583–585.
36. Culpitt SV, Maziak W, Loukidis S, Nightingale JA, Matthews JL, Barnes PJ. Effect of high dose inhaled steroid on cells, cytokines and proteases in induced sputum in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999;160:1635–1639.
37. Culpitt SV, de Matos C, Russell RE, Donnelly LE, Rogers DF, Barnes PJ. Effect of theophylline on induced sputum inflammatory indices and neutrophil chemotaxis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002;165:1371–1376.
38. Wright CD, Kuipers PJ, Kobylarz D, Singer L, Devall J, Klinkefus BA, Weishaar RE. Differential inhibition of human neutrophil functions: role of cyclic AMP-specific, cyclic GMP-insensitive phosphodiesterase. *Biochem Pharmacol* 1990;40:699–707.
39. Maestrelli P, Saetta M, Di Stefano A, Calcagni PG, Turato G, Ruggieri MP, Roggeri A, Mapp CE, Fabbri LM. Comparison of leukocyte counts in sputum, bronchial biopsies, and bronchoalveolar lavage. *Am J Respir Crit Care Med* 1995;152:1926–1931.
40. Pouw EM, Schols AMWJ, Deutz NEP, Wouters EFM. Plasma and muscle amino acid levels in relation to resting energy expenditure and inflammation in stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;158:797–801.
41. Schols AMWJ, Buurman WA, Staal-van den Brekel AJ, Dentener MA, Wouters EFM. Evidence for a relation between metabolic derangements and increased levels of inflammatory mediators in a subgroup of patients with chronic obstructive pulmonary disease. *Thorax* 1996; 51:819–824.
42. de Godoy I, Donahoe M, Calhoun WJ, Mancino J, Rogers RM. Elevated TNF-α production by peripheral blood monocytes of weight-losing COPD patients. *Am J Respir Crit Care Med* 1996;153:633–637.
43. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, Yernault JC, Decramer M, Higenbottam T, Postma DS, *et al.* Optimal assessment and management of chronic obstructive pulmonary disease (COPD). *Eur Respir J* 1995;8:1398–1420.