Relationship between airway inflammation and the frequency of exacerbations in patients with smoking related COPD

S Gompertz, D L Bayley, S L Hill, R A Stockley

Abstract

Background—Patients with more frequent exacerbations of chronic obstructive pulmonary disease (COPD) may have increased bronchial inflammation. Airway inflammation was measured in patients who had been thoroughly investigated with full pulmonary function testing, thoracic HRCT scanning, and sputum microbiology to examine further the relationship between exacerbation frequency and bronchial inflammation.

Methods—Airway inflammation (spontaneous sputum sol phase myeloperoxidase (MPO), elastase, leukotriene (LT)B4, interleukin (IL)-8, secretary leukoprotease inhibitor (SLPI), protein leakage) and serum levels of C reactive protein (CRP) were compared in 40 patients with stable, smoking related COPD, divided into those with frequent (≥3/year) or infrequent (<2/year) exacerbations according to the number of primary care consultations during the preceding year. The comparisons were repeated after excluding eight otherwise clinically indistinguishable patients who had tubular bronchiectasis on the HRCT scan.

Results—Patients with frequent (n=12) and infrequent (n=28) exacerbations were indistinguishable in terms of their clinical, pulmonary function, and sputum characteristics, CRP concentrations, and all of their bronchial inflammatory parameters (p>0.05). The patients without evidence of tubular bronchiectasis (n=32) were equally well matched but the sputum concentrations of SLPI were significantly lower in the frequent exacerbators (n=8) in this subset analysis (p<0.05).

Conclusions—There are several clinical features that directly influence bronchial inflammation in COPD. When these were carefully controlled for, patients with more frequent reported exacerbations had lower sputum concentrations of SLPI.

This important antiproteinase is also known to possess antibacterial and anti-viral activity. Further studies are required into the nature of recurrent exacerbations and, in particular, the regulation and role of SLPI in affected individuals.

(Thorax 2001;56:36–41)

Keywords: chronic obstructive pulmonary disease; inflammation; secretary leukoprotease inhibitor (SLPI)
the stable clinical state and collected evidence of the number of reported exacerbations in the 12 months before and after our study. We have assessed bronchial and systemic inflammation in those patients with a clear physiological diagnosis of COPD and related this to the number of exacerbations over 12 months as defined by Bhowmik et al. The results of this analysis form the basis of the present study.

Methods

STUDY SUBJECTS

Seventy patients who were able to provide a spontaneous sputum sample in the stable clinical state, at least eight weeks after their index exacerbation, were selected from a study designed to characterise the nature of COPD and exacerbations in primary care. All had a clinical diagnosis of COPD and chronic bronchitis (daily sputum production for at least three months of two consecutive years) made in the community and had presented to their primary care physician with an acute exacerbation (any combination of worsening respiratory symptoms including one or more of: increased sputum volume, purulence, or breathlessness, with or without other minor symptoms, including increased sputum viscosity, cough, wheeze, chest pain, malaise, fever or rigors). None of the patients were receiving oral corticosteroids at the time of the index exacerbation.

EXACERBATION FREQUENCY

The primary care records were available for 58 patients (83%) and were examined for the 12 months before and after their index exacerbation by an experienced specialist respiratory research nurse. Discrete episodes of worsening respiratory symptoms were identified regardless of whether the consultation resulted in an increase in bronchodilator use or the prescription of an antibiotic or an oral steroid. The patients were divided into those who had presented to their general practitioner with an acute exacerbation (acute exacerbators) and those who had suffered two or less exacerbations (infrequent exacerbators).

All 58 patients underwent full pulmonary function testing with reversibility and high resolution computed tomographic CT (HRCT) scanning (1 mm slices) in the stable state to characterise the nature of their lung disease as described elsewhere. Forced expiratory volume in one second (FEV1), forced vital capacity (VC), residual volume (RV), total lung capacity (TLC), carbon monoxide transfer factor (TLCO) and transfer coefficient (Kco) were determined according to national guidelines as described previously and are expressed as percentages of the predicted normal reference values. FEV1/VC and RV/TLC were expressed as ratios and multiplied by 100. Spirometric tests were performed before and 20 minutes after inhalation of 400 µg salbutamol. Forty one of the patients (71%) had physiological evidence of COPD with both an FEV1 of <80% predicted for age, sex, and height and an FEV1/VC ratio of <70%. Data from the 17 patients (five frequent, 12 infrequent exacerbators) who did not have airflow obstruction by these criteria were excluded, as were the data from a further patient who had never smoked.

SPUTUM CLASSIFICATION AND PROCESSING

Spontaneous sputum samples were obtained over four hours from rising and the macroscopic appearance was allocated a number by an experienced microbiology technician with reference to a standard colour chart. Values of 0–2 were allocated to samples which were colourless through to white (mucoid), and 3–8 to increasingly purulent samples. Quantitative sputum culture was performed on an aliquot of the sample as described previously. The remaining sample was centrifuged at 50 000g at 4°C for 90 minutes and the sputum pellet was harvested and stored at −70°C until analysed. Ten ml of blood was also obtained, allowed to clot, centrifuged at 3000 rpm for 10 minutes, and the serum was stored at −70°C until required.

Myeloperoxidase (MPO) activity was measured by chromogenic substrate assay relative to a standard preparation of lysed neutrophils (reference of 3.5 arbitrary units/ml of sputum) and neutrophil elastase activity was assessed spectrophotometrically using the synthetic substrate M-OsAAPVpNa (Sigma-Aldrich Company Ltd, Poole, Dorset, UK). The lower limit of detection of the assay was 0.8 nM and sample results below this level were taken as zero for statistical purposes.

The neutrophil chemotactants IL-8 and leukotriene B4 (LTB4) and the bronchial protease inhibitor secretory leukoprotease inhibitor (SLPI) were measured using commercially available ELISA kits (IL-8 and SLPI: R&D Systems Europe Ltd, Abingdon, UK; LTB4; Amersham International plc, Buckinghamshire, UK). The characteristics and recovery of these assays have been described previously.

Sputum sol and serum albumin were measured by radial immunodiffusion using a commercially available kit (The Binding Site Ltd, Birmingham, UK). Sputum sol/serum ratios (expressed as a percentage) were calculated, providing an assessment of protein “leakage” from the blood into the lung.

SERUM C REACTIVE PROTEIN

Serum levels of C reactive protein (CRP) were measured in 35 of the 40 patients (11 frequent, 24 infrequent exacerbators) by radial immunodiffusion using a commercially available kit (The Binding Site Limited, Birmingham, UK) with an interassay coefficient of variation of 2.2%. Five µl of standard or sample was added to each well and the plate was incubated for at least 72 hours at room temperature. Precipitation ring diameters were measured using an eye piece graticule and a regression curve was constructed of the square of the diameter of the precipitation ring versus concentration of the
The number of exacerbations in the year after the index exacerbation (Fig 1) had very few exacerbations (0–1) in the year preceding the index exacerbation, indicating that the selection criteria did not exclude those patients with the lowest exacerbation frequencies.

All patients (by the selection criteria described) had smoked, and both groups had a similar history of cigarette consumption with a median (IQR) pack year history of 35 (25–56) and 57 (33–77) for the frequent and infrequent exacerbators, respectively (p>0.05). Although there were no differences in age or in the proportions of men or of current smokers between the two groups, a higher proportion (p<0.05) of frequent exacerbators were receiving inhaled corticosteroids at the onset of the index exacerbation. The demographic data for both groups are summarised in table 1.

Table 1  Demographic and pulmonary function data (all patients)

<table>
<thead>
<tr>
<th></th>
<th>Frequent exacerbators (n=12)</th>
<th>Infrequent exacerbators (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>67.3 (4.9)</td>
<td>66.7 (7.2)</td>
</tr>
<tr>
<td>Male sex†</td>
<td>6 (50%)</td>
<td>18 (64%)</td>
</tr>
<tr>
<td>Current smokers†</td>
<td>5 (42%)</td>
<td>15 (54%)</td>
</tr>
<tr>
<td>Inhaled steroids†</td>
<td>10 (83%)*</td>
<td>13 (46%)</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>1.24 (0.48)</td>
<td>1.19 (0.35)</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>52.3 (13.8)</td>
<td>48.2 (15.4)</td>
</tr>
<tr>
<td>Response to inhaled β₂ agonists (ml)</td>
<td>225 (130)</td>
<td>175 (145)</td>
</tr>
<tr>
<td>FEV₁/VC (%)</td>
<td>43.5 (10.3)</td>
<td>43.3 (12.6)</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>118.8 (28.3)</td>
<td>124.8 (25.2)</td>
</tr>
<tr>
<td>KCO (%)</td>
<td>93.7 (33.8)</td>
<td>91.0 (26.5)</td>
</tr>
</tbody>
</table>

FEV₁ = forced expiratory volume in one second; VC = vital capacity; RV = residual volume; TLC = total lung capacity; KCO = carbon monoxide transfer coefficient.

Frequent exacerbators had suffered ≥3 and infrequent exacerbators ≤2 exacerbations in the previous 12 months.

†Clinical characteristics expressed as number (%); the remainder of the data are shown as mean (SD).

*Significant difference (p<0.05) in the proportion of patients receiving inhaled corticosteroids.

ent relationship (r=0.32, p<0.03) between the number of exacerbations reported each year (fig 1). The 28 patients (70%) who had experienced ≤2 exacerbations in the 12 months preceding the study (1.0 (0.0–2.0)) had a similar number (p>0.05) in the second year (1.0 (0.0–2.8)). However, the 12 patients (30%) who had ≥3 exacerbations in the year before the study (4.0 (3.0–5.8)) had fewer (p<0.05) in the subsequent 12 months (2.0 (1.0–4.0)). Twenty of the 28 patients with ≤2 exacerbations (71%) had very few exacerbations (0–1) in the year preceding the index exacerbation, indicating that the selection criteria did not exclude those patients with the lowest exacerbation frequencies.

Previous work by our group (unpublished data) has indicated that, although clinically indistinguishable, patients with tubular bronchiectasis have evidence of increased airway inflammation (neutrophil elastase, IL-8 and LTB₄). An a priori decision was therefore taken to perform a subset analysis having excluded these individuals.

The study was approved by the South Birmingham Health Authority ethics committee and all subjects provided written informed consent.

Results

ALL PATIENTS

The 40 patients eventually studied had consulted their primary care physician with up to 10 exacerbations in the 12 months before the study (median (IQR) 1.5 (1.0–3.0)) and this was similar (p>0.05) to the exacerbation rate in the 12 months after the study (median (IQR) 2.0 (0.0–3.0)). In general there was a consistent relationship (r=0.32, p<0.03) between the number of exacerbations reported each year (fig 1). The 28 patients (70%) who had experienced ≤2 exacerbations in the 12 months preceding the study (1.0 (0.0–2.0)) had a similar number (p>0.05) in the second year (1.0 (0.0–2.8)). However, the 12 patients (30%) who had ≥3 exacerbations in the year before the study (4.0 (3.0–5.8)) had fewer (p<0.05) in the subsequent 12 months (2.0 (1.0–4.0)). Twenty of the 28 patients with ≤2 exacerbations (71%) had very few exacerbations (0–1) in the year preceding the index exacerbation, indicating that the selection criteria did not exclude those patients with the lowest exacerbation frequencies.

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The study was approved by the South Birmingham Health Authority ethics committee and all subjects provided written informed consent.
The concentrations of the inflammatory mediators are shown as median (IQR).

There were no significant differences between the frequent and infrequent exacerbators (p>0.05).

### Table 2: Bronchial inflammation according to exacerbation frequency (all patients)

<table>
<thead>
<tr>
<th></th>
<th>MPO (units/ml)</th>
<th>Elastase (nM)</th>
<th>LTB4 (nM)</th>
<th>IL-8 (nM)</th>
<th>SLPI (µM)</th>
<th>Sputum albumin ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequent (n=12)</td>
<td>0.41 (0.31–0.59)</td>
<td>3.48 (1.6–5.7)</td>
<td>3.1 (1.6–6.8)</td>
<td>2.9 (1.9–6.0)</td>
<td>0.35 (0.30–0.93)</td>
<td>0.35 (0.28–0.86)</td>
</tr>
<tr>
<td>Infrequent (n=28)</td>
<td>0.37 (0.14–0.66)</td>
<td>4.5 (1.9–7.1)</td>
<td>3.8 (1.0–6.9)</td>
<td>4.4 (3.2–5.5)</td>
<td>0.59 (0.28–1.19)</td>
<td>0.37 (0.28–0.86)</td>
</tr>
</tbody>
</table>

MPO = myeloperoxidase; LTB4 = leukotriene B4; IL-8 = interleukin 8; SLPI = secretory leukoprotease inhibitor.

### Table 3: Demographic and pulmonary function data (patients with no bronchial dilatation on high resolution CT scan)

<table>
<thead>
<tr>
<th></th>
<th>Frequent exacerbators (n=8)</th>
<th>Infrequent exacerbators (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.4 (5.0)</td>
<td>66.7 (6.9)</td>
</tr>
<tr>
<td>male sex†</td>
<td>5 (63%)</td>
<td>16 (67%)</td>
</tr>
<tr>
<td>Current smokers†</td>
<td>4 (50%)</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>Inhaled steroids†</td>
<td>7 (88%)*</td>
<td>12 (50%)</td>
</tr>
<tr>
<td>FEV1 (l)</td>
<td>1.25 (0.50)</td>
<td>1.15 (0.33)</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>50.5 (14.9)</td>
<td>46.5 (14.6)</td>
</tr>
<tr>
<td>Response to inhaled β2 agonist (ml)</td>
<td>225 (95)</td>
<td>170 (135)</td>
</tr>
<tr>
<td>FEV1/VC (%)</td>
<td>41.6 (11.5)</td>
<td>42.3 (12.5)</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>120 (27)</td>
<td>125 (23)</td>
</tr>
<tr>
<td>Kco (%)</td>
<td>91.0 (28.7)</td>
<td>90.8 (27.2)</td>
</tr>
</tbody>
</table>

FEV1 = forced expiratory volume in one second; VC = vital capacity; RV = residual volume; TLC = total lung capacity; Kco = carbon monoxide transfer coefficient.

The two groups were similar with no differences in any of the demographic or pulmonary function data.

The concentrations of sputum inflammatory parameters were shown in table 2. There were no significant differences in the concentrations of MPO, elastase, LTB4, IL-8, SLPI, or protein leakage between the two groups. Five (42%) of the frequent exacerbators had detectable sputum elastase activity, as did nine (32%) of the infrequent exacerbators (p>0.05). Furthermore, there were no significant differences in the concentrations of CRP: 3.0 (1.3–4.9) mg/l and 2.7 (1.0–7.4) mg/l, respectively, in those with frequent and infrequent exacerbations.

### Table 4: Bronchial inflammation according to exacerbation frequency (patients with no bronchial dilatation)

<table>
<thead>
<tr>
<th></th>
<th>MPO (units/ml)</th>
<th>Elastase (nM)</th>
<th>LTB4 (nM)</th>
<th>IL-8 (nM)</th>
<th>SLPI (µM)</th>
<th>Sputum albumin ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequent (n=8)</td>
<td>0.37 (0.16–0.49)</td>
<td>0.00</td>
<td>2.7 (0.9–6.4)</td>
<td>2.2 (1.9–6.0)</td>
<td>2.2 (1.9–6.0)</td>
<td>0.30 (0.30–0.68)</td>
</tr>
<tr>
<td>Infrequent (n=24)</td>
<td>0.30 (0.14–0.66)</td>
<td>0.00</td>
<td>4.5 (1.9–6.8)</td>
<td>2.7 (1.9–6.5)</td>
<td>4.5* (3.2–5.5)</td>
<td>0.59 (0.28–1.21)</td>
</tr>
</tbody>
</table>

MPO = myeloperoxidase; LTB4 = leukotriene B4; IL-8 = interleukin 8; SLPI = secretory leukoprotease inhibitor.

The concentrations of sputum IL-8 were higher (p=0.05). There were no differences in any of the inflammatory parameters between the two groups (p>0.05).

**Influence of Bronchiectasis**

Eight (20%) of the patients with a clinical and physiological diagnosis of COPD (four with frequent and four with infrequent exacerbations) had radiological evidence of mild tubular bronchiectasis on the HRCT scan, as described previously, although they were indistinguishable clinically from those without bronchial dilatation. These patients were excluded and a further comparison between frequent and infrequent exacerbators was carried out in the remaining subgroup, as per our a priori decision. The demographic and pulmonary function data of these remaining 32 individuals (eight frequent and 24 infrequent exacerbators) are summarised in table 3.

The two groups were similar with no differences in age, lung function, the proportion of men, or current smokers between them (table 3). Pack year smoking histories were also similar: 31 (25–70) in the group with frequent exacerbations and 61 (33–91) in those with infrequent exacerbations (p>0.05). However, the proportion of frequent exacerbators receiving inhaled corticosteroids at the onset of the index exacerbation remained higher (88% vs 50%).

The sputum characteristics of the two subgroups also remained the same (p>0.05 for all parameters). The mean (SD) sputum colour was 2.3 (0.7) in the frequent exacerbators and 2.6 (1.0) in the infrequent exacerbators. Potential respiratory pathogens were isolated from the sputum samples of two (25%) of those with frequent exacerbations and from nine (38%) of the patients with infrequent exacerbations and the proportion in whom a high bacterial load (>10³ cfu/ml) was recovered was also the same (one (13%) and five (21%), respectively).

In this subgroup analysis there were still no differences in the sputum sol MPO, elastase, LTB4, IL-8 levels or protein leakage between the two groups (p>0.05, table 4). Two of the frequent exacerbators (25%) had detectable sputum elastase activity, as did seven (29%) of the infrequent exacerbators (p>0.05). CRP concentrations were no different (p>0.05) between the two groups of patients, being 2.8 (1.3–4.8) mg/l and 3.1 (1.0–8.6) mg/l, respectively. However, those who had experienced ≥3 exacerbations over the preceding 12 months had lower sputum SLPI concentrations (p<0.05; table 4 and fig 2). Furthermore, there was a significant inverse (Spearman) correlation between exacerbation frequency (determined over the preceding year) and sputum SLPI concentration (r=−0.49, p<0.005).
who had found raised levels of IL-6 and IL-8 in those patients with COPD in whom sputum inductions.

Corticosteroids are also known to reduce both the number and severity of exacerbations in COPD, and the difference observed in sputum bacterial load and HRCT appearances, which may remain unreported, and possibly increasing SLPI concentrations. Inhaled corticosteroids are known to influence bronchial inflammation but found no difference in inflammation may have been related to unreported, undetected, or uncontrolled for clinical differences between the two groups of patients, or even to a different response to sputum induction which is known to be a pro-inflammatory stimulus.

Bhowmik et al collected their data relating to exacerbation numbers prospectively from a highly trained outpatient population who filled in symptom diary cards on a daily basis, whereas our data were obtained retrospectively from analysis of primary care records. Furthermore, other workers have shown that reconsultation rates for acute lower respiratory tract infection in the general population are closely related to past consulting behaviour. It is possible that either of these factors could explain the lack of a difference in inflammation between the two patient groups in our population. Nevertheless, the design of the study determined that only exacerbation symptoms reported to the primary care physician were identified, rather than the 50% or so of exacerbations which may remain unreported, and these were therefore likely to be of more clinical significance.

In our subgroup analysis we excluded patients with bronchial dilatation seen on HRCT scanning whom we have found previously to have increased bronchial inflammation. In the remaining 32 patients the concentrations of SLPI were significantly lower in the sputum of those with frequent exacerbations. This observation merits further study because SLPI is not only a protease inhibitor, but also has antiviral and antibacterial activity.

Factors influencing SLPI concentrations in the airway are largely unknown, although elastase and bacterial endotoxin (unpublished data) will reduce its release from epithelial cells. However, it is unlikely that either mechanism explains our findings as there was little detectable elastase activity in either group of patients in the stable state and the proportion colonised with bacteria was also similar. Steroids can increase both SLPI production by epithelial cells in vitro and concentrations in lung secretions in vivo. However, the group with low SLPI levels were frequent exacerbators and more of this group were receiving inhaled steroids, which would (if anything) reduce the difference observed.
At present we cannot exclude a chance finding of differences in SLPI concentration between our two groups of patients. However, it is tempting to speculate that a reduction in the antibacterial/antiviral function of SLPI would predispose to more frequent exacerbations related to these organisms. In addition, low concentrations of SLPI would result in greater local elastase activity during exacerbations, leading to increased epithelial damage and reduced ciliary beat frequency which might also predispose to more bacterial exacerbations. Clearly, further studies are indicated to try to reproduce these findings in a larger number of patients.

In summary, there are several clinical features that directly influence bronchial inflammation in COPD. When these were carefully controlled for patients with more frequent reported exacerbations did not have any significantly greater bronchial inflammation. Clearly, the relationship between frequent exacerbations, bronchial inflammation, and lung defence is of major importance in understanding the pathogenesis of COPD. Further studies are required into the nature of recurrent exacerbations and also the regulation and role of SLPI in larger numbers of affected individuals.

This study was funded by GlaxoWellcome who provided an unrestricted educational grant. The authors also wish to acknowledge all the hard work of M Henson and H Whitehouse (specialist research nurses) who collected the exacerbation frequency data.


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