Cystic fibrosis

ORIGINAL ARTICLE

Distinct patterns of inflammation in the airway lumen and bronchial mucosa of children with cystic fibrosis

Nicolas Regamey,1,2,3 Lemonia Tsartsali,1 Tom N Hilliard,1,2 Oliver Fuchs,3 Hui-Leng Tan,1,2 Jie Zhu,2 Yu-Sheng Qiu,2 Eric W F W Alton,2 Peter K Jeffery,2 Andrew Bush,1 Jane C Davies1,2

ABSTRACT

Background Studies in cystic fibrosis (CF) generally focus on inflammation present in the airway lumen. Little is known about inflammation occurring in the airway wall, the site ultimately destroyed in end-stage disease.

Objective To test the hypothesis that inflammatory patterns in the lumen do not reflect those in the airway wall of children with CF.

Methods Bronchoalveolar lavage (BAL) fluid and endobronchial biopsies were obtained from 46 children with CF and 16 disease-free controls. BAL cell differential was assessed using May-Grunewald-stained cytocins. Area profile counts of bronchial tissue immunopositive inflammatory cells were determined.

Results BAL fluid from children with CF had a predominance of neutrophils compared with controls (median 810 × 10^3/ml vs 1 × 10^3/ml, p < 0.0001). In contrast, subepithelial bronchial tissue from children with CF was characterised by a predominance of lymphocytes (median 961 vs 717 cells/mm², p = 0.014), of which 82% were (CD3) T lymphocytes. In chest exacerbations, BAL fluid from children with CF had more inflammatory cells of all types compared with those with stable disease whereas, in biopsies, only the numbers of lymphocytes and macrophages, but not of neutrophils, were higher.

A positive culture of Pseudomonas aeruginosa was associated with higher numbers of T lymphocytes in subepithelial bronchial tissue (median 1174 vs 714 cells/mm², p = 0.029), but no changes were seen in BAL fluid. Cell counts in BAL fluid and biopsies were positively correlated with age but were unrelated to each other.

Conclusion The inflammatory response in the CF airway is compartmentalised. In contrast to the neutrophil-dominated inflammation present in the airway lumen, the bronchial mucosa is characterised by the recruitment and accumulation of lymphocytes.

INTRODUCTION

Pulmonary disease is the most significant cause of morbidity and mortality in cystic fibrosis (CF).1 A hallmark of CF lung disease is chronic bacterial infection and the predominance of large numbers of neutrophils in the airway lumen. Neutrophils—which release an array of mediators, oxidants and proteases, including neutrophil elastase—are thus considered to play a major role in ensuing tissue damage and CF disease progression. In contrast to the large number of studies that have used bronchoalveolar lavage (BAL) to focus on the lumen, the CF airway wall has been underinvestigated. Indeed, little is known about the nature of inflammation in the airway wall, particularly that which occurs in the bronchial mucosa.

Studies in asthma have shown poor agreement between the patterns of inflammation in the airway lumen and that occurring in the bronchial mucosa,2 suggesting that BAL and endobronchial biopsy measure dissimilar patterns of inflammation in these distinct compartments. In the few studies that have assessed airway wall inflammation in CF, the predominance of neutrophils seen in BAL fluid was not present in the bronchial wall.3–5 Quantitative assessment of inflammatory cells in the bronchial mucosa has shown an accumulation of lymphocytes, especially at the distal level where intense tissue damage is observed. In contrast, neutrophils appeared to accumulate preferentially in the surface epithelium, suggesting migration of these cells towards the airway lumen.4 As these studies have used end-stage CF lung tissue obtained at autopsy or transplantation, data on the pattern of inflammation in the bronchial mucosa in earlier stages of CF lung disease are lacking.

Airway disease is present early in life in CF even in asymptomatic infants. Infection and inflammation can be detected in the BAL fluid of infants with CF even at a few weeks of age.5 Structural airway wall changes, referred to as airway remodelling, also begin early in life in CF. CT scanning demonstrates the

Key messages

What is the key question?

Do inflammatory patterns in the airway lumen of children with cystic fibrosis (CF) reflect those in the airway wall, the site ultimately destroyed in end-stage disease?

What is the bottom line?

The inflammatory patterns and responses to infective stimuli in the airway lumen and the airway wall of children with CF are distinct, and thus bronchoalveolar lavage and endobronchial biopsy provide different but complementary information.

Why read on?

This paper provides a detailed description of inflammatory cellular processes in the airways of subjects with CF in early disease stages.

Received 8 June 2011
Accepted 21 September 2011
Published Online First
18 October 2011

1Department of Paediatric Respiratory Medicine, Royal Brompton Hospital, London, UK
2Department of Gene Therapy, National Heart and Lung Institute, Imperial College London, London, UK
3Division of Paediatric Respiratory Medicine, Department of Paediatrics, University Hospital of Bern, Bern, Switzerland

Correspondence to
Nicolas Regamey, Division of Respiratory Medicine, Department of Paediatrics, University Hospital of Bern, 3010 Inselspital, Bern, Switzerland; nicolas.regamey@insel.ch

Additional materials are published online only. To view these files please visit the journal online (http://thorax.bmj.com/ content/67/2.toc).
presence of thickened airway walls, narrowed airway lumina, air trapping and bronchiectasis in young children and infants, and lung function has been shown to be diminished in infants with CF. However, the sequence of inflammation and remodelling events in the disease process is poorly understood. Airway remodelling seems, at least in part, to be unrelated to airway lumen inflammation, but its relationship to the inflammatory infiltrate in the bronchial mucosa has not been explored. Endobronchial studies during early stages of CF lung disease could assess to what extent—if at all—the patterns of inflammation in the airway lumen reflect airway wall changes, shed light on pathophysiological processes occurring in the airway wall and possibly allow assessment of longitudinal changes.

Based on the findings in asthma which show that differing yet complementary information is gleaned from BAL and endobronchial biopsy, we hypothesised that the CF airway inflammatory processes would be compartmentalised and that the luminal patterns of inflammation would not reflect changes in the bronchial mucosa. We tested this hypothesis using BAL fluid and endobronchial biopsies obtained from children with CF and disease-free controls.

METHODS

Subjects

Subjects were prospectively recruited between March 2003 and June 2007 from children with CF diagnosed using standard criteria who were undergoing bronchoscopy for a clinical indication and control children who had been referred to a tertiary centre, but in whom no lower airway disease was found upon investigations that included clinically indicated flexible bronchoscopy. Specifically, inclusion criteria for control children were: (1) no prior history of lower airway disease; (2) no medication taken; (3) no macroscopic signs of inflammation seen on bronchoscopy (ie, no abnormal secretion, mucosal oedema or redness); (4) no viral detection or growth of pathogenic organisms seen on bronchoscopy (ie, no abnormal secretion, mucosal oedema or redness); (5) no prior history of lower airway disease; (2) no medication taken; (3) no macroscopic signs of inflammation seen on bronchoscopy (ie, no abnormal secretion, mucosal oedema or redness); (4) no viral detection or growth of pathogens seen on bronchoscopy (ie, no abnormal secretion, mucosal oedema or redness); (5) no prior history of lower airway disease; (2) no medication taken; (3) no macroscopic signs of inflammation seen on bronchoscopy (ie, no abnormal secretion, mucosal oedema or redness); (4) no viral detection or growth of pathogens seen on bronchoscopy (ie, no abnormal secretion, mucosal oedema or redness); (5) BAL fluid with normal differential cell counts (ie, >80% macrophages). One control was a child undergoing cardiac catheterisation where informed consent was given to perform bronchoscopy for research reasons. Exclusion criteria for both subject groups were bleeding tendency or treatment with anticoagulants and preterm birth before 36 weeks of gestation. Data from some of the children included in the present study have been previously reported. The study was approved by the Royal Brompton Harefield and NHLI ethics committee. Informed consent and age-appropriate assent was obtained from parents and children respectively.

Flexible bronchoscopy

Bronchoscopy was performed as previously described under general anaesthesia. BAL was performed using three aliquots of 1 ml/kg 0.9% saline at room temperature instilled separately into one or two lobes (usually the right middle lobe and the clinically most affected lobe), and the returns pooled. Up to five endobronchial biopsies (median 3, range 1–5) were taken from a standardised site (subsegmental bronchi of the right lower lobe) and processed into paraffin wax blocks as described previously. In order to be included in the study, each child was a priori required to have at least one evaluable biopsy with at least 0.1 mm² of subepithelial tissue.

BAL fluid analysis

BAL fluid was processed as described previously. Viable cells were counted and cell differentials were assessed on cytospin preparations using May-Grunewald-Giemsa staining. Bacterial and fungal growth was assessed and viruses (respiratory syncytial virus, parainfluenza 1, 2 and 3, influenza A and B, adenovirus and cytomegalovirus) were sought using direct immunofluorescence and/or rapid viral tissue culture.

Immuno-histochemistry

Endobronchial biopsies were immunostained for neutrophils (neutrophil elastase), T lymphocytes (CD3) and B lymphocytes (CD20) lymphocytes, macrophages (CD68), eosinophils (EG2) and mast cells (tryptase). Area profile counts of immunopositive cells in subepithelial tissue were performed by one of two investigators (NR and LT) blinded to the study groups.

Tissue morphometry

Morphometry was performed on 3 μm thick haematoxylin and eosin-stained biopsy sections as described previously. Reticular basement membrane (RBM) thickness was measured on coded sections by taking the geometric mean of 40 measurements at 20 μm intervals. The airway smooth muscle (ASM) volume fraction was measured, applying point and line intersection counting using previously recommended and validated methods of design-based stereology.

Power calculation

Biopsy data from a study in atopic adults indicate that a sample size between 13 and 48 subjects is needed to detect at least one doubling difference in cell number per 0.1 mm² for a particular inflammatory cell type in a study using a parallel design such as ours, with α=0.05 and power of 0.80.

Statistical analysis

Categorical data were examined using a χ² test. For normally distributed data, shown as mean (SD), between-group comparisons were performed with Student t tests and associations tested by Pearson correlation. For non-normally distributed data, shown as median (range or IQR), between-group comparisons were performed with Mann–Whitney U-tests and associations tested by Spearman rank correlation. Multivariable linear regression models were used to adjust for possible confounders. Additional details are provided in the online supplement.

RESULTS

Patients

The study included 62 children of median age 7.3 years (range 0.2–16.8). Forty-six children had CF and underwent bronchoscopy for a clinical indication, and there were 16 controls without lower airway disease (table 1).

Neutrophilic inflammation in BAL fluid

Inflammatory cell counts in BAL fluid were available in 33 (72%) of the children with CF and in 10 (63%) controls, and cell differentials were available in 35 (76%) of the CF children and in 12 (75%) controls.

BAL fluid from children with CF contained more inflammatory cells and more of every cell type compared with controls (table 2). There was a predominance of neutrophils and macrophages, with moderate numbers of lymphocytes (figure 1A). In contrast, most of the inflammatory cells in control BAL fluid were macrophages. Mast cells were rarely seen in either CF or control BAL fluid (data not shown).

Biopsy analysis

A total of 181 biopsies were taken from the 62 children; 121 (67%) of these biopsies were considered evaluable, according to...
Pathogens in BAL fluid

- **Viruses:**
  - Parainfluenza virus 3 (n = 2; 4%)
  - Bacteria:
    - Pseudomonas aeruginosa (n = 11; 24%)
    - Other (n = 8; 18%)
- **Fungi:**
  - Aspergillus fumigatus (n = 12; 28%)
  - Candida albicans (n = 4; 9%)

**Indication for bronchoscopy (n)**

- Microbiological surveillance at the time of chest exacerbation (n = 23)
- Routine microbiological surveillance at the time of new CF diagnosis; child well (n = 8) or during chest exacerbation (n = 7)
- Microbiological surveillance at the time of line or gastrostomy insertion or removal; child well (n = 2) or during chest exacerbation (n = 6)

<table>
<thead>
<tr>
<th>Pathogens in BAL fluid</th>
<th>Cystic fibrosis (CF) [n = 46]</th>
<th>Controls (n = 16)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Viruses:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
<td>2 (n = 2; 4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacteria:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>11 (n = 24%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>8 (18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fungi:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>12 (28%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida albicans</td>
<td>4 (9%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cystic fibrosis**

<table>
<thead>
<tr>
<th>Subject characteristics (n = 62)</th>
<th>Cystic fibrosis (CF) [n = 46]</th>
<th>Controls (n = 16)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex, F:M</strong></td>
<td>27:19</td>
<td>10:6</td>
<td>0.789</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>7.8 (0.2–16.8)</td>
<td>6.3 (0.2–16.4)</td>
<td>0.334</td>
</tr>
<tr>
<td><strong>FEV1, % predicted</strong></td>
<td>53 (29–84)</td>
<td>98 (61–131)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>FVC, % predicted</strong></td>
<td>74 (32–89)</td>
<td>93 (85–137)</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>CFTR genotype (n, %)</strong></td>
<td>F508del/F508del (n = 27, 59%);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F508del/F504X (n = 2, 4%);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F508del/1717-1G&gt;A (n = 2, 4%);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F508del/other (n = 6, 13%);</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>or other (n = 9, 20%).</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Relationship of airway inflammation with age, pulmonary function tests and CFTR genotype**

Within the CF group, the number of BAL inflammatory cells (r = 0.68, p < 0.0001), neutrophils (r = 0.64, p < 0.0001), macrophages (r = 0.44, p = 0.011) and eosinophils (r = 0.64, p < 0.0001) correlated positively with age. In contrast, in the bronchial mucosa, only macrophage counts were positively related to age (r = 0.52, p = 0.042).

In the 29 children old enough to perform reliable spirometry, inflammatory cell counts in BAL fluid or biopsies were not related to any test value of pulmonary function (forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), or FEV1/FVC). There was no relationship between CFTR genotype and any inflammatory cell counts in BAL fluid or biopsies.

**Different patterns of inflammation during chest exacerbations and phases of clinical stability in BAL fluid and biopsies**

Bronchoscopic sampling had been performed during chest exacerbations—defined in terms of the decision to hospitalise and treat with intravenous antibiotics—in 36 (78%) of the children with CF. The other 10 children underwent the procedure while well and did not require intravenous antibiotics either before or after the procedure. All inflammatory cell types in BAL fluid were higher in children investigated during chest exacerbations than in those who were well (table 3). These differences remained significant after adjustment for age for all cell types except for eosinophils. In contrast, in bronchial subepithelial tissue, only T lymphocytes and macrophages were significantly higher during chest exacerbations compared with those who were well. These differences remained significant after adjustment for age.
Pathogenic organisms: distinct associations with luminal and mucosal inflammation

Pathogenic organisms were identified in 26 (56%) of the 46 children with CF (table 1). The most frequent pathogens were Aspergillus spp (n=13) and Pseudomonas aeruginosa (n=11). In one child both Aspergillus spp and P. aeruginosa were identified.

The presence of Aspergillus spp was associated with higher numbers of BAL fluid inflammatory cells (median (IQR) 2898 (1577–6737) vs 1098 (670–2193) ×10^3/ml, p=0.014) and neutrophils (median (IQR) 1198 (861–2087) vs 918 (720–1423) cells/mm², p=0.056) and these were of the T lymphocyte subset (median (IQR) 1606 (861–2087) vs 918 (720–1423) cells/mm², p=0.056 and these were of the T lymphocyte subset (median (IQR) 1174 (812–1956) vs 714 (575–1192) cells/mm², p=0.029). These differences remained significant after adjustment for age. In contrast, no differences were seen in BAL fluid.

Inflammatory cell counts in BAL fluid or biopsies were unrelated to the presence of other pathogens.

Relationship of airway inflammation and markers of airway remodelling

As we have previously reported in a smaller CF group including some of these cases,15 RBM was thicker in children with CF than in controls (mean (SD) 4.8 (1.3) μm vs 3.9 (1.0) μm, p=0.008; figure E1 in online supplement). Within the CF group, RBM thickness was related to age (r=0.62, p<0.0001; figure E2 in online supplement), as shown in children without pulmonary disease.27 After adjustment for age in a multivariable analysis, inflammatory cell counts in BAL fluid or biopsies were unrelated to RBM thickness (data not shown).

As also reported in a smaller group including some of these cases,15 the volume fraction of ASM in bronchial tissue was larger in children with CF compared with controls (median (IQR) 0.11 (0.06–0.17) vs 0.06 (0.02–0.08), p=0.008; figure E4 in online supplement). Inflammatory cell counts in BAL fluid or biopsies were unrelated to the ASM volume fraction (data not shown).

DISCUSSION

Because of ease of access, most of the studies on inflammation and airway remodelling in CF have been performed on BAL fluid.28 29 In contrast, the airway wall—the site of the destructive changes—has thus far been underinvestigated. This has been mainly due to difficulties in obtaining adequate material and also possibly to an assumption that the patterns of inflammation in the CF lumen provide a complete and reliable picture of those occurring in the airway wall per se. Recent advances in bronchoscopic techniques have allowed safe investigation of inflammatory and structural changes to the airway wall in CF, even in young children and infants.30 31 In this study we used both BAL fluid and endobronchial biopsy to study inflammation in the airway lumen and the airway wall in children with CF as young as 2 months of age in order to

Table 2  Total and differential inflammatory cell counts in bronchoalveolar lavage (BAL) fluid and in subepithelial bronchial tissue obtained from children with cystic fibrosis (CF) (n=46) and controls (n=16)

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>CF (cells ×10^3 per ml)</th>
<th>Controls (cells/mm²)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total inflammatory cells</td>
<td>1382 (753–2951)</td>
<td>102 (78–190)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>810 (285–1200)</td>
<td>1 (0–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>37 (11–126)</td>
<td>3 (1–5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Macrophages</td>
<td>641 (328–1042)</td>
<td>96 (73–168)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>11 (1–41)</td>
<td>0 (0–1)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biopsies (cells/mm²)</th>
<th>Total inflammatory cells</th>
<th>Neutrophils</th>
<th>T lymphocytes</th>
<th>B lymphocytes</th>
<th>Macrophages</th>
<th>Eosinophils</th>
<th>Mast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF</td>
<td>1773 (1256–2626)</td>
<td>13 (4–26)</td>
<td>786 (648–1364)</td>
<td>125 (52–259)</td>
<td>487 (225–793)</td>
<td>0 (0–0)</td>
<td>40 (14–78)</td>
</tr>
<tr>
<td>Controls</td>
<td>1045 (760–1317)</td>
<td>0 (0–1)</td>
<td>624 (439–788)</td>
<td>62 (28–179)</td>
<td>255 (102–446)</td>
<td>5 (0–12)</td>
<td>52 (26–82)</td>
</tr>
<tr>
<td>p Value</td>
<td>0.008</td>
<td>0.100</td>
<td>0.035</td>
<td>0.100</td>
<td>0.017</td>
<td>0.279</td>
<td>0.641</td>
</tr>
</tbody>
</table>

BAL cell counts and differentials available from 33 children with CF and 10 controls; biopsy cell counts available from 39–42 children with CF and from 13–16 controls, depending on cell type analysed; median (IQR) cell counts and percentage of total inflammatory cells shown.

Figure 1  Representative high power view of a BAL sample obtained from a 10-year-old CF child showing neutrophilic infiltration (A; May–Grünewald Giemsa, original×400) and of an endobronchial biopsy sample obtained from the same child showing dense lymphocytic infiltration with only few neutrophils in subepithelial tissue (B; top image: anti-NE staining for neutrophils; bottom image: anti-CD3 staining for T-lymphocytes, original×400). L, lymphocyte; M, macrophage; N, neutrophils; RBM, reticular basement membrane.
compare the two. We demonstrate that inflammation is present in both compartments early in the course of the disease and that, as hypothesised, the patterns of inflammation in the lumen and bronchial mucosa are different. In contrast to the neutrophil-dominated inflammation in the airway lumen, CF is characterised by a lymphocytic inflammation of the bronchial mucosa. Furthermore, inflammatory associations with periods of exacerbation or CF-specific pathogens also differ at these two sites. These data demonstrate that BAL fluid and endobronchial biopsy provide different but complementary information, and both need to be sampled in order to be able to understand fully the pathophysiological processes in CF.

Our finding of the presence of large numbers of neutrophils in the BAL fluid is as expected and in agreement with multiple previous studies, whereas the scarcity of neutrophils in the bronchial mucosa sampled by biopsy is novel. Our interpretation is that there is likely to be an intensive neutrophil recruitment from the bronchial vasculature followed by rapid migration through the bronchial wall with their accumulation in the airway lumen where they can be identified in high numbers in BAL fluid. Alternatively, neutrophils may enter the airway luminal compartment via tissue migration occurring more distally in the smaller membranous bronchioli. The data of Hubeau et al, however, who investigated the inflammatory infiltrate of the CF bronchial mucosa in transplantation tissue, would argue against this last speculation. The neutrophils identified in their study were particularly numerous at the segmental level compared with the lobar or distal level of the bronchial tree.

Previous studies have described a lymphocytic infiltrate consisting mainly of T lymphocytes in the CF bronchial mucosa in autopsy or explant tissues recovered at end-stage disease. Our findings in biopsy tissue of higher numbers of lymphocytes in the bronchial mucosa of children indicate clearly that their presence is not just a feature of end-stage disease but part of the ongoing inflammatory process of CF. Indeed, enhanced chemotaxis of CF compared with control lymphocytes towards interleukin 8, a predominant chemokine found in BAL fluid of subjects with CF has recently been shown in an in vitro setting. The pathophysiological function of these infiltrating lymphocytes is unclear, but it could be speculated that T lymphocytes, the numbers of which we found to be higher during exacerbations and in the presence of *Pseudomonas*, may be involved in disease progression—for instance, through the induction of matrix metalloproteinase expression, known to be present in BAL fluid of subjects with CF and believed to be involved in airway tissue destruction. In the study by Hubeau et al there were more T lymphocytes, particularly at the distal level of the bronchial tree where intense tissue damage was observed. We are currently investigating further their role in early CF lung disease.

Attention has recently turned to the role of alveolar macrophages in progression of CF lung disease. Brennan et al reported higher absolute macrophage numbers but lower percentages in BAL fluid obtained from preschool children with CF with evidence of bacterial infection compared with uninfected children, suggesting that relatively fewer macrophages present during a pulmonary infection may have a diminished ability to phagocytise the increasing population of dying neutrophils. Our findings of both higher macrophage numbers and percentages in the airway lumen and mucosa during CF exacerbations would argue against this hypothesis. The contribution of these cells to the disease process requires further investigation, but data obtained from CF fetal tissue suggest their possible involvement in the early onset of inflammation in infants with CF.

We observed that chest exacerbations manifest differently in the bronchial lumen (higher numbers of all inflammatory cell types) and in the mucosa (higher numbers of lymphocytes and macrophages, but not of neutrophils). The higher numbers of submucosal lymphocytes during exacerbations are reminiscent of observations made in mild asthma exacerbations, but the lack of higher numbers of submucosal neutrophils contrasts with findings reported in exacerbations of severe asthma and chronic obstructive pulmonary disease, suggesting that mechanisms of exacerbation are disease-specific. It could be speculated that the main source of neutrophil chemotactants in the CF lung derives from the airway lumen (eg, soluble bacterial products, complement fragments or substances produced by airway luminal cellular components) or from the apical part of the airway epithelium whereas, in other chronic airway diseases, neutrophil chemotactants such as

### Table 3

Total and differential inflammatory cell counts in bronchoalveolar lavage (BAL) fluid and in subepithelial bronchial tissue obtained from children with cystic fibrosis (CF) during chest exacerbation or while well (n=36) or while well (n=10).

<table>
<thead>
<tr>
<th></th>
<th>Exacerbation</th>
<th>Well</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BAL (cells ×10⁶ per ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total inflammatory cells</td>
<td>1914 (1098–3199)</td>
<td>512 (119–588)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>970 (287–1584), 51%</td>
<td>222 (21–380), 56%</td>
<td>0.004</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>48 (18–150), 3%</td>
<td>10 (5–21), 3%</td>
<td>0.019</td>
</tr>
<tr>
<td>Macrophages</td>
<td>884 (433–1158), 46%</td>
<td>164 (94–319), 41%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>17 (5–52), 1%</td>
<td>1 (0–3), 0%</td>
<td>0.007*</td>
</tr>
<tr>
<td><strong>Biopsies (cells/mm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total inflammatory cells</td>
<td>1864 (1296–2634)</td>
<td>1263 (742–1549)</td>
<td>0.067</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>14 (5–23), 1%</td>
<td>15 (0–31), 1%</td>
<td>0.964</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>912 (693–1411), 55%</td>
<td>563 (398–963), 59%</td>
<td>0.034</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>126 (66–197), 7%</td>
<td>102 (38–288), 11%</td>
<td>0.931</td>
</tr>
<tr>
<td>Macrophages</td>
<td>570 (318–881), 34%</td>
<td>187 (56–473), 20%</td>
<td>0.005</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0 (0–10), 0%</td>
<td>0 (0–4), 0%</td>
<td>0.215</td>
</tr>
<tr>
<td>Mast cells</td>
<td>45 (21–73), 3%</td>
<td>82 (15–178), 9%</td>
<td>0.379</td>
</tr>
</tbody>
</table>

BAL cell counts and differentials available from 27 children with CF during chest exacerbations and from six children with CF while well; biopsy cell counts available from 29–32 children with CF during chest exacerbations and from 8–10 children with CF while well, depending on cell type analysed; median (IQR) cell counts and percentage of total inflammatory cells shown.

*This significance was lost after adjustment for age (p=0.227).
CXCL8 (interleukin 8) or CXCL5 (ENA-78) originate from the submucosa.\textsuperscript{31, 42} We also found that different organisms had differential associations with luminal and mucosal inflammation. While the presence of \textit{P. aeruginosa} was associated with higher numbers of lymphocytes in the bronchial mucosa, no cell count differences between \textit{Pseudomonas}-positive and \textit{Pseudomonas}-negative patients were seen in BAL fluid, suggesting a role for submucosal lymphocytes in host defence against this pathogen as shown in animal models.\textsuperscript{43, 44} Chronic infection with \textit{P. aeruginosa} has clear detrimental effects on lung function and prognosis, perhaps underscores the role of submucosal lymphocytic inflammation in disease progression. In contrast, we observed higher numbers of neutrophils in BAL fluid in the presence of \textit{Aspergillus} spp, but no cell count differences between \textit{Aspergillus}-positive and \textit{Aspergillus}-negative patients were seen in the biopsies. However, the association in BAL fluid was lost after correction for age, suggesting that the presence of \textit{Aspergillus} spp in BAL fluid is rather a marker of more severe inflammation and advanced or prolonged lung disease (ie, older age) than independently associated with disease progression, as recently proposed.\textsuperscript{45} In our study we could not differentiate between colonisation and infection with pathogens; further work is needed to clarify their relationship with both luminal and mucosal inflammation.

**Study limitations**

We acknowledge that one reason for the lack of correlation of cells in BAL fluid with cells in biopsies could be technical, as endobronchial biopsy and BAL sample different areas of the airways. Endobronchial biopsy samples come from relatively proximal large airways and the airway mucosa of bronchial bifurcations (carinae) and they do not represent the full airway wall thickness. Our data may therefore not be representative of the entirety of the conducting airways, and we may have missed changes occurring in subsegmental airways and therefore associations with, for example, spirometry, even though spirometry is probably too insensitive to detect subtle changes.\textsuperscript{46} Endobronchial biopsies are further limited in size and there is considerable variability between samples of a given individual, so multiple biopsies per subject should ideally be analysed.\textsuperscript{47, 48} Endobronchial biopsy in children is challenging,\textsuperscript{49, 50} and we were only able to analyse 1–2 biopsies of adequate morphology per child. Although our study was powered sufficiently to detect between-group differences,\textsuperscript{24} some relationships within individuals may thus have been missed due to inadequate sampling. The findings of our study need to be interpreted in light of these limitations. However, the strength of our data lies in the relatively large number of biopsies studied and in the inclusion of disease-free controls.

**Conclusions**

Our data show that inflammation is present in both the airway lumen and the bronchial mucosa but that, in support of our hypothesis, the patterns of inflammation and the responses to infective stimuli observed in BAL fluid and biopsies obtained from children with CF are dissimilar. In contrast to the neutrophil-dominated inflammation present in the airway lumen, CF is characterised by a lymphocytic infiltration and accumulation of the bronchial mucosa, the function of which merits further investigation. BAL and endobronchial biopsy provide different but complementary information, underscoring the need to apply both techniques in the study of the pathophysiological processes in the CF airway. Considering that progressive airway wall changes ultimately lead to bronchiec- 

tasis, we suggest that further research should focus on understanding the pathogenetic mechanisms at this tissue site.

**Acknowledgments**

We thank Chloe Dunn, Bernie Ortega, Carmen Lacruz, Eleanor Singh, Gemma Moody and the staff of the Department of Anaesthesia, Royal Brompton Hospital, for their assistance with bronchoscopies; Mark Rosenthal and Ian Balfour-Lynn for performing some of the bronchoscopies; Nikki Comish for excellent technical assistance; and Andrew Nicholson and the Department of Pathology, Royal Brompton Hospital for their preparation of biopsy material. We also gratefully acknowledge the patients and families who agreed to take part in the study.

**Funding**

NR is the recipient of a European Respiratory Society Fellowship (Nr. 64) and a grant from the Swiss National Science Foundation (SSMBS Nr. 1172).

**Competing interests**

None.

**Patient consent**

Obtained.

**Ethics approval**

Ethics approval was provided by Royal Brompton Harefield and NHLI ethics committee.

**Contributors**

Conception and design of the study: NR, TNH, EWFWA, PKJ, AB, JCD. Acquisition of data: NR, LT, TNH, HT, JZ, Y-SQ, AB, JCD. Analysis and interpretation of data: NR, OF, EWFWA, PKJ, AB, JCD. Drafting and revising: NR, OF, PKJ, AB, JCD. Important intellectual content: NR, TNH, EWFWA, PKJ, AB, JCD. Final approval: all authors.

**Provenance and peer review**

Not commissioned; externally peer reviewed.

**REFERENCES**

Cystic fibrosis


Role of kinase suppressor of Ras-1 in Pseudomonas aeruginosa infections

Respiratory infection with Pseudomonas aeruginosa can have serious implications, particularly on a background of immunodeficiency, cystic fibrosis and mechanical ventilation. In this study, by conducting a series of experiments on mice, the authors identified the key role of the kinase suppressor of Ras-1 (Ksr1), an enzymatic protein, in the innate host response to Pseudomonas aeruginosa infection.

Ksr1 deficiency impairs the bactericidal activity of alveolar macrophages and, as a consequence, Ksr1-deficient mice were found to die of sepsis from failed clearance of Pseudomonas. The bactericidal activity of alveolar macrophages and neutrophils is mediated by the formation and release of nitric oxide (NO) and peroxynitrite, which is triggered by Ksr1. This occurs through a previously unidentified pathway where Ksr1 functions as a unique scaffold and mediates the interaction between inducible NO synthase (iNOS) and heat shock protein 90, thereby activating iNOS and releasing NO, which kills the bacteria.

The authors concluded that this study identifies a unique role of Ksr1 in bacterial infection and they have shown a link between Ksr1 and the regulation of bacterial pneumonia and sepsis.

Syed Huq

Correspondence to Dr Syed Huq, ST5 Respiratory Medicine, Liverpool Heart and Chest Hospital, Liverpool, UK; syedhuq@nhs.net

Published Online First 14 May 2011

Distinct patterns of inflammation in the airway lumen and bronchial mucosa of children with cystic fibrosis

Nicolas Regamey, Lemonia Tsartsali, Tom N Hilliard, Oliver Fuchs, Hui-Leng Tan, Jie Zhu, Yu-Sheng Qiu, Eric W F W Alton, Peter K Jeffery, Andrew Bush and Jane C Davies

Thorax 2012 67: 164-170 originally published online October 18, 2011
doi: 10.1136/thoraxjnl-2011-200585

Updated information and services can be found at:
http://thorax.bmj.com/content/67/2/164

These include:

Supplementary Material
Supplementary material can be found at:
http://thorax.bmj.com/content/suppl/2011/10/18/thoraxjnl-2011-200585.DC1

References
This article cites 84 articles, 67 of which you can access for free at:
http://thorax.bmj.com/content/67/2/164#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Inflammation (1020)
Cystic fibrosis (525)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/