Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids

R H Green, C E Brightling, G Woltmann, D Parker, A J Wardlaw, I D Pavord

Background: The debate as to whether asthma is a single or heterogeneous disease remains unresolved although pathological studies, mostly using fiberoptic bronchoscopy on small numbers of subjects, have emphasised the similarities between different clinical phenotypes.

Methods: Lower airway inflammation was assessed non-invasively using induced sputum in 34 normal controls and 259 adults with symptomatic asthma receiving treatment at steps 1–3 of the British Thoracic Society (BTS) guidelines. A subgroup of 49 patients treated with as required β2 agonists only who met BTS criteria for a step up in treatment were studied before and 2 months after treatment with inhaled budesonide 400 µg twice daily.

Results: There was considerable heterogeneity in induced sputum cell counts, particularly in non-atopic patients. A subgroup of 60 patients had a distinctive sputum cell profile with a neutrophil count higher than our normal range (>65.3%) and a normal sputum eosinophil count (<1.9%). These patients were older, predominantly female, and were more likely to be non-atopic but otherwise had similar clinical and physiological features to the group as a whole. Among the 49 subjects studied before and after inhaled budesonide, 11 patients had an isolated sputum neutrophilia. Following treatment, these patients showed significantly less improvement in visual analogue symptom scores (–5.5 v –19.4 mm; mean difference 13.9; 95% CI 0.7 to 27.0), forced expiratory volume in 1 second (FEV1) (–0.08 v 0.13 l; mean difference 0.21; 95% CI 0.03 to 0.39), and concentration of methacholine provoking a fall in FEV1 of 20% or more (PC20) (0.15 v 2.15) than the remaining 38 patients.

Conclusions: These results suggest the presence of a distinct subgroup of patients with mild to moderate asthma who have predominantly neutrophilic lower airway inflammation and who respond less well to treatment with inhaled corticosteroids.
Earlier study no symptom (0 mm) to the worst ever symptom (100 mm). Assessed using a 100 mm visual analogue scales (VAS) from breathlessness, wheeze, or cough and the severity of this was months. These patients identified their predominant symptom day, having nocturnal wakening or limitations in activities, previously.

Scores were repeated 12 hours after the last dose of treatment. Metric tests, methacholine inhalation test, and VAS symptom induction were standard. A subgroup of patients taking as twice daily as the best of three blows over a 14 day period.

The patients then attended for a third visit when the spirometric and sputum evidence of eosinophilic airway inflammation was measured using the tidal breathing method followed, after recovery, by sputum induction and processing as previously described. The duration of inhalation of hypertonic saline was standard. A subgroup of patients taking as required β2 agonists only who met the BTS criteria for a step up in treatment (using rescue β2 agonists more than once per day, having nocturnal waking or limitations in activities, peak flow variability ≥20%, or PEF ≤80% of predicted or best) were given inhaled budesonide 400 µg twice daily for 2 months. These patients identified their predominant symptom (breathlessness, wheeze, or cough) and the severity of this was assessed using a 100 mm visual analogue scales (VAS) from no symptom (0 mm) to the worst ever symptom (100 mm). This scale was the most responsive outcome measure in our earlier study and has been validated.

The patients then attended for a third visit when the spirometric tests, methacholine inhalation test, and VAS symptom scores were repeated 12 hours after the last dose of treatment. Data from some of these patients have been presented previously.

### Analysis of data
Normal ranges were derived from the eosinophil and neutrophil counts of the control subjects as the mean ± 2SD and the mean ± 1.75D using one tailed and two tailed tests, respectively. One tailed tests were used for eosinophil counts since there is no lower reference limit. Spirometric values, induced sputum macrophage, neutrophil, lymphocyte and epithelial differential cell counts and maximum PEF amplitude % mean were described as mean (SE) values. Methacholine PC20 results were log normally distributed and were log transformed and described as geometric mean (log SE) values. Sputum eosinophil counts were expressed as median and interquartile range (IQR). Differences between groups were analysed for normally distributed variables using the independent t test and for variables not observing a normal distribution using the Mann-Whitney U test. The correlation between sputum eosinophils and methacholine PC20, PEF amplitude % mean (A%M), and FEV1, were assessed using the Spearman rank test. Differences in methacholine PC20 were expressed as doubling doses. The χ2 test was used to compare the percentage of patients taking inhaled steroids and the percentage of atopic patients between groups.

### Study design and protocol
Patients and controls attended on two occasions. On the first occasion allergen sensitivity was measured by radioallergosorbent tests for specific IgE or skin prick testing to Dermatophagoides pteronyssinus, cat fur, grass pollen, and Aspergillus fumigatus and atopy was defined as one or more positive skin tests (weal >2 mm larger than negative control) or raised specific IgE (>0.34 kU/l) to one or more antigen. Spirometric tests before and after inhaled salbutamol and chest radiography were performed. Subjects recorded PEF percentage of atopic patients between groups.

### RESULTS
#### All subjects
Normal ranges derived from normal subjects were <65.3% for sputum neutrophil counts and <1.9% for sputum eosinophil counts. 143 patients had intermittent asthma treated with required β2 agonists only (step 1 of the BTS guidelines); 116 had more persistent symptoms requiring regular inhaled corticosteroids (steps 2 and 3) (table 1). Twenty patients (11 steroid naïve, nine atopic) were current smokers and 78 (42 steroid naïve, 26 atopic) were ex-smokers, but all had a history of <10 pack years. Patient details categorised according to atopic status and use of inhaled corticosteroids are shown in table 1. The mean (SE) daily dose of inhaled steroid (in beclomethasone equivalent doses) for atopic and non-atopic subjects was 424 (56) µg and 416 (50) µg, respectively. Non-atopic asthma was associated with less methacholine airway responsiveness (methacholine PC20 <0.68 mg/ml; geometric mean difference 1.0 doubling doses; 95% CI of difference 0.4 to 1.6; p=0.002) and higher mean neutrophil count (54.1% v 45.0%; mean difference 9.1%; 95% CI of difference 2.3 to 15.8; p=0.008).

Sputum evidence of eosinophilic airway inflammation was the most common abnormality in the group as a whole with 135 patients (52%) having an induced sputum eosinophil count outside our normal range (fig 1). The median sputum eosinophil count was significantly lower in atopic subjects receiving inhaled corticosteroids (1.1%) than in similarly treated non-atopic subjects. (3.3%, p<0.05; table 1). Among the whole study population there was no correlation between the sputum eosinophil count and the methacholine PC20 (ρ=0.03, p>0.05), the maximum PEF amplitude % mean (ρ=−0.02, p>0.05), or the % predicted FEV1, (ρ=−0.03, p>0.05). Among the 114 atopic patients a weakly negative

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### Table 1 Patient details and sputum counts in normal controls, atopic and non-atopic subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atopic</th>
<th>Non-atopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>34</td>
<td>114</td>
<td>53</td>
</tr>
<tr>
<td>Male (%)</td>
<td>47</td>
<td>63.8**</td>
<td>34.0</td>
</tr>
<tr>
<td>Age</td>
<td>34 [16]</td>
<td>39 [21]**</td>
<td>38 [21]**</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>16 [3]**</td>
<td>27 [8]**</td>
<td>11 [9]**</td>
</tr>
<tr>
<td>FEV1 (% predicted)</td>
<td>98.5 (5.6)</td>
<td>86.5 (1.4)</td>
<td>80.7 (2.4)</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>85.3 (7.1)</td>
<td>75.1 (0.9)</td>
<td>73.8 (1.7)</td>
</tr>
<tr>
<td>Methacholine PC20 (mg/ml)†</td>
<td>&gt;16</td>
<td>6.0 (0.7)**</td>
<td>6.1 (0.7)**</td>
</tr>
<tr>
<td>PEF amplitude % mean</td>
<td>2.8 (0.5)</td>
<td>2.7 (0.4)</td>
<td>2.1 (0.5)</td>
</tr>
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<td>Squamous cells (%)</td>
<td>5.1 (14.4)</td>
<td>4.4 (13.8)</td>
<td>8.1 (16.6)</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>60.8 (2.8)</td>
<td>61.7 (2.6)</td>
<td>60.2 (2.6)</td>
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<td>Eosinophils (%)‡</td>
<td>3.8 (0.9)</td>
<td>3.2 (0.5)</td>
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<td>Neutrophils (%)</td>
<td>40.4 (14.0)</td>
<td>37.7 (14.8)</td>
<td>45.7 (19.2)</td>
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<td>Lymphocytes (%)</td>
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FEV1=forced expiratory volume in 1 second; FVC=forced vital capacity; PC20=concentration of methacholine provoking a fall in FEV1 of 20% or more; TCC=total cell count.

Values are mean (SE) except for †geometric mean (log SE) and ‡median (IQR)

*p<0.05 atopic v non-atopic subjects; **p<0.01 atopic v non-atopic subjects.
We have analysed the extent and nature of airway inflammation in induced sputum in normal controls and in a large population of well characterised patients with asthma. Our estimates of normal ranges, although derived from small numbers, are very similar to findings in larger populations. In the adults with asthma receiving treatment at BTS stages 1–3 and with relatively normal spirometric parameters, we found considerable heterogeneity in induced sputum inflammatory cell counts. Importantly, a number of predominantly female, non-atopic patients with adult onset asthma had a distinctive sputum inflammatory cell profile consisting of sputum neutrophilia and a normal sputum eosinophil count. Furthermore, a subgroup of steroid naive subjects with this isolated neutrophilic inflammation had an impaired response to treatment with inhaled corticosteroids.

Previous studies have noted sputum evidence of isolated neutrophilic airway inflammation in some patients with severe asthma and in a minority of adults studied during asthma exacerbations. Gibson et al used induced sputum to assess 56 patients with persistent asthma taking high doses of inhaled corticosteroids and found that 59% of patients had suppressed sputum eosinophil counts but evidence of neutrophilic inflammation. Wenzel et al used bronchoscopic techniques to characterise the underlying airway immunopathology of a group of patients with severe refractory asthma who had severely impaired lung function and were treated with high dose inhaled steroids and oral prednisolone and have suggested the presence of a subgroup who have a predominant neutrophilic airway inflammation, absence of eosinophils, and normal basement membrane thickness. It is not clear whether the findings are peculiar to severe asthma or reflect the effects of treatment with high doses of corticosteroids. Our results provide support for the presence of such a distinct asthma phenotype and, for the first time, show that it is a relatively common finding in patients with milder asthma and, in some subjects at least, that it is not an artefact due to corticosteroid treatment. The incidence of neutrophilic inflammation was higher in the population studied by Gibson et al and in the patients with severe asthma studied by Wenzel et al and it remains possible that this phenotype is particularly associated with more severe disease. We have further extended these earlier findings by showing a significantly impaired response to inhaled corticosteroids in a subgroup of

**DISCUSSION**

**Correlation between these two measurements (r=0.22, p<0.05).**

**Subgroup with isolated neutrophilic inflammation**

There was considerable heterogeneity in induced sputum eosinophil and neutrophil cell counts, even among those patients treated with as required β2 agonists alone. A subgroup of 60 patients, including 35 who were steroid naïve, had a distinctive sputum cell profile with a sputum neutrophil count outside the normal range and a normal sputum eosinophil count. Five of these were current smokers and 20 were ex-smokers. These patients were older, tended to develop asthma later, and were more likely to be female and non-atopic than the group as a whole. Clinical and physiological features were otherwise similar (table 2).

**Patients studied before and after treatment with inhaled corticosteroids**

Ninety two of the patients treated with as required β2 agonists only met the BTS criteria for a step up in treatment. Forty nine such patients were randomly selected and agreed to attend again 2 months after treatment with inhaled budesonide 400 µg twice daily. Of these subjects, 11 were included in the subgroup described above, having an isolated sputum neutrophilia with a normal sputum eosinophil count (table 3). Compared with the other 38 patients studied before and after treatment, these subjects had significantly less improvement in VAS scores (−5.5 v –19.4 mm; mean difference 13.9; 95% CI 0.7 to 27.0; p=0.04), FEV1 (−0.08 v 0.13 l; mean difference 0.21; 95% CI 0.03 to 0.39; p=0.026), and PC20 (0.15 v 1.29 doubling doses; mean difference 1.11; 95% CI 0.13 to 2.15; p=0.029; fig 1).
the subjects with an isolated neutrophilia. The poor response to inhaled corticosteroid is not only of obvious clinical significance, but it also provides a possible mechanism by which subjects might be particularly likely to evolve into more severe refractory cases.

We do not have a clear explanation for the development of neutrophilic airway inflammation in these patients. All patients had a smoking history of less than 10 pack years, only patients had a smoking history of less than 10 pack years, only an isolated neutrophilia. The poor response to inhaled corticosteroid is not only of obvious clinical significance, but it also provides a possible mechanism by which subjects might be particularly likely to evolve into more severe refractory cases.

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with inhaled corticosteroids and other treatments are also required to assess the efficacy of these interventions fully. Our findings raise the possibility of a distinct phenotype of asthma, with active neutrophilic and suppressed eosinophilic airway inflammation, across the range of severity of asthma that differs in response to treatment and could have important implications for our understanding and treatment of the disease.

**ACKNOWLEDGEMENTS**

We thank William Monteiro and Richard Ward for help with sputum processing and members of the department of respiratory physiology for performing the sputum inductions. This work was supported by grants from Astra Zeneca, Trent Region and Glenfield Hospital Research fund. Ruth Green is supported by a National Asthma Campaign grant.

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Thorax 2002 57: 875-879
doi: 10.1136/thorax.57.10.875

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