

1 **TITLE PAGE**

2 **Letter to the Editor**

3 **Severe Asthma: differential chemokine response of airway epithelial cells**

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29 **Funding:**

30 C.E.B. obtained support from the Leicester National Institute for Health Research (NIHR)  
31 Respiratory Biomedical Research Unit (BRU), Wellcome Trust, Asthma UK, and GlaxoSmithKline,  
32 which funded this study in part. Bench experiments were performed in C.O.'s laboratory and were  
33 not supported by these sources. C. E. Brightling serves on advisory boards for GlaxoSmithKline,  
34 Astra-Zeneca, MedImmune, Roche, and Aerovance; receives honoraria from Novartis; and receives  
35 research support from GlaxoSmithKline, AstraZeneca, and MedImmune. Since completion of this  
36 research, C O'Callaghan has received research support from Glaxo SmithKline. The rest of the  
37 authors have declared that they have no conflict of interest. The views expressed are those of the  
38 authors and not necessarily those of the NHS (UK), the NIHR or the Department of Health (UK).

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42 **Keywords**

43 Asthma, allergy, cytokine, chemokine, *Streptococcus pneumoniae*, *Dermatophagoides*  
44 *pteronyssinus*, Der p 1

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47 **Capsule summary**

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49 The differential chemokine response of airway basal cells of severe asthma patients to  
50 *Streptococcus pneumoniae* and *Dermatophagoides pteronyssinus* allergen may be of significance  
51 in the context of developing novel immunomodulatory therapeutic strategies for atopic asthma.

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59 To the Editor:

60 Approximately 10-15% of asthmatic adults belong to a group with severe refractory asthma and  
61 suffer from debilitating chronic symptoms, despite optimal standard asthma treatment.<sup>1</sup> Unraveling  
62 the complex pathophysiology of severe asthma has proven to be a major research challenge.<sup>1</sup>  
63 There is growing interest in the role of airway epithelium and its interactions with inhaled  
64 aeroallergens and pathogens, in the pathogenesis of severe asthma.

65

66 In a study of patients with severe asthma and healthy controls, we have recently shown that  
67 profound ciliary dysfunction and marked ultrastructural abnormalities of the airway epithelium are  
68 features of severe asthma.<sup>2</sup> One potential consequence of these abnormalities is prolonged and  
69 more intense exposure of the airway epithelium to inhaled aeroallergens and pathogens. Moreover,  
70 given the marked epithelial disintegrity seen in patients with severe asthma and the ability of the  
71 proteolytically active substances such as the *Dermatophagoides pteronyssinus* allergens to cause  
72 disruption of the intercellular tight junctions, resulting in increased transepithelial permeability,<sup>3</sup> the  
73 airway basal cells could also be exposed to inhaled allergens and pathogens. In this regard, we  
74 studied the effect (in terms of cytokine and chemokine release) of a common respiratory pathogen  
75 (*Streptococcus pneumoniae*) on primary airway basal cells of patients with atopic severe asthma  
76 and compared that to healthy controls. As a positive control, the cytokine and chemokine release in  
77 response to a common inhaled allergen (*Dermatophagoides pteronyssinus* allergen 1 [Der p 1]) by  
78 primary airway basal cells was also studied.

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80 Detailed methodology is given in this article's Online Repository. Briefly, we studied 8 subjects with  
81 severe asthma and 6 healthy controls. Subjects with severe asthma met the American Thoracic  
82 Society criteria for refractory asthma,<sup>1</sup> were current non smokers and had a smoking history of less  
83 than 10 pack years. Healthy controls were non smokers, had no history of respiratory disease and  
84 had normal lung function and PC<sub>20</sub>. Demographics and clinical detail were collected. All subjects  
85 underwent flexible bronchoscopy and using epithelial brushings taken from the bronchus  
86 intermedius, confluent monolayers of basal cell cultures were developed. The basal cells were

87 incubated with wild type *Streptococcus pneumoniae* (strain D39) at concentrations of  $10^6$  cfu/ml and  
88  $10^7$  cfu/ml for up to 4 hours at 37°C. For the control, basal cells were incubated with 400µl bronchial  
89 epithelial base medium (BEBM) (Clonetics, UK). The supernatants were harvested at one hour and  
90 four hours after incubation and stored at -70°C. Similarly, confluent monolayers of basal cells were  
91 incubated with LoTox™ Natural Der p 1 (Indoor Biotechnologies) at concentrations of 1 µg/ml and 5  
92 µg/ml for up to 24 hours. The supernatants were harvested at eight hours and 24 hours after  
93 incubation and stored at -70°C. Chemokines and cytokines in the supernatant were measured using  
94 a 96-well multispot assay (Meso Scale Discovery [MSD], Maryland, USA) using a high band  
95 MS6000 10 spot plate, using SECTOR Imager 6000 (MSD, Maryland, USA) according to the  
96 manufacturer's instructions. The lower limit of detection was 1 pg/ml.

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98 The baseline characteristics of the subjects and the data on chemokine and cytokine release in  
99 response to *Streptococcus pneumoniae* and Der p 1, are given in the online repository tables E1 –  
100 E5. The release of cytokines and chemokines by airway basal cells of patients with severe asthma  
101 and healthy controls in response to *Streptococcus pneumoniae* and Der p 1 was time and dose  
102 dependent. The magnitude of release of chemokines CXCL8 (IL8), CCL11 (Eotaxin) and CCL26  
103 Eotaxin\_3) in response to *S pneumoniae* by basal cells from healthy controls, was significantly  
104 higher ( $p<0.05$ ), compared to that from severe asthma patients (see Figure 1). In contrast, the  
105 magnitude of release of chemokines CXCL8 (IL8), CCL11 (Eotaxin), CCL26 (Eotaxin\_3) (see  
106 Figure 2); as well as CCL4 (MIP 1b), CCL5 (RANTES), CCL13 (MCP 4), CCL17 (TARC) and  
107 CCL22 (MDC) in response to Der p 1 by basal cells from patients with severe asthma, was  
108 significantly higher ( $p<0.05$ ) compared to that from healthy controls. We observed a similar  
109 differential cytokine response (IL6 and IL1b) of basal cells from severe asthma patients and healthy  
110 controls, to Der p 1 and *Streptococcus pneumoniae* (Online repository table E4 & E5, Figure E3).

111

112 In the context of profound ciliary dysfunction and epithelial disintegrity seen in patients with severe  
113 asthma,<sup>2</sup> the differential chemokine response of severe asthma patients' airway basal cells to Der p  
114 1 and *Streptococcus pneumoniae* that we observed in this study is of great interest due to two main

115 reasons. Firstly, asthma has been shown to be an independent risk factor for invasive  
116 pneumococcal disease.<sup>4, 5</sup> It remains to be determined if the reduced CXCL8 release by asthmatic  
117 airway epithelium compared to that of healthy controls leads to a reduction in neutrophil influx and  
118 delayed bacterial clearance, thereby increasing the risk of invasive pneumococcal disease in  
119 patients with severe asthma. Secondly, it has been suggested that in individuals with atopic  
120 sensitization to aeroallergens, there may be an altered mucosal immune response to bacterial  
121 antigens.<sup>6, 7</sup> In recent studies, using a mouse model of allergic asthma, immunomodulatory therapy  
122 with *Streptococcus pneumoniae* vaccine has been shown to attenuate both Th1 and Th2 cytokine  
123 production.<sup>8, 9</sup>

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125 In this study we did not attempt to elucidate the mechanisms underlying the basal cell response to  
126 Der p 1 or *Streptococcus pneumoniae*. It would be of interest to investigate the effect of aberrant  
127 chemokine milieu on epithelial injury-repair mechanisms and whether prior exposure of asthmatic  
128 airway epithelium to *Streptococcus pneumoniae* leads to an attenuated response to Der p 1. As we  
129 used different time points for assessing the epithelial response to *Streptococcus pneumoniae* and  
130 Der p 1, there remains the possibility that alterations in the epithelial response kinetics may be  
131 contributory to the differential response that we showed and this needs further investigation.

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133 In summary, our study shows that airway basal cells of patients with atopic severe asthma and  
134 healthy controls are capable of releasing chemokines and cytokines in response to Der p 1 and  
135 *Streptococcus pneumoniae* in a dose and time dependent manner. Though no major conclusions  
136 may be drawn from this small pilot study, the differential response of the asthmatic epithelium is of  
137 interest and may be further explored in the context of developing novel immunomodulatory  
138 therapeutic strategies for the treatment of allergic airway inflammation.

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206 **Figure legends**

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208 **Figure 1.** Release of CXCL8 (Fig 1 A & B), CCL11 (Fig 1 C & D) and CCL26 (Fig 1 E & F) by  
209 primary respiratory basal cells of patients with severe asthma and healthy controls, in response to  
210 *Streptococcus pneumoniae* (D39) at  $10^6$  cfu/ml and  $10^7$  cfu/ml. A, C & E- CXCL8 response of basal  
211 cells at 1 hour post exposure; B, D & F- CXCL8 response of basal cells at 4 hours post exposure.  
212 Data expressed as median (IQR).

213 †  $p < 0.01$  compared to corresponding values for severe asthma.

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215

216 **Figure 2.** Release of CXCL8 (Fig 1 A & B), CCL11 (Fig 1 C & D) and CCL26 (Fig 1 E & F) by  
217 primary respiratory basal cells of patients with severe asthma and healthy controls, in response to  
218 LoTox Der p 1, 1 $\mu$ g/ml and 5 $\mu$ g/ml. A, C & E- response of basal cells at 8 hours post exposure; B, D  
219 & F- response of basal cells at 24 hours post exposure. Data expressed as median (IQR).

220 †  $p < 0.01$  compared to corresponding values for healthy controls.

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