Letter to the Editor

Severe Asthma: differential chemokine response of airway epithelial cells

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Funding:

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

Keywords

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

Capsule summary

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

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

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

Detailed methodology is given in this article's Online Repository. Briefly, we studied 8 subjects with severe asthma and 6 healthy controls. Subjects with severe asthma met the American Thoracic Society criteria for refractory asthma,¹ were current non smokers and had a smoking history of less than 10 pack years. Healthy controls were non smokers, had no history of respiratory disease and had normal lung function and PC₂₀. Demographics and clinical detail were collected. All subjects underwent flexible bronchoscopy and using epithelial brushings taken from the bronchus intermedius, confluent monolayers of basal cell cultures were developed. The basal cells were
incubated with wild type *Streptococcus pneumoniae* (strain D39) at concentrations of $10^6$ cfu/ml and
$10^7$ cfu/ml for up to 4 hours at 37°C. For the control, basal cells were incubated with 400µl bronchial
epithelial base medium (BEBM) (Clonetics, UK). The supernatants were harvested at one hour and
four hours after incubation and stored at -70°C. Similarly, confluent monolayers of basal cells were
incubated with LoTox™ Natural Der p 1 (Indoor Biotechnologies) at concentrations of 1 µg/ml and 5 µg/ml for up to 24 hours. The supernatants were harvested at eight hours and 24 hours after
incubation and stored at -70°C. Chemokines and cytokines in the supernatant were measured using
a 96-well multispot assay (Meso Scale Discovery [MSD], Maryland, USA) using a high band
MS6000 10 spot plate, using SECTOR Imager 6000 (MSD, Maryland, USA) according to the
manufacturer’s instructions. The lower limit of detection was 1 pg/ml.

The baseline characteristics of the subjects and the data on chemokine and cytokine release in
response to *Streptococcus pneumoniae* and Der p 1, are given in the online repository tables E1 –
E5. The release of cytokines and chemokines by airway basal cells of patients with severe asthma
and healthy controls in response to *Streptococcus pneumoniae* and Der p 1 was time and dose
dependent. The magnitude of release of chemokines CXCL8 (IL8), CCL11 (Eotaxin) and CCL26
Eotaxin_3) in response to *S pneumoniae* by basal cells from healthy controls, was significantly
higher (p<0.05), compared to that from severe asthma patients (see Figure 1). In contrast, the
magnitude of release of chemokines CXCL8 (IL8), CCL11 (Eotaxin), CCL26 (Eotaxin_3) (see
Figure 2); as well as CCL4 (MIP 1b), CCL5 (RANTES), CCL13 (MCP 4), CCL17 (TARC) and
CCL22 (MDC) in response to Der p 1 by basal cells from patients with severe asthma, was
significantly higher (p<0.05) compared to that from healthy controls. We observed a similar
differential cytokine response (IL6 and IL1b) of basal cells from severe asthma patients and healthy
controls, to Der p 1 and *Streptococcus pneumoniae* (Online repository table E4 & E5, Figure E3).

In the context of profound ciliary dysfunction and epithelial disintegrity seen in patients with severe
asthma, the differential chemokine response of severe asthma patients’ airway basal cells to Der p
1 and *Streptococcus pneumoniae* that we observed in this study is of great interest due to two main
reasons. Firstly, asthma has been shown to be an independent risk factor for invasive pneumococcal disease.\textsuperscript{4,5} It remains to be determined if the reduced CXCL8 release by asthmatic airway epithelium compared to that of healthy controls leads to a reduction in neutrophil influx and delayed bacterial clearance, thereby increasing the risk of invasive pneumococcal disease in patients with severe asthma. Secondly, it has been suggested that in individuals with atopic sensitization to aeroallergens, there may be an altered mucosal immune response to bacterial antigens.\textsuperscript{6,7} In recent studies, using a mouse model of allergic asthma, immunomodulatory therapy with \textit{Streptococcus pneumoniae} vaccine has been shown to attenuate both Th1 and Th2 cytokine production.\textsuperscript{8,9}

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

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

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170 References

171


Figure legends

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

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

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

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