

Asthma during pregnancy alters immune cell profile and airway epithelial chemokine release

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Abstract

Objective Pregnancy can influence the course of maternal asthma, but the mechanisms are presently unknown. The aim of the present study was to access maternal immune cell profiles in the presence and absence of asthma and to determine the effect of pregnancy-derived factors on epithelial cell function.

Methods Cells from the human bronchial epithelial cell line BEAS-2B were treated with plasma from pregnant or nonpregnant asthmatic and nonasthmatic subjects. Cell culture supernatants were collected after 24 h and assayed for IL-6, IL-8, eotaxin, RANTES and sICAM-1 protein using ELISA. Maternal immune cell count and peripheral blood chemotactic response to plasma from pregnant and non-pregnant asthmatic subjects were also assessed.

Results The presence of maternal asthma during pregnancy was associated with increased monocyte and neutrophil numbers, increased BEAS-2B cell production of IL-8 and sICAM-1 ($P < 0.05$) and increased chemotactic capacity relative to pregnant women without asthma.

Conclusion The results of this study suggest that circulating pregnancy-related factors enhance chemotactic mediators in epithelial cells in the presence of asthma. This may be one mechanism that contributes to pregnancy-induced changes in asthma.

Keywords Chemokine · Immune cells · Chemotaxis · Bronchial epithelium · Pregnancy

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Introduction

Changes in the course of maternal asthma have been observed during pregnancy with at least one-third of women experiencing worsening asthma particularly in the last trimester [1, 2]. The “one-third dogma” is likely to be an underestimation as a recent prospective study reported that 55% of women experience at least one asthma exacerbation during pregnancy [3]. The mechanisms that contribute to worsening of asthma with pregnancy are not fully characterised. We have been interested in how asthma during pregnancy and pregnancy-related factors alter maternal inflammatory pathways in the circulation and the airways.

We have previously found that maternal asthma during pregnancy is associated with numerous changes in circulating proteins. A proteomic study of plasma collected during pregnancy in asthmatic and non-asthmatic women has shown that there are pregnancy- and asthma-specific changes in the maternal and foetal protein profile [4].

These circulating, unidentified, pregnancy-specific factors may influence worsening asthma during pregnancy.

More recently we have demonstrated in bronchial smooth muscle that maternal and foetal plasma from patients with pregnancies complicated by asthma upregulates inflammatory pathways by increasing cytokine and chemokine production [5]. In particular, bronchial smooth muscle cell production of RANTES (regulated on activation, normal T cell expressed and secreted), interleukin (IL)-6, IL-8, sICAM-1 and eotaxin increased in response to pregnancy-derived factors [5]. These inflammatory mediators may promote immune cell recruitment, survival and chemotaxis [6–9] and may in part contribute to worsening asthma. The present study aims to extend this research in the airway by examining changes observed in the bronchial epithelium in response to pregnancy-derived factors from asthmatics.

The epithelium is fundamentally disordered in chronic asthma, manifested by increased fragility and an altered phenotype to one that secretes mucus, mediators, cytokines, chemokines and growth factors [10, 11]. For many years, epithelial cells were considered to have the simple role of a barrier participating in mucous secretion and removal of noxious agents by their cilia. More recently these cells were found to have a much broader activity including the release of eicosanoids [10, 12], the degrading of neuropeptides (NEP) by neural endopeptidases [13], participation in the regeneration of normal epithelium, production of cytokines [11, 14] and in addition, they might have an immune function related to their capability to present antigens [15].

IL-8, IL-6 and granulocyte–macrophage colony-stimulating factor (GM-CSF) are produced by human bronchial epithelial cells in response to IL-1 β and tumour necrosis factor- α (TNF- α) in vitro [11]. Current studies by Yoon et al. [16] have shown that epithelial cell line BEAS-2B cells infected with respiratory syncytial virus (RSV) produced high concentrations of IL-6, IL-8 and RANTES in cell culture medium.

During pregnancy, alterations in circulating immune cells and chemotactic capacity may also affect maternal asthma. Sacks et al. [17] and others [18, 19] have reported changes in maternal immune cell profiles during normal pregnancy with increased numbers of circulating neutrophils and monocytes and activation of the innate immune system [17, 20–23]. In non-pregnant asthmatic subjects, increased numbers of monocytes, neutrophils and eosinophils have been reported in the circulation [24, 25] and are known to be associated with asthma severity. We have previously found that monocyte concentrations are increased with maternal asthma [26], suggesting that changes in the maternal innate immune system during

pregnancy may contribute to worsening asthma. Our previous work suggests that the lung may increase chemokine production in the response to pregnancy-derived factors [5] and therefore activate chemotactic pathways. We hypothesize that the changes in maternal immune cell profile, chemotactic capacity and airway synthetic function in the presence of pregnancy-derived factors may influence maternal asthma severity during pregnancy. In the present study, we examined maternal immune cell profiles in whole blood of asthmatic and non-asthmatic pregnant women and then extracted the plasma to examine the effect of pregnancy-derived factors on bronchial epithelial cell synthetic function and immune cell chemotaxis in the presence and absence of asthma. This study is an initial investigation into the mechanisms that may contribute to worsening maternal asthma during pregnancy.

Methods

Assessment of glucocorticoid use

Cumulative, inhaled glucocorticoid dose was calculated for each trimester and summarized as the mean daily dose of beclomethasone dipropionate or equivalent used during the pregnancy, where 1 μ g beclomethasone dipropionate was considered equal to 1 μ g budesonide and to 0.5 μ g fluticasone propionate. Subjects were then grouped into the following categories, based on glucocorticoid dosage: Nil, no glucocorticoid use; Glu, low to high dose glucocorticoid, with daily dose during pregnancy between 400 and 1,500 μ g. Asthmatic women in the Nil group used a β 2 agonist (ventolin) when required.

Assessment of asthma severity

Clinical asthma severity was rated as mild, moderate or severe using the integrated severity score described in the Australian Asthma Management Guidelines which closely approximates the National Heart, Lungs and Blood Institute Guidelines [27]. Measurements of current daytime symptoms, nocturnal and morning symptoms due to asthma, bronchodilator use, forced expiratory volume at 1 s (FEV₁), peak expiratory flow (PEF) and hospitalisations were used to assign the patient a severity rating of mild, moderate or severe. For each category, the patient was assigned the most severe grade that she experienced during her pregnancy. Subjects with mild persistent asthma exhibited any of the following characteristics: FEV₁ greater than 80% predicted, no night-time asthma symptoms, no asthma symptoms on awakening, infrequent

short-acting bronchodilator use, no severe attacks in the past year, and only occasional daytime symptoms (fewer than four times per week). Moderate asthma was defined by symptoms on most days, short-acting β_2 agonist requirements most days, night symptoms up to once per week, FEV₁ greater than 60% and less than 80% predicted or PEF variability less than 25%. In severe asthma, there were daily symptoms, limited physical activity, frequent night-time symptoms (more than once per week) and asthma on awakening, FEV₁ less than 60% predicted or PEF variability greater than 25%. Subjects were managed according to a standard treatment protocol (Asthma Management Guidelines) in a combined antenatal/asthma management clinic. Control subjects had spirometry monitored at 18 weeks gestation, whereas asthmatic women had two to eight visits at the asthma management service, depending on severity of asthma and individual needs. At the first visit, a history of asthma control was recorded, including number of emergency presentations, hospital admissions, and oral glucocorticoid use in the previous 2 years before pregnancy.

Each asthmatic study subject received instruction with an asthma educator at 18 and 30 weeks gestation in asthma control, management skills, and a crisis plan. The asthma education visits allowed for the assessment of drug compliance and asthma stability and control throughout pregnancy [28].

Human bronchial epithelial cell line culture (BEAS-2B)

BEAS-2B, an SV40 adenovirus-transformed human bronchial epithelial cell line, was used in this study. The cells were maintained in 75 cm² flasks at 37°C and 5% CO₂ in RPMI/F12 medium containing 1% antibiotics and 2 mM L-glutamine and 10% foetal calf serum. Medium was replaced every second day, and cells were passaged when >85% confluent by washing with Ca- and Mg-free PBS and dislodging with 0.05% trypsin (Life Technologies, Melbourne, Australia). BEAS-2B cells cultured between passages 40–60 were used for experimentation.

Stimulation of epithelial cells (BEAS-2B) in culture

Cells were seeded at a density of 5×10^5 cells on six-well cluster plates (Costar, Cambridge, MA). When cells were about 85% confluent, they were growth arrested in serum-free medium for 24 h and treated with 20% plasma from pregnant asthmatics, pregnant non-asthmatics, non-pregnant asthmatics and non-pregnant non-asthmatics. Maternal plasma samples were collected as previously described [5]. Cells in plasma-free medium were used as control treatment.

Cytokine measurement

Measurement of RANTES, eotaxin, IL-6, IL-8 and sICAM-1

Cell culture supernatants were assayed for RANTES, eotaxin, IL-6, IL-8 and sICAM-1 using commercial enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN and Amersham Pharmacia Biotech, England, UK). All ELISAs were performed using matched antibody pairs according to the manufacturer's instructions. The sensitivity of the assay was 6.2 pg/ml for eotaxin, 1.6 ng/ml for sICAM-1, 3.1 pg/ml for IL-8, 15.6 pg/ml for RANTES and 4.7 pg/ml for IL-6.

Maternal peripheral blood immune cell count

Venous blood was collected from women in the first trimester (<15 weeks), second trimester (16–24 weeks) and third trimester (>25 weeks) of gestation during their antenatal visit. Blood samples were sent to the Hunter Area Pathology Services (HAPS, Newcastle, Australia) for the determination of the different number of white blood cells including monocytes, lymphocytes, eosinophils, neutrophils and basophils.

Chemotaxis assay

Venous blood was obtained from non-pregnant ($n = 6$) and pregnant ($n = 10$) subjects with or without asthma and used for chemotaxis experiments. Blood samples were collected into lithium-heparin tubes and centrifuged at 1,000 g for 15 min at 4°C to obtain plasma. Plasma samples were pooled together for each group. Blood collected from the pregnant subjects in the third trimester was used. Peripheral blood mononuclear (PBMN) cells were isolated from a healthy non-asthmatic donor with no history of allergy as previously described [29]. PBMN cells were isolated by density-gradient centrifugation using ficoll. PBMN cell chemotaxis was measured by using a 24-well Micro Chemotaxis Transwell (Corning Costar, Cambridge, MA, USA). The cells were resuspended at 1×10^6 /ml RPMI medium then loaded into the upper chamber of the Micro Chemotaxis chamber. The chemoattractant which was the maternal plasma from our study subject group was added to the lower chamber. In separate wells, FMLP (Sigma-Aldrich, St Louis, MO, USA) and medium alone were put in the lower chamber as a positive control and to account for random movement of cells, respectively. The lower and upper chambers were separated by a polycarbonate membrane (5- μ m pore size). The transwell set up was left for 3 h at 37°C in a humidified incubator with 5% CO₂. After the incubation period, the number of PBMN cells that migrated to the lower compartment was

determined by counting the cells with a haemocytometer. PBMN cells counted in plasma were expressed as a percentage of cells that migrated into medium alone.

Statistical analysis

Data on maternal characteristics are expressed as median with inter-quartile range or as mean with standard error of the mean (SEM). Cytokine release from bronchial epithelial cells and maternal immune cell count data are expressed as mean and SEM. If data were normally distributed, a one-way ANOVA with post-hoc *t*-test using Bonferroni correction was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). However, if data were not normally distributed, then Kruskal–Wallis with Dunnett's multiple comparison post test was used.

Results

Maternal characteristics

The details of the subject characteristics are outlined in Table 1. The forced expiratory volume at 1 s (FEV_1),

which is an indicator of lung function, was significantly lower in the asthmatic group using inhaled glucocorticoids compared with the non-asthmatic control (ANOVA, $P < 0.05$). The dose of inhaled glucocorticoid was significantly increased through pregnancy from first to third trimester (Table 1).

The effect of maternal plasma on IL-6, IL-8, eotaxin, s-ICAM-1 and RANTES

To examine the effect of pregnancy-derived factors on BEAS-2B cell synthetic function, plasma from non-pregnant and pregnant women with or without asthma was incubated with BEAS-2B cells in culture for 24 h. The constitutive production of IL-6, IL-8, eotaxin, sICAM-1 and RANTES was detectable in culture supernatant after 24 h without any plasma treatment.

The production of IL-6 was significantly increased by plasma from pregnant non-asthmatics [2,919 pg/ml (2,805–3,200); $P < 0.05$] and from pregnant asthmatics [3,385 pg/ml (2,154–3,880); $P < 0.01$] compared with cells in plasma-free medium [87.7 pg/ml (54.8–120.9)] as seen in Fig. 1a. Plasma from non-pregnant women did not significantly increase IL-6 production relative to the constitutive expression (Fig. 1a). Post-hoc analyses showed a

Table 1 Maternal and neonatal characteristics

	Non-asthmatic (control)	Asthmatics using inhaled glucocorticoid	Asthmatics not using inhaled glucocorticoids
Maternal age (years)	28 (25–31) ($n = 137$)	27 (23–31) ($n = 198$)	25 (21–29) ($n = 163$)
Parity	1 (0–1) ($n = 161$)	1 (0–2) ($n = 197$)	1 (0–2) ($n = 133$)
Gravidity	2 (2–3) ($n = 133$)	2 (1–3) ($n = 197$)	2 (1–3) ($n = 161$)
VC (l)	3.9 ± 0.08 ($n = 51$)	3.7 ± 0.05 ($n = 184$)	3.8 ± 0.04 ($n = 143$)
FEV_1 (l)	3.3 ± 0.07 ($n = 152$)	$3.0 \pm 0.04^*$ ($n = 192$)	3.1 ± 0.4 ($n = 144$)
Inhaled glucocorticoid dose ($\mu\text{g/day}$)	–	1st trimester: 583 ± 42 ($n = 196$)	–
	–	2nd trimester: $763 \pm 46^{\S}$ ($n = 196$)	–
	–	3rd trimester: $920 \pm 43^{\S\S}$ ($n = 195$)	–
With mild asthma (%)	–	17	31
With moderate asthma (%)	–	11	3
With severe asthma (%)	–	29	9
Smokers (%)	2	9	12
Non-smokers (%)	23	32	22
Gestational age at delivery (weeks)	39.4 (38.4–42.2) ($n = 135$)	39.4 (39–40) ($n = 194$)	40 (39–40) ($n = 158$)
Birth weight (g)	$3,509 \pm 58$ ($n = 135$)	$3,465 \pm 42$ ($n = 191$)	$3,482 \pm 43$ ($n = 158$)
Ratio of female:male babies	1:1.1	1:1.1	1:0.9

VC Vital capacity, FEV_1 forced expiratory volume at 1 s

Data are reported as median (inter-quartile range) or as mean $\pm$ standard error of the mean (SEM)

$\S$ Significantly different from 1st to 2nd trimester (ANOVA, $P < 0.01$)

$\S\S$ Significantly different from 1st to 3rd trimester (ANOVA, $P < 0.001$)

* Significantly different between groups (Kruskal–Wallis, $P < 0.01$)

The information presented in this table is pertinent to data presented in Fig. 3a–c

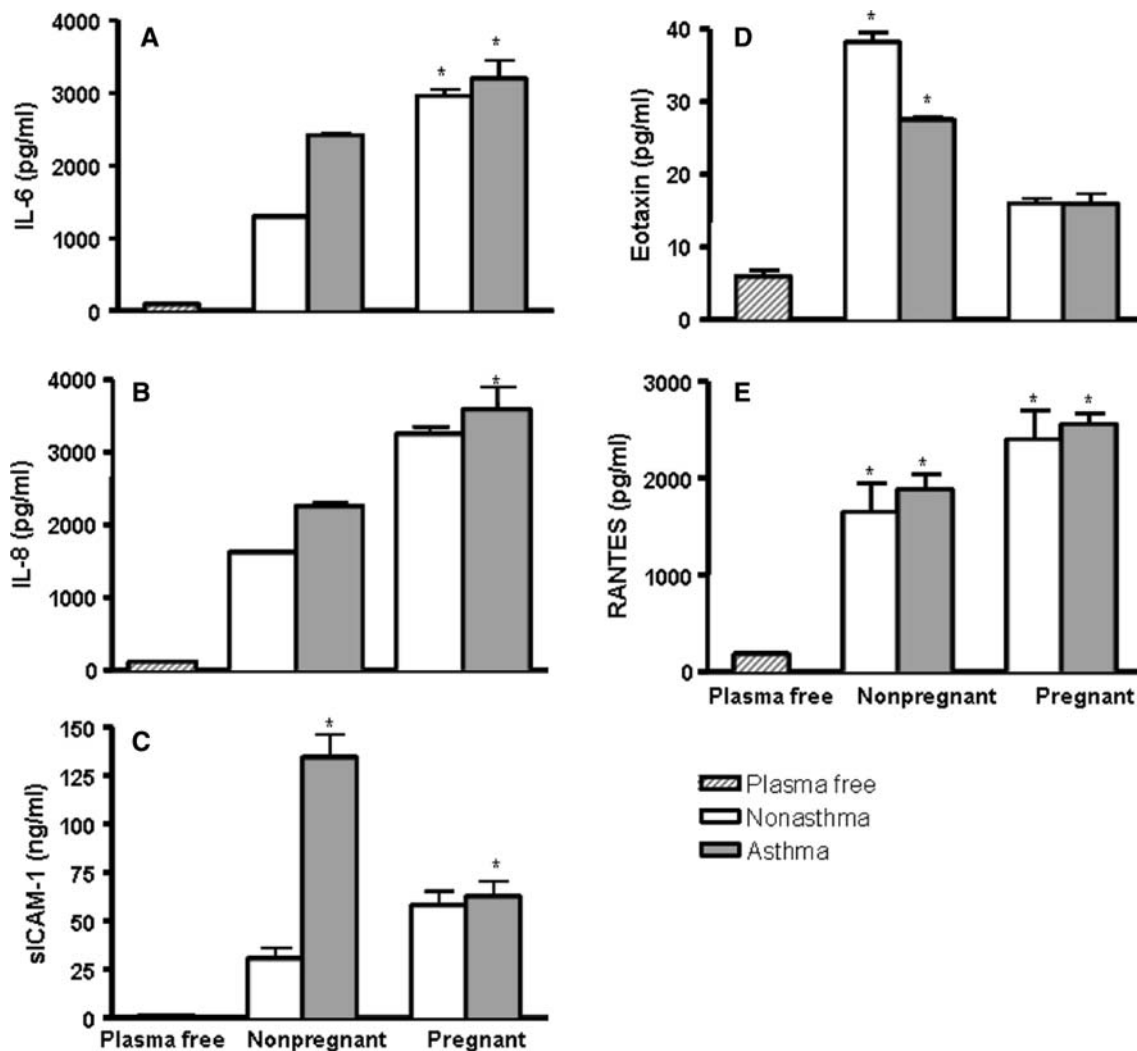


Fig. 1 The release of **a** IL-6, **b** IL-8, **c** sICAM-1, **d** eotaxin and **e** RANTES protein by BEAS-2B cells in plasma-free medium (striped bars, $n = 6$); cells stimulated with plasma from non-pregnant ($n = 3$) and pregnant ($n = 10$) non-asthmatic subjects (open bars); and cells stimulated with plasma from non-pregnant ($n = 3$) and pregnant

($n = 20$) subjects with maternal asthma (shaded bars). This experiment was repeated in two cell lines. Data are expressed as mean $\pm$ standard error of mean from triplicate wells and two experiments. *Significant compared with plasma-free media (ANOVA and Dunnett's multiple comparison test)

significant increase in IL-6 production by plasma from pregnant asthmatics compared with pregnant non-asthmatics ($P < 0.01$). Similarly, in the non-asthmatic group, plasma from pregnant women significantly increased IL-6 production compared with plasma from the non-pregnant group ($P < 0.001$).

IL-8 was also significantly increased by plasma from asthmatic pregnant subjects [3,763 pg/ml (2,268–4,685); $P < 0.05$] relative to the constitutive expression [109 pg/ml (100–109.8)] (Fig. 1b). Plasma from non-asthmatic pregnant and non-pregnant subjects did not significantly increase IL-8 production. Post-hoc analyses identified a significant increase in IL-8 production by plasma from pregnant asthmatics compared with pregnant non-asthmatics ($P < 0.001$). In the non-asthmatic group, plasma from pregnant women

significantly increased IL-8 production compared with plasma from the non-pregnant group ($P < 0.001$).

The production of sICAM-1 (Fig. 1c) was significantly increased in the presence of plasma from both pregnant [52.9 ng/ml (44.9–98.4); $P < 0.05$] and non-pregnant [134 ng/ml (122–146); $P < 0.01$] women with asthma compared with cells in plasma-free medium [0.8 ng/ml (0.2–1.7)]. Interestingly sICAM-1 production was significantly increased in response to the plasma from non-pregnant asthmatics compared with plasma from pregnant asthmatics ($P < 0.05$). Plasma from non-asthmatic pregnant and non-pregnant subjects did not significantly alter sICAM-1 production.

Eotaxin production was increased by plasma from non-pregnant non-asthmatics [38 pg/ml (36.8–39.5); $P < 0.01$]

and asthmatics [27.4 pg/ml (27–27.9); $P < 0.05$] and not significantly altered in the presence of plasma from pregnant subjects (Fig. 1d).

RANTES secretion (Fig. 1e) was significantly increased by plasma from pregnant non-asthmatics [1,609 pg/ml (1,138–2,238); $P < 0.01$] and pregnant asthmatics [1,750 pg/ml (1,496–2,898); $P < 0.001$] in comparison to cells in plasma-free medium [181 pg/ml (166–196.8)]. Similarly, RANTES was increased by treatment with plasma from non-pregnant non-asthmatics [2,398 pg/ml (2,098–2,698); $P < 0.001$] and asthmatics [2,553 pg/ml (2,439–2,667); $P < 0.001$].

Chemotactic response of PBMN cells to plasma from pregnant and non-pregnant of asthmatics and non-asthmatics

PBMN cells from a normal non-pregnant donor were used to examine the chemotactic response to plasma obtained from pregnant and non-pregnant women with or without asthma. The presence of pregnancy-derived factors in plasma of non-asthmatic subjects significantly inhibited the chemotactic response of PBMN cells (Fig. 2) when compared to the other plasma samples (ANOVA $P = 0.006$). Post-hoc t -test identified that the presence of asthma during pregnancy did not significantly reduce the chemotactic response of PBMN cells, and the response was comparable to the non-pregnant samples ($P > 0.05$).

Immune cell profile during pregnancy

Total white blood cells were counted and subdivided into the various cell types identifiable in the blood. Data in this

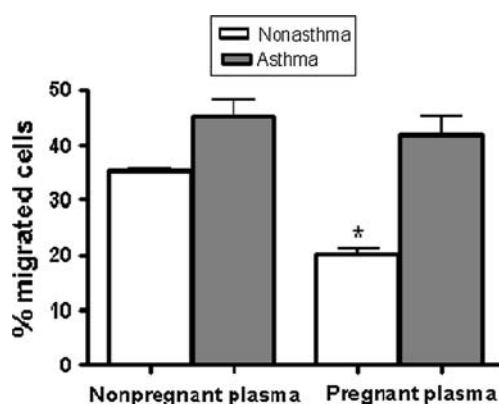


Fig. 2 Chemotactic response of peripheral blood mononuclear cells to plasma from non-pregnant and pregnant women with asthma (shaded bars) and without asthma (open bars). Plasma samples were pooled from at least 5 subjects per group. Data are expressed as mean $\pm$ standard error of the mean of percentage of migrated cells relative to medium alone (* $P = 0.006$, ANOVA). Bonferroni post test, $P < 0.05$, pregnant non-asthma compared with pregnant asthma

section are reported as median number of cells with the inter-quartile range. Monocytes (Fig. 3a) were significantly increased in number from the first trimester [$0.5 (0.4–0.7) \times 10^9/l$] to the third trimester [$0.6 (0.6–0.9) \times 10^9/l$] of pregnancy in asthmatic women using inhaled glucocorticoid (Kruskal–Wallis ANOVA, $P = 0.03$). Within the asthmatic group that were not using inhaled glucocorticoid treatment, there was also an increase in monocytes with pregnancy from first to third trimester [$0.6 (0.4–0.7)$, Kruskal–Wallis $P < 0.001$]. There was also a progressive increase from second to third trimester ($P < 0.01$).

There was a significant increase in neutrophil numbers (Fig. 3b) in control subjects from the first [$6.4 (5.6–7.7) \times 10^9/l$] to third trimester [$7.3 (6.6–9.7) \times 10^9/l$; $P < 0.001$]. Similarly, neutrophil numbers increased in asthmatic glucocorticoid group [$6.2 (5.2–7.3)$ to $7.7 (6.8–9.1) \times 10^9/l$; $P < 0.001$] and no glucocorticoid group [$6.3 (5.2–7.8)$ to $8.0 (6.5–9.9) \times 10^9/l$; $P < 0.01$] throughout pregnancy.

Eosinophil numbers (Fig. 3c) decreased significantly in asthmatic women using inhaled glucocorticoid [$0.3 (0.1–0.4)$ to $0.1 (0.1–0.2) \times 10^9/l$; $P < 0.001$] and those not using inhaled glucocorticoid treatment [$0.2 (0.1–0.2)$ to $0.1 (0.1–0.2) \times 10^9/l$; $P < 0.001$] as pregnancy progressed. Eosinophil numbers were significantly higher in asthmatics than non-asthmatics throughout pregnancy ($P < 0.001$).

No changes were observed in lymphocyte and basophil numbers throughout pregnancy in the asthmatic and non-asthmatic groups (data not shown).

Discussion

In the present study, we have examined the maternal immune cell profile in whole blood and then extracted the plasma to examine the influence of pregnancy-derived factors on chemotactic capacity and secretory function of bronchial epithelial cell line in relation to pregnancy and asthma. The presence of maternal asthma was associated with increased concentrations of eosinophils, monocytes and neutrophils relative to non-asthmatics during pregnancy. Exposure of BEAS-2B cells to plasma from normal pregnancies resulted in an increased production of IL-6 and RANTES suggesting that pregnancy alone can activate synthetic function of cytokines and chemokines in the airway. The production of chemokines from bronchial epithelial cells was further enhanced in the presence of asthma during pregnancy. We then assessed the chemotactic response to pregnancy-derived factors in the presence and absence of asthma and found chemotaxis was suppressed by pregnancy-derived factors of normal pregnancies but enhanced in the presence of asthma and pregnancy. These data suggest that pregnancy may

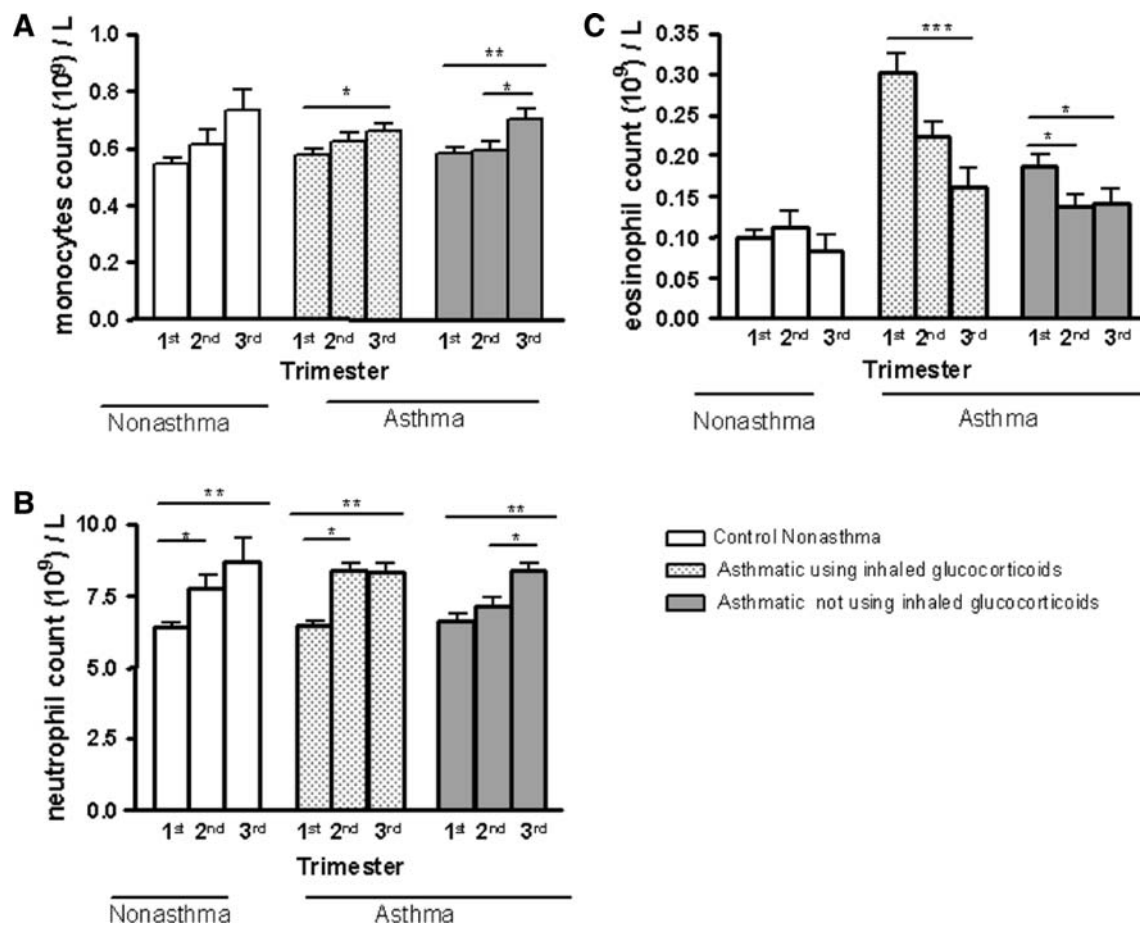


Fig. 3 Circulating maternal **a** monocytes, **b** neutrophils and **c** eosinophils during pregnancy in control non-asthmatic subjects (open bars; 1st trimester $n = 59$, 2nd trimester $n = 23$, 3rd trimester $n = 19$), asthmatic subjects taking inhaled glucocorticoid (dotted bars; 1st trimester $n = 119$, 2nd trimester $n = 56$, 3rd trimester

$n = 59$) and asthmatic subjects using β_2 agonist only (shaded bars; 1st trimester $n = 115$, 2nd trimester $n = 49$, 3rd trimester $n = 56$). Data are expressed as mean $\pm$ standard error of the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between trimesters (Dunnett's multiple comparison test)

promote worsening asthma via enhanced production of systemic inflammatory cells of the innate immune system in combination with increased chemotactic capacity and local production of chemokines from airway cells.

Bronchial epithelial cells in vitro have been shown to produce a number of Th-2-derived cytokines and chemokines in response to a variety of stimuli including allergens, viral particles, air pollutants and proinflammatory cytokines [30–33]. Studies have shown that air pollutants such as ozone, nitrogen dioxide and diesel exhaust particles [31, 32] can increase IL-8, RANTES and sICAM-1 production in human bronchial epithelial cells. In addition bronchial epithelial cells increase IL-6 and IL-8 release in response to RSV [16]. Airway epithelial cells stimulated with IL-1 β or TNF- α [34, 35] or neutrophil elastase [36] produce IL-8. Th2 cells and their derived cytokines and chemokines play an important role in airway inflammation. These not only recruit eosinophils, neutrophils and other immune cell types but also prolong their survival within the tissue [11, 37].

Both exogenous and external stimuli can trigger and activate the bronchial epithelium to release inflammatory factors. Our study is novel in that it suggests that systemic circulating factors during pregnancy may influence airway epithelial function via delivery of inflammatory stimuli from maternal circulation. This can have implications for women with asthma in enhancing the inflammatory process. The inflammation occurring in asthma results in focal or diffuse desquamation of the epithelial lining of the airways [38, 39], vasodilatation and increased capillary permeability of the blood supply to the airways. This leads to increased plasma leakage [38, 40, 41]. The accumulation of enriched plasma in the environment surrounding airways may promote smooth muscle mitogenesis and hyperplasia and cytokine release [42] and perpetuate the inflammatory response in the airways. When stimulated, the BEAS-2B epithelial cell line has a cytokine production profile similar to isolated primary human epithelial cells in vitro [11, 16]. Certainly, a limitation of using BEAS-2B in culture is that

these cells do not reflect the entire *in vivo* micro-environment or function of lung epithelial cells including mucous production, but these cells are still a tool for defining mechanisms such as the influence of human pregnancy on airway cell function, which can then be addressed *in vivo* with relevant animal models.

Chemotactic inflammatory mediators cause infiltration of the airway walls with eosinophils and neutrophils which produce epithelial injury, abnormal neural regulation of airway tone, increased airway smooth muscle responsiveness, and airway obstruction [39, 43]. IL-8 is recognised principally as a neutrophil chemoattractant and activator [44] and has been shown to induce neutrophil adherence and transendothelial migration [45]. IL-8 is also chemotactic for T lymphocytes [46]. In the present study, asthma with pregnancy was associated with increased secretion of IL-8 by epithelial cells and an increased number of circulating neutrophils. In non-pregnant asthmatic subjects, activated neutrophils have been reported in the circulation [20]. We propose that IL-8 and neutrophils may be contributing factors to the pathology of asthma during pregnancy.

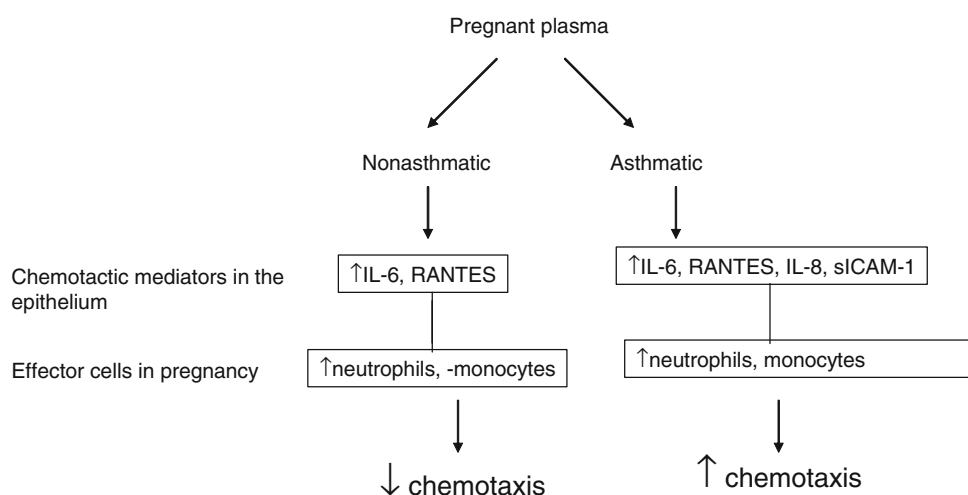
We have previously shown that pregnant plasma from both asthmatic and non-asthmatic women can increase bronchial smooth muscle production of RANTES [5]. This effect was also observed in the bronchial epithelium. Increased levels of RANTES have been reported in bronchoalveolar lavage (BAL) fluid from asthmatic subjects, and the eosinophil chemoattractant activity of BAL fluid of asthmatic subjects was blocked by antibodies to RANTES [47, 48]. Epithelial cells preferentially secrete RANTES through the apical cell surface [38], thereby establishing a gradient which may potentiate chemotaxis across the epithelium in asthmatic women. The increased RANTES secretion during pregnancy may specifically promote monocyte chemotaxis.

While RANTES is a potent chemotactic factor for multiple immune cells, eotaxin triggers eosinophil-selective chemotaxis [6] and also induces release of eosinophils and their progenitors from the bone marrow [49]. Eotaxin immunoreactivity in the bronchial epithelium is increased in patients with asthma, and stimulation of epithelial cells with IL-1 β or TNF- α induces eotaxin release [50]. However, in the current study, there were no changes in the release of eotaxin in epithelial cells by pregnancy-derived factors. Interestingly, the level of circulating eosinophils was significantly elevated in the asthmatic group in the first trimester of pregnancy. In this study, the effects of smoking alone did not affect the number of circulating leukocytes. The highest number of circulating eosinophils was observed in the women who were on inhaled glucocorticoid treatment in the beginning of pregnancy. Pre-pregnancy eosinophil numbers are associated with asthma severity [51]. Therefore, the increased number of eosinophils observed at the beginning of pregnancy may perhaps be explained by the high number of severe asthmatic patients within this cohort. By the late stages of pregnancy, in the asthmatic women that were using inhaled glucocorticoids for treatment, the levels of eosinophils had significantly dropped to that observed in the non-asthmatic group. These data suggest that towards the end of pregnancy, non-eosinophilic pathways may in part induce worsening asthma.

Epithelial cells constitutively express ICAM-1, and surface expression is enhanced with cytokine priming with TNF- α , IFN- γ or IL-1 β [52]. We have found that sICAM-1, which has the capacity to bind to activated immune cells, was greatly increased by plasma from asthmatic women. These data suggest the airway epithelium may have an increased binding capacity for monocytes during pregnancy in women with asthma.

In the present study, we have shown that sICAM-1, IL-8 and RANTES were increased by plasma from asthmatic

Fig. 4 Plasma from pregnant asthmatic women initiates the release of multiple chemotactic mediators in the airway epithelium. In addition, pregnancy complicated by asthma is associated with increased circulating monocytes resulting in possible recruitment and activation of inflammatory and immune effector cells. These alterations may contribute to worsening of asthma during pregnancy in susceptible individuals



women. Increased circulating maternal monocytes and neutrophils and increased production of adhesion molecules and chemotactic factors by the bronchial epithelium with pregnancy may contribute in part to changes in maternal asthma (Fig. 4). The results of this study provide evidence that systemic differences in innate immune cell profiles between asthmatic and non-asthmatic women in combination with alterations in airway epithelial synthetic function and chemotactic capacity may contribute to worsening of asthma during pregnancy in susceptible individuals.

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References

- Schatz M, Harden K, Forsythe A, Chilingar L, Hoffman C, Sperling W, et al. The course of asthma during pregnancy, post partum, and with successive pregnancies: a prospective analysis. *J Allergy Clin Immunol.* 1988;81:509–17.
- Jana N, Vasishtha K, Saha SC, Khunnu B. Effect of bronchial asthma on the course of pregnancy, labour and perinatal outcome. *J Obstet Gynaecol.* 1995;21:227–32.
- Murphy VE, Gibson P, Talbot PI, Clifton VL. Severe asthma exacerbations during pregnancy. *Obstet Gynecol.* 2005;106:1046–54.
- Murphy VE, Johnson RF, Wang YC, Akinsanya K, Gibson PG, Smith R, et al. Proteomic study of plasma proteins in pregnant women with asthma. *Respirology.* 2006;11:41–8.
- Osei-Kumah A, Ammit AJ, Smith R, Ge Q, Clifton VL. Inflammatory mediator release in normal bronchial smooth muscle cells is altered by pregnant maternal and fetal plasma independent of asthma. *Placenta.* 2006;27:847–52.
- Rothenberg ME, Ownbey R, Mehlhop PD, Loiselle PM, van de Rijn M, Bonventre JV, et al. Eotaxin triggers eosinophil-selective chemotaxis and calcium flux via a distinct receptor and induces pulmonary eosinophilia in the presence of interleukin 5 in mice. *Mol Med.* 1996;2:334–48.
- Garcia-Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P, Luster AD. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat Med.* 1996;2:449–56.
- Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol.* 2003;111:450–63. quiz 464.
- Lazaar AL, Albelda SM, Pilewski JM, Brennan B, Pure E, Panettieri RA Jr. T lymphocytes adhere to airway smooth muscle cells via integrins and CD44 and induce smooth muscle cell DNA synthesis. *J Exp Med.* 1994;180:807–16.
- Campbell AM, Chanez P, Vignola AM, Bousquet J, Couret I, Michel FB, et al. Functional characteristics of bronchial epithelium obtained by brushing from asthmatic and normal subjects. *Am Rev Respir Dis.* 1993;147:529–34.
- Cromwell O, Hamid Q, Corrigan CJ, Barkans J, Meng Q, Collins PD, et al. Expression and generation of interleukin-8, IL-6 and granulocyte-macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 beta and tumour necrosis factor-alpha. *Immunology.* 1992;77:330–7.
- Kumlin M, Hamberg M, Granstrom E, Bjorck T, Dahlen B, Matsuda H, et al. 15(S)-hydroxyeicosatetraenoic acid is the major arachidonic acid metabolite in human bronchi: association with airway epithelium. *Arch Biochem Biophys.* 1990;282:254–62.
- Nadel JA. Neurogenic inflammation in airways and its modulation by peptidases. *Ann N Y Acad Sci.* 1992;664:408–14.
- Sousa AR, Poston RN, Lane SJ, Nakhosteen JA, Lee TH. Detection of GM-CSF in asthmatic bronchial epithelium and decrease by inhaled corticosteroids. *Am Rev Respir Dis.* 1993;147:1557–61.
- Rossi GA, Sacco O, Balbi B, Oddera S, Mattioni T, Corte G, et al. Human ciliated bronchial epithelial cells: expression of the HLA-DR antigens and of the HLA-DR alpha gene, modulation of the HLA-DR antigens by gamma-interferon and antigen-presenting function in the mixed leukocyte reaction. *Am J Respir Cell Mol Biol.* 1990;3:431–9.
- Yoon JS, Kim HH, Lee Y, Lee JS. Cytokine induction by respiratory syncytial virus and adenovirus in bronchial epithelial cells. *Pediatr Pulmonol.* 2007;42:277–82.
- Sacks GP, Studena K, Sargent K, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol.* 1998;179:80–6.
- Siegel I, Gleicher N. Peripheral white blood cell alterations in early labor. *Diagn Gynecol Obstet.* 1981;3:123–6.
- Valdimarsson H, Mulholland C, Fridriksdottir V, Coleman DV. A longitudinal study of leucocyte blood counts and lymphocyte responses in pregnancy: a marked early increase of monocyte-lymphocyte ratio. *Clin Exp Immunol.* 1983;53:437–43.
- Borzychowski AM, Croy BA, Chan WL, Redman CW, Sargent IL. Changes in systemic type 1 and type 2 immunity in normal pregnancy and pre-eclampsia may be mediated by natural killer cells. *Eur J Immunol.* 2005;35:3054–63.
- Sacks G, Sargent I, Redman C. Innate immunity in pregnancy. *Immunol Today.* 2000;21:200–1.
- Sacks G, Sargent I, Redman C. An innate view of human pregnancy. *Immunol Today.* 1999;20:114–8.
- Sacks GP, Redman CW, Sargent IL. Monocytes are primed to produce the Th1 type cytokine IL-12 in normal human pregnancy: an intracellular flow cytometric analysis of peripheral blood mononuclear cells. *Clin Exp Immunol.* 2003;131:490–7.
- Mann BS, Chung KF. Blood neutrophil activation markers in severe asthma: lack of inhibition by prednisolone therapy. *Respir Res.* 2006;7:59.
- Silvestri M, Bontempelli M, Giacomelli M, Malerba M, Rossi GA, Di Stefano A, et al. High serum levels of tumour necrosis factor-alpha and interleukin-8 in severe asthma: markers of systemic inflammation? *Clin Exp Allergy.* 2006;36:1373–81.
- Murphy VE, Gibson PG, Giles WB, Zakar T, Smith R, Bisits AM, et al. Maternal asthma is associated with reduced female fetal growth. *Am J Respir Crit Care Med.* 2003;168:1317–23.
- National Institute of Health. Guidelines for the diagnosis and management of asthma. NIH publication no. 97-4051. Bethesda, MD: National Heart, Lung and Blood Institute; 1997.
- Murphy VE, Gibson PG, Talbot PI, Kessell CG, Clifton VL. Asthma self-management skills and the use of asthma education during pregnancy. *Eur Respir J.* 2005;26:435–41.
- Hung CH, Li CY, Hua YM, Chen CJ, Yang KD, Jong YJ. Effects of leukotriene receptor antagonists on monocyte chemotaxis, p38 and cytoplasmic calcium. *Pediatr Allergy Immunol.* 2006;17:250–8.
- Bayram H, Devalia JL, Khair OA, Abdelaziz MM, Sapsford RJ, Sagai M, et al. Comparison of ciliary activity and inflammatory

- mediator release from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients and the effect of diesel exhaust particles in vitro. *J Allergy Clin Immunol.* 1998;102:771–82.
31. Bayram H, Devalia JL, Sapsford RJ, Ohtoshi T, Miyabara Y, Sagai M, et al. The effect of diesel exhaust particles on cell function and release of inflammatory mediators from human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol.* 1998;18:441–8.
 32. Bayram H, Sapsford RJ, Abdelaziz MM, Khair OA. Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro. *J Allergy Clin Immunol.* 2001;107:287–94.
 33. Erzurum SC. Inhibition of tumor necrosis factor alpha for refractory asthma. *N Engl J Med.* 2006;354:754–8.
 34. Kwon OJ, Au BT, Collins PD, Adcock IM, Mak JC, Robbins RR, et al. Tumor necrosis factor-induced interleukin-8 expression in cultured human airway epithelial cells. *Am J Physiol.* 1994;267:L398–405.
 35. Standiford TJ, Kunkel SL, Basha MA, Chensue SW, Lynch JP 3rd, Toews GB, et al. Interleukin-8 gene expression by a pulmonary epithelial cell line. A model for cytokine networks in the lung. *J Clin Invest.* 1990;86:1945–53.
 36. Nakamura H, Yoshimura K, McElvaney NG, Crystal RG. Neutrophil elastase in respiratory epithelial lining fluid of individuals with cystic fibrosis induces interleukin-8 gene expression in a human bronchial epithelial cell line. *J Clin Invest.* 1992;89:1478–84.
 37. Brown JR, Kleimberg J, Marini M, Sun G, Bellini A, Mattoli S. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin Exp Immunol.* 1998;114:137–46.
 38. Holgate ST. Epithelial damage and response. *Clin Exp Allergy.* 2000;30(Suppl 1):37–41.
 39. Holgate ST. Inflammatory and structural changes in the airways of patients with asthma. *Respir Med.* 2000;94(Suppl D):S3–6.
 40. Pueringer RJ, Hunninghake GW. Inflammation and airway reactivity in asthma. *Am J Med.* 1992;92:32S–8S.
 41. Shiels IA, Bowler SD, Taylor SM. Airway smooth muscle proliferation in asthma: the potential of vascular leakage to contribute to pathogenesis. *Med Hypotheses.* 1995;45:37–40.
 42. Sukkar MB, Hughes JM, Johnson PR, Armour CL. GM-CSF production from human airway smooth muscle cells is potentiated by human serum. *Mediat Inflamm.* 2000;9:161–8.
 43. Holgate ST, Lackie PM, Davies DE, Roche WR, Walls AF. The bronchial epithelium as a key regulator of airway inflammation and remodelling in asthma. *Clin Exp Allergy.* 1999;29(Suppl 2):90–5.
 44. Leonard EJ, Yoshimura T. Neutrophil attractant/activation protein-1 (NAP-1 [interleukin-8]). *Am J Respir Cell Mol Biol.* 1990;2:479–86.
 45. Smith WB, Gamble JR, Clark-Lewis I, Vadas MA. Interleukin-8 induces neutrophil transendothelial migration. *Immunology.* 1991;72:65–72.
 46. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science.* 1989;243:1464–6.
 47. Alam R, York J, Boyars M, Stafford S, Grant JA, Lee J, et al. Increased MCP-1, RANTES, and MIP-1alpha in bronchoalveolar lavage fluid of allergic asthmatic patients. *Am J Respir Crit Care Med.* 1996;153:1398–404.
 48. Venge J, Lampinen M, Hakansson L, Rak S, Venge P. Identification of IL-5 and RANTES as the major eosinophil chemoattractants in the asthmatic lung. *J Allergy Clin Immunol.* 1996;97:1110–5.
 49. Palframan RT, Collins PD, Severs NJ, Rothery S, Williams TJ, Rankin SM. Mechanisms of acute eosinophil mobilization from the bone marrow stimulated by interleukin 5: the role of specific adhesion molecules and phosphatidylinositol 3-kinase. *J Exp Med.* 1998;188:1621–32.
 50. Lilly CM, Nakamura H, Kesselman H, Nagler-Anderson C, Asano K, Garcia-Zepeda EA, et al. Expression of eotaxin by human lung epithelial cells: induction by cytokines and inhibition by glucocorticoids. *J Clin Invest.* 1997;99:1767–73.
 51. Sampson AP. The role of eosinophils and neutrophils in inflammation. *Clin Exp Allergy.* 2000;30(Suppl 1):22–7.
 52. Arnold R, Konig W. ICAM-1 expression and low-molecular-weight G-protein activation of human bronchial epithelial cells (A549) infected with RSV. *J Leukoc Biol.* 1996;60:766–71.