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The effects of corticosteroids on cytokine production from asthma lung lymphocytes

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ABSTRACT

Background: Lymphocytes play a central role in the pathophysiology of asthma. Corticosteroids have a limited effect in severe asthma and we hypothesise that lymphocytes play a central role in corticosteroid insensitivity. We investigated the effects of corticosteroids on cytokine production from lung lymphocytes obtained from patients with moderate severe asthma (MSA) compared to mild asthma (MA) and healthy non-smokers (HNS).
Methods: Bronchoalveolar lavage (BAL) cells obtained by bronchoscopy from patients with MSA and MA ($n = 11$ and $n = 14$ respectively) and HNS ($n = 7$) were stimulated with CD2/3/28 beads to activate the lymphocytes, in the presence or absence of dexamethasone (0.01–1 μM). Supernatants were assayed for IL-2, IFNγ, IL-17, IL-13 and IL-10 production.
Results: Dexamethasone caused variable inhibition of cytokines; 1 μM inhibited IL-10 and IL-17 by 50% or lower, while inhibition >50% was observed for IL-2, IL-13 and IFNγ. The effect of dexamethasone on IL-13 production was reduced in MSA.
Conclusion: These findings suggest that the production of specific lymphocyte derived cytokines is poorly suppressed by corticosteroids in MSA, which may be responsible for persistent airway inflammation in these patients

1. Introduction

Asthma is characterised by airway inflammation, bronchial hyperresponsiveness and variable airflow obstruction [1]. Lymphocytes play a central role in the pathophysiology of asthma. Allergic asthma is mediated by CD4 T helper (Th) 2 cells [2] through the secretion of cytokines including interleukin (IL)-4, IL-5 and IL-13; these cause diverse effects including eosinophil recruitment, B-cell IgE synthesis and fibroblast activation [3]. Patients with severe asthma (SA) may have neutrophilic airway inflammation and skew towards a more Th1 type response [4] that is characterised by the production of cytokines such as IFNγ and IL-2. More recently, Th17 lymphocytes have also been implicated in severe asthma [5]; these cells produce IL-17 which stimulates bronchial epithelial cells, smooth muscle cells and fibroblasts to secrete neutrophil chemoattractants such as CXCL8 [6].

Inhaled corticosteroids (ICS) are the mainstay of anti-inflammatory therapy for asthma. However, many patients with moderate to severe asthma have poor control associated with persistent inflammation despite high ICS doses [7,8]. This suggests that ICS exert sub-optimal anti-inflammatory effects on immune cells in the airways of moderate to severe asthma patients. The effects of corticosteroids have been studied using peripheral blood lymphocytes from asthma patients [9,10]. However, we are unaware of studies that have investigated the more relevant issue of the effects of corticosteroids on airway lymphocytes from asthma patients.

We have investigated the effects of corticosteroids on cytokine production from lung lymphocytes obtained from patients with asthma. The aims of this study were to determine whether the effects of corticosteroids are reduced in patients with more severe asthma, and to investigate whether corticosteroids have different effects on the production of Th1, Th2 and Th17 cytokines released from lung lymphocytes.

2. Methods

2.1. Study subjects

We recruited 11 moderate to severe asthma (MSA) patients, 14 mild asthma (MA) patients who were not using ICS and 7 healthy subjects (demographics shown in table I). All subjects were required to be non-smokers. The inclusion criteria for MSA were FEV1 <80% predicted, ICS use >800 mcg beclomethasone equivalent/day, and ACQ score >1. All asthma patients were required to demonstrate reversibility of 200 ml or 12% to salbutamol, or a methacholine PD20 <16 mg/ml. The study was approved by the local South Manchester research ethics

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committee (08/51006/54 & 06/Q1403/156) and all subjects provided written informed consent.

2.2. Cell collection

Bronchoalveolar lavage (BAL) was collected from the upper lobes; the bronchoscope was wedged in the bronchus and a maximum of 4 × 60 ml aliquots of pre-warmed sterile 0.9% NaCl solution were instilled into each lobe. The aspirated fluid was stored on ice before filtration (100 μm filter, Becton Dickenson). The filtrate was centrifuged (400 *g*/10 min at 4 °C) and the cell pellet resuspended in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Viable cell counts were determined by trypan blue exclusion (Neubauer haemocytometer). Total cell count was adjusted to 1×10^6 cells/ml in supplemented RPMI 1640 medium with additional 10% (vol/vol) foetal calf serum and used for cell culture.

2.3. Cell culture

1×10^5 total BAL cells were seeded in a 96 well plate in RPMI-1640 media supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin and 10% FCS (v/v). Cells were incubated with dexamethasone (Sigma Aldrich) (1, 0.1 & 0.01 μM) for 1 h at 37 °C, 5% CO2 prior to the addition of CD2/3/28 activation beads (Miltenyi Biotec) to specifically activate the lymphocytes for 24 h at a ratio of 1 bead to 2 cells. Supernatants were harvested by plate centrifugation 10 min, 400 *g*, and 4 °C and stored at −20 °C prior to ELISA & Luminex analysis.

2.4. Cytokine and chemokine quantification

Release of IL-2 was assayed on cell culture supernatants (either undiluted or up to 1:5 dilution with RPMI containing 10% FCS as required) using R&D systems ELISA duo sets according to manufacturer's instructions (lower level of detection 15.25 pg/ml). The release of IFNγ, IL-13, IL-17, and IL-10 (lower level of detection 0.17 pg/ml) were measured using the Luminex 100 system.

2.5. Data analysis

Stimulated cytokine levels in the absence of drugs were not normally distributed; Friedman Test (nonparametric repeated measures ANOVA) followed by Kruskal Wallis were performed to compare cytokine levels between groups. The percentage inhibition of the stimulated cytokine levels was normally distributed. Maximum inhibition was defined as the effect of the highest concentration of dexamethasone. ANOVA was performed to compare the maximum inhibition caused by, dexamethasone, within each subject group. $p < 0.05$ was considered significant. Analysis was carried out using GraphPad Prism version 5 (GraphPad Software, Inc., San Diego, CA, USA).

3. Results

Patients with MSA were older compared to MA, and had a lower FEV1, greater bronchodilator reversibility and worse ACQ (Table 1).

3.1. Cytokine production

Unstimulated levels of IL-10, IL-13, IL-17, IL-2 and IFNγ from BAL lymphocytes were low, with the majority of samples below the immunoassay detection limits (data not shown). CD2/3/28 stimulation increased cytokine production from BAL lymphocytes from all 3 subject groups (Fig. 1); IL-2 production was greater in MSA compared to healthy subjects and MA ($p = 0.001$ and $p = 0.038$ respectively), while IFNγ production showed the opposite pattern with increased levels in healthy subjects compared to MA and MSA ($p = 0.003$ and 0.06 respectively). IL-10 production was greater in MA ($p = 0.04$ and $p = 0.05$ for MA compared to MSA and healthy subjects respectively). In the case of IL-13 and IL-17 there was no difference in cytokine production between the three groups examined.

3.2. Dexamethasone effects

The inhibitory effect of dexamethasone varied between cytokines (Fig. 2); dexamethasone at the highest concentration (1 μM) inhibited IL-17 by only approximately 50% (51.56% HNS, 49.61% MA & 42.33% MSA) and had no effect on IL-10 production (7.25% HNS, 7.04% MA & 6.01% MSA), while greater than 50% inhibition was achieved for IL-2 (71.33% HNS, 84.5% ± MA & 51.04 MSA), IL-13 (89.61% HNS, 88.88% MA & 67.83% MSA) and IFNγ (61.43% HNS, 43.04% MA & 50.9%). The effect of dexamethasone on IL-13 production was reduced in the MSA group ($p = 0.01$ and $p = 0.008$ compared to HNS and MA respectively at 1 μM). IL-2 and IL-17 also showed trends towards reduced sensitivity to dexamethasone but these were not statistically significant. IFNγ inhibition showed no significant difference between groups.

4. Discussion

Corticosteroids had a reduced effect on IL-13 production from MSA airway lymphocytes. This finding may be a significant contributing factor to persistent airway inflammation in asthma patients who have symptoms despite treatment with ICS. We also observed a marked difference in the effects of corticosteroids between cytokines in both asthma patients and controls; notably, corticosteroid suppression of IL-17 production was limited, with approximately 50% suppression

Table 1
Subject demographics.
Data were normally distributed and are expressed as mean (SD).

	Healthy non-smokers (HNS)	Mild asthma (MA)	Severe asthma (SA)	ANOVA
	n = 7	n = 14	n = 11	p Value
Sex (M/F)	7/0	11/3	6/5	
Age (years)	30.7 (13.6)	37.3 (9.4)	49.5 (11.8)	0.004
FEV_1 (L)	4.2 (0.6)	3.6 (0.9)	1.96 (0.5)	<0.0001
FEV_1 % predicted	98.7 (11.8)	94.3 (13.4)	64.4 (13.0)	<0.0001
FVC (L)	5.3 (0.5)	4.7 (1.1)	3.2 (0.8)	<0.0001
Beclomethasone Equivalent dose (mcg)	0 (0)	0 (0)	1000 (800–2000)	NA
Reversibility (%)	5.17 (3.51)	6.57 (4.65)	24.16 (14.72)	<0.0001
Reversibility (mls)	214.28 (137.34)	221.42 (166.18)	453.81 (297.53)	0.002
ACQ-7 score	NA	0.70 (0.50)	2.08 (0.76)	<0.0001
BAL yield (mls)	226.5 (86.36)	171.2 (92.55)	128.3 (33.67)	0.03
Total cells/ml ($\times 10^6$)	0.05	0.11	0.07	ns