

Rapid communication

Effect of inhaled glucocorticoids on IL-1 β and IL-1 receptor antagonist (IL-1ra) expression in asthmatic bronchial epithelium

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Abstract

Background—Accumulating evidence suggests that the cytokine network is central to the immunopathology of bronchial asthma and the existence of naturally occurring cytokine antagonists has added to this complexity. Upregulation of both interleukin 1 β (IL-1 β) and its naturally occurring receptor antagonist, interleukin 1 receptor antagonist (IL-1ra), has previously been observed on asthmatic bronchial epithelium compared with normal airways.

Methods—The effect of inhaled beclomethasone dipropionate (BDP) on asthmatic bronchial epithelial expression of IL-1 β and IL-1ra was studied. Frozen bronchial biopsy specimens from nine asthmatic subjects receiving 1000 μ g BDP daily for eight weeks and from six asthmatic subjects receiving matching placebo were stained with anti-IL-1 β and anti-IL-1ra antibodies. Hue-saturation-intensity (HSI) colour image analysis was used to quantify the brown immunoperoxidase reaction colour present on the bronchial epithelium.

Results—There was a significant twofold decrease in the epithelial expression of IL-1 β after treatment with BDP but no significant change was seen in IL-1ra ($p=0.175$).

Conclusion—The selective inhibition of IL-1 β , without effect on IL-1ra, provides a novel mechanism for the anti-inflammatory action of glucocorticosteroids.

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Keywords: asthma, bronchial epithelium, interleukin 1 β (IL-1 β), interleukin 1 receptor antagonist (IL-1ra), steroids.

Bronchial epithelial cells are major contributors to the complex network of cytokines such as IL-1, IL-8, IL-6, GM-CSF, RANTES, and MCP-1 that are believed to be important in the immunopathology of bronchial asthma.¹⁻⁴ Recent evidence for the existence of naturally occurring cytokine antagonists has added to this complexity. A naturally occurring receptor antagonist for IL-1 β known as IL-1 receptor antagonist (IL-1ra) has been identified which inhibits IL-1 β activity by binding to cellular IL-1 receptors without activating them.^{5,6}

IL-1 β is produced by many different cell types such as monocytes, tissue macrophages, alveolar macrophages, lymphocytes, mast cells or basophilic cell lines, smooth muscle and endothelium.⁵ It has a wide spectrum of activities but, of particular importance in asthma, is its ability to stimulate granulocytes, B and T lymphocytes, endothelium, epithelium, and haemopoietic cells.⁷

Evidence that IL-1 β contributes directly to the pathology of asthma comes from studies of the intratracheal administration of IL-1 β in Brown Norway rats which resulted in inflammatory changes, including an increased neutrophil count in bronchoalveolar lavage (BAL) fluid and increased airway responsiveness to bradykinin.⁸ Work on alveolar macrophages of asthmatic subjects has shown that IL-1 β expression is upregulated and that the level of IL-1 β in the BAL fluid of subjects with symptomatic asthma is higher than that of normal subjects or subjects with asymptomatic asthma.⁹ In addition, BAL fluid from symptomatic, non-allergic asthmatic subjects has increased levels of IL-1, as well as tumour necrosis factor (TNF) and IL-6.

IL-1ra is produced by a similar range of cell types to IL-1 β —mainly monocytes, macrophages, neutrophils, fibroblasts, and keratinocytes.⁶ It may protect against bronchial asthma as exposure of ovalbumin sensitised guinea pigs to an aerosol of IL-1ra immediately before antigen challenge results in a marked protection against bronchial hyperreactivity and accumulation of pulmonary eosinophils.¹⁰ TNF bioreactivity in the BAL fluid of antigen challenged animals was also significantly reduced in animals exposed to aerosolised IL-1ra before challenge. Pretreatment of guinea pigs with IL-1ra reduced the generation of a late asthmatic response (LAR) in terms of pulmonary resistance and reduced the percentage of hypodense eosinophils in the BAL fluid, although the cellular components remained unchanged.¹¹

In a previous study we have shown that there is an upregulation of both IL-1 β and IL-1ra protein expression on asthmatic bronchial epithelium.⁴ These data, together with data showing upregulation of IL-1ra in various diseases such as idiopathic pulmonary fibrosis (IPF), sarcoidosis and rheumatoid arthritis, suggest

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that antagonism of IL-1 is part of the host's natural defensive response. Disease might result, at least in part, from a failure to produce sufficient amounts of IL-1ra in order to compensate for the increased expression of IL-1 itself.

Corticosteroids are effective in the treatment of bronchial asthma. In this study we have examined the effect of inhaled beclomethasone dipropionate (BDP) on both IL-1 β and IL-1ra protein expression on asthmatic bronchial epithelium in order to establish if the anti-inflammatory effect of steroids is due to a decrease in the IL-1 β /IL-1ra ratio.

Methods

SUBJECTS

To assess the effects of inhaled BDP on bronchial hyperresponsiveness to carbachol, lung function and airways immunopathology, 15 atopic asthmatic subjects participated in a double-blind, placebo controlled trial. They had never taken corticosteroids and were using β_2 stimulants only when necessary. Carbachol airway responsiveness was assessed as previously described.³ Following a two week run in period nine of the asthmatic subjects (three men) of mean age 27 years (range 22–41) inhaled 500 μ g BDP twice daily and six of the asthmatic subjects (four men) of mean age 27 years (range 22–34) inhaled matching placebo for eight weeks. Forced expiratory volume in one second (FEV₁) and airways carbachol responsiveness were measured at the end of the run in and treatment periods.

The mean baseline dose of carbachol provoking a fall in FEV₁ of 20% (PD₂₀ FEV₁) was 0.32 mg in the group on BDP (range 0.10–0.70) and 0.65 mg in the group on placebo (range 0.50–1.00). The mean baseline values for FEV₁ were 3.47 l in the group on BDP (range 2.50–4.45) and 3.52 l for the group

on placebo (range 2.74–4.70). Mean baseline values for % predicted FEV₁ was 94% in the group on BDP (range 71–108) and 88.5% in the group on placebo (range 73–105). There was no significant difference in these baseline values between the two groups.

All individuals were subjected to fiberoptic bronchoscopy as previously described,³ before and after the eight week course of BDP or placebo. Bronchoscopy was performed 24 hours after assessment of carbachol airways responsiveness and after β_2 agonists had been discontinued for 12 hours. Biopsy samples were obtained from the junction of the right upper lobe bronchus and bronchus intermedius, or at the junction of the bronchus intermedius and the right middle lobe. They were immediately snap frozen and mounted in OCT (Miles Inc, Elkhart, USA) to be used later for immunohistochemical examination.

The study was performed with the subjects' informed consent and was approved by the ethics committee of the Augusta Teaching Hospital.

IMMUNOHISTOCHEMISTRY

Double immunohistochemistry was performed by the avidin-biotin complex and the alkaline phosphatase techniques combined as previously described.¹² Either affinity purified rabbit polyclonal anti-IL-1 β (R&D, Minneapolis, Minnesota, USA) in a dilution of 1/200 or rabbit anti-IL-1ra¹³ in a dilution of 1/400 was used, together with the mouse monoclonal antibody EBM11 (Dako, Carpinteria, California, USA), a specific CD68 macrophage marker used in a dilution of 1/100.

The polyclonals were developed with a swine anti-rabbit biotinylated secondary antibody (Dako) 1/200 and diaminobenzidine to give a brown precipitate. The monoclonal antibody was developed by an alkaline phosphatase con-

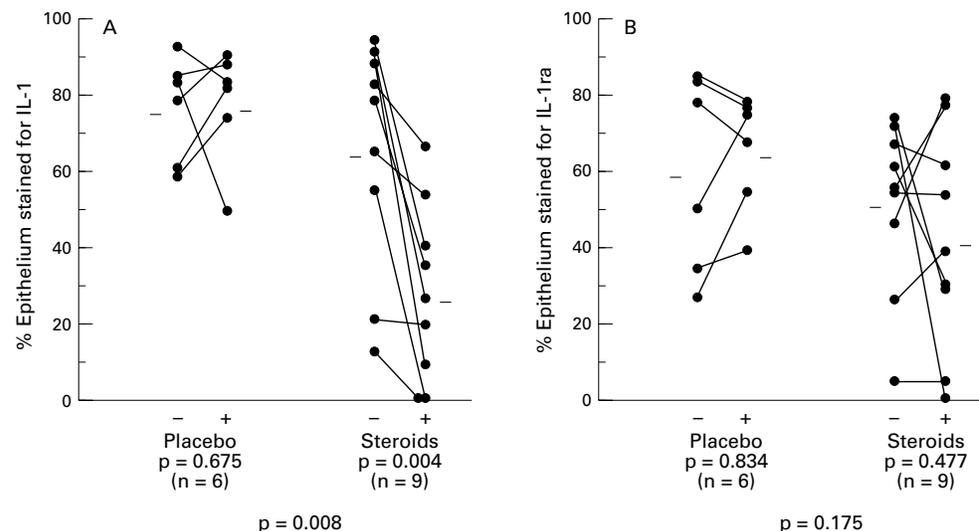


Figure 1 Percentage of bronchial epithelium stained for (A) IL-1 β and (B) IL-1ra with polyclonal antibodies before and after inhalation of 1000 μ g beclomethasone dipropionate (BDP) or placebo for eight weeks in nine and six asthmatic subjects, respectively. Mean values are indicated beside each group. Statistical differences before and after treatment were analysed by the Wilcoxon signed rank test for matched pairs and the differences in the percentage change in cytokine expression between the BDP and placebo groups were analysed by the Mann-Whitney U test.

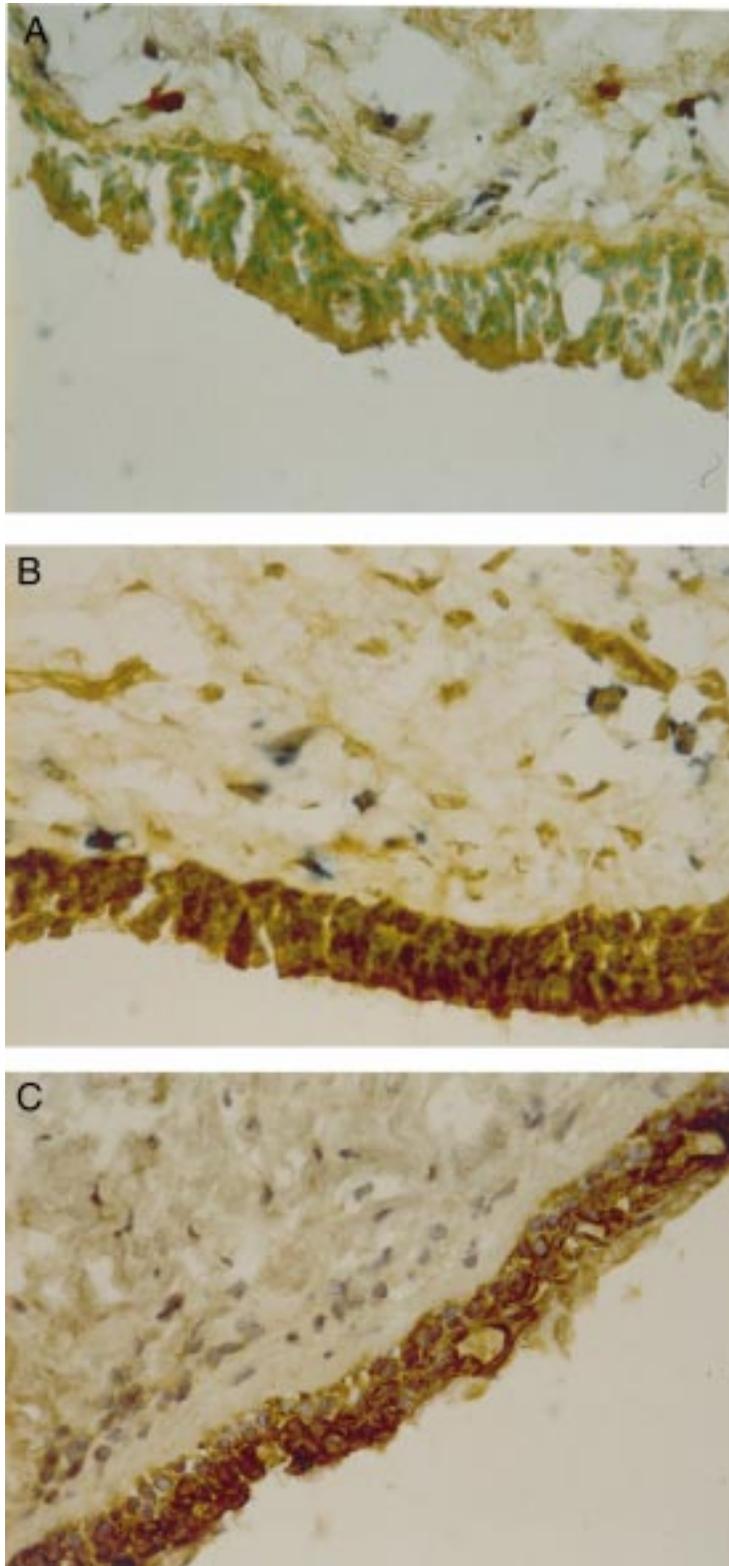


Figure 2 Microphotographs of frozen asthmatic bronchial biopsy specimens stained for (A) IL-1 β , (B) IL-1ra, and (C) CAM 5.2 anti-cytokeratin antibody to localise product in epithelial cells.

jugated goat anti-mouse secondary antibody (Seralab, Crawley Down, Sussex, UK) 1/100 and BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium) to give a blue precipitate. The sections were counterstained in methyl green which stains nuclei green.

IMAGE ANALYSIS

The hue-saturation-intensity (HSI) method of colour image analysis¹⁴ was adopted for detection of the brown immunoperoxidase reaction product as previously described.²³ This was performed using an image analyser consisting of a PC computer containing a DT2871 frame grabber and DT2858 frame processor boards (Data Translation, Massachusetts, USA), with Colour Freelance software (Foster-Findlay Associates, Newcastle on Tyne, UK). This method allows for reliable and reproducible detection of a particular colour, as variation in light intensity across a field or with time is otherwise often a problem in image analysis. The use of HSI has been validated and used in previous studies by our group and others and has proved to be the most reliable method to quantify immunohistochemistry.^{1-4,14} The total epithelial area present on each biopsy specimen was measured.

DATA ANALYSIS

Statistical analysis of the baseline levels of IL-1 β and IL-1ra and of the percentage change in cytokine expression between subjects receiving BDP and those on placebo was performed by the Mann-Whitney U test using Minitab software (Minitab Inc).

Statistical analysis of the levels of IL-1 β and IL-1ra and of the difference in the clinical data before and after treatment with BDP or placebo was performed by the Wilcoxon matched pairs signed rank test, also using Minitab software (Minitab Inc). The results are expressed as mean (SE).

Results

Following placebo administration there was no significant change in the epithelial expression of either IL-1 β or IL-1ra (from 76.51 (5.52)% to 78.25 (6.14)%, $p=0.675$ (fig 1A) and from 59.70 (10.60)% to 65.5 (6.26)%, $p=0.834$ (fig 1B), respectively).

Administration of inhaled corticosteroids for eight weeks led to a significant reduction in the epithelial area stained with IL-1 β (from 65.6 (10.1)% to 28.8 (7.74)%, $p=0.009$ (fig 1A)) but had no effect on the area stained with IL-1ra (from 51.62 (7.51)% to 41.88 (9.53)%, $p=0.477$ (fig 1B)). The reduction in epithelial area stained with IL-1 β was significantly ($p=0.008$) greater after steroid administration than after placebo which was not observed for IL-1ra staining ($p=0.175$).

Staining of the asthmatic bronchial biopsy specimens with CAM 5.2 anti-cytokeratin antibody confirmed localisation of IL-1 β and IL-1ra in the epithelial cells (fig 2). Staining of epithelial macrophages was excluded by using double staining for macrophages.

Clinically, BDP produced a significant increase in the mean PD₂₀ FEV₁ values from 0.32 (0.09) mg to 0.78 (0.27) mg carbachol ($p=0.036$) which was not observed in the group on placebo in which the mean PD₂₀ FEV₁ values changed from 0.65 (0.08) mg carbachol to 0.80 (0.25) mg carbachol ($p=0.893$).

BDP also produced a significant increase in the mean FEV₁ values from 3.47 (0.20) l to 3.72 (0.21) l ($p=0.009$) which was not observed in the group on placebo in whom FEV₁ values changed from 3.52 (0.31) l to 3.43 (0.36) l ($p=1.000$). There was a weak correlation of $r=-0.381$ ($p<0.05$) between FEV₁ values and epithelial expression of IL-1ra. No other relevant significant correlation was found between PD₂₀ FEV₁ or % predicted FEV₁ and epithelial expression of either IL-1 β or IL-1ra. In addition, morphologically there was a significant improvement in the integrity of the bronchial epithelium of the group on steroids.

Discussion

There is increasing interest in the possible role of cytokines in the amplification and modulation of the underlying inflammatory process present in bronchial asthma. The sources of these cytokines, as well as the target cells for their actions, are pleomorphic and include resident cells and recruited inflammatory cells which together are central to the asthmatic process. The accessibility of airway epithelial cells to inhaled substances further suggests that they could respond to exogenous factors and regulate airway function by producing inflammatory cytokines such as IL-1 β .

IL-1 β is synthesised as a larger precursor molecule with a molecular weight of 31 kD. The molecular weight of the mature (active) form is 17.5 kD.⁵ IL-1 β is released into the extracellular space and the circulation. The mechanism of release includes exocytosis from vesicles, active transport by carrier proteins, and cell death. The most active form of IL-1 β is its cleaved mature form. The enzyme involved specifically in cleaving the IL-1 β precursor into its active mature form is known as the IL-1 converting enzyme.

IL-1ra is a 22–25 kD protein^{5,6} that binds to cellular IL-1 receptors without causing cellular activation. Soluble IL-1 receptors can function as naturally occurring IL-1 inhibitors and achieve the same inhibiting result by binding to IL-1 before it can bind to the cell receptors. In previous in vitro work the production of IL-1 and IL-1ra has been shown to be differentially regulated even though the same cell may be synthesising both cytokines. This differential regulation is likely to contribute to the balance of IL-1 and IL-1ra production during diseases such as asthma. Over 100 times excess IL-1ra is needed to inhibit IL-1. This may be explained in part by the observation that only about 5% of IL-1 receptors need to be bound to IL-1 in order to give rise to a biological response.

The present results extend previous data on the inhibition of cytokine products by corticosteroids. These studies show an inhibitory effect of steroids on IL-1 β levels in nasal secretions after allergen challenges, in PHA stimulated and *Der p* I stimulated blood mononuclear cell cultures, on cultured epithelial cells from normal nasal mucosa and nasal polyps, on human monocytes stimulated with lipopolysaccharide (LPS), on asthmatic alveolar

macrophages, and on peripheral blood mononuclear cells of corticosteroid responsive asthmatic subjects. In turn, corticosteroids have been implicated in the upregulation of IL-1ra – for example, they have been shown to induce keratinocyte derived IL-1ra. Prednisolone in the culture medium at physiological concentrations enhanced the release of IL-1 inhibitory activity and suppressed the release of IL-1 by macrophages in the BAL fluid.

Corticosteroids are very effective in the treatment of bronchial asthma. There is previous in vitro and in vivo evidence that corticosteroids downregulate bronchial epithelial expression of cytokines such as GM-CSF, RANTES and IL-8.^{1,2,15} In this study we provide direct in vivo evidence that inhaled corticosteroids reduce the expression of IL-1 β by asthmatic bronchial epithelial cells. Inhaled corticosteroids did not inhibit bronchial epithelial expression of IL-1ra with a net effect of decreasing the IL-1 β /IL-1ra ratio. These data suggest a novel mechanism for the efficacy of inhaled corticosteroids in the treatment of bronchial asthma.

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- 1 Wang JH, Trigg CJ, Devalia JL, Jordans S, Davies RJ. Effect of inhaled beclomethasone dipropionate on expression of proinflammatory cytokines and activated eosinophils in the bronchial epithelium of patients with mild asthma. *J Allergy Clin Immunol* 1994;**93**:1025–34.
- 2 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.
- 3 Sousa AR, Lane SJ, Nakhosteen JA, Yoshimura T, Lee TH, Poston RN. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am J Respir Cell Mol Biol* 1994;**10**:142–7.
- 4 Sousa AR, Lane SJ, Nakhosteen JA, Lee TH, Poston RN. The expression of interleukin 1 beta (IL-1 β) and interleukin 1 receptor antagonist (IL-1ra) on asthmatic bronchial epithelium. *Am J Respir Crit Care Med* 1996;**154**:1061–6.
- 5 Dinarello C, Wolff SM. The role of interleukin-1 in disease. *N Engl J Med* 1993;**328**:106–13.
- 6 Arend WP. Interleukin-1 receptor antagonist. *Adv Immunol* 1993;**54**:167–227.
- 7 Oppenheim JJ, Kovacs EJ, Matsushima K, Durum SK. There is more than one interleukin 1. *Immunol Today* 1986;**7**:45–56.
- 8 Tsukagoshi H, Sakamoto T, Xu W, Barnes PJ, Chung KF. Effect of interleukin-1 β on airway hyperresponsiveness and inflammation in sensitized and nonsensitized Brown-Norway rats. *J Allergy Clin Immunol* 1994;**93**:464–9.
- 9 Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthma airways. *J Allergy Clin Immunol* 1992;**89**:958–67.
- 10 Watson ML, Smith D, Bourne AD, Thompson RC, Westwick J. Cytokines contribute to airway dysfunction in antigen-challenged guinea pigs: inhibition of airway hyperreactivity, pulmonary eosinophil accumulation, and tumour necrosis factor generation by pretreatment with an interleukin-1 receptor antagonist. *Am J Respir Cell Mol Biol* 1993;**8**:365–9.
- 11 Okada S, Inoue H, Yamauchi K, Iijima H, Ohkawara Y, Takishima T, et al. Potential role of interleukin-1 in allergen-induced late asthmatic reactions in guinea pigs: suppressive effect of interleukin receptor antagonist on late asthmatic reaction. *J Allergy Clin Immunol* 1995;**95**:1236–45.
- 12 Nasser SMS, Pfister R, Christie PE, Sousa AR, Barker J, Schmitz-Schumann M, et al. Inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. *Am J Respir Crit Care Med* 1996;**153**:90–6.
- 13 Malyak M, Joslin FG, Verderber EL, Eisenberg SP, Arend WP. IL-1ra ELISA: reduction and alkylation of synovial fluid eliminates interference by IgM rheumatoid factors. *J Immunol Methods* 1991;**140**:281–8.
- 14 Poston RN, Gall NP. Hue-saturation-intensity colour image analysis for the quantitation of immunoperoxidase staining. In: Elder H Y, ed. *Micro 90*. Bristol, Philadelphia, New York: Adam Hilger, 1990: 525–8.
- 15 Kwon OJ, PJ Jose, RA Robbins, TJ Schall, TJ Williams, PJ Barnes. Glucocorticoid inhibition of RANTES expression in human lung epithelial cells. *Am J Respir Cell Mol Biol* 1995;**12**:488–96.