Effect of a leukotriene B₄ receptor antagonist, LY293111, on allergen induced responses in asthma

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Abstract

Background – Leukotriene (LT) B₄ is a potent neutrophil chemoattractant and also stimulates eosinophils in vitro, but its role in asthmatic inflammation is unknown.

Methods – The effect of the novel LTB₄ receptor antagonist, LY293111, was examined using allergen challenge as a model for asthmatic inflammation in 12 atopic asthmatic subjects in a double blind placebo controlled crossover trial. Subjects with an established early (EAR) and late asthmatic response (LAR) to allergen screening received oral LY293111 in a dose of 112 mg three times daily for seven days or placebo before further allergen challenge. Each treatment was separated by a washout period of 28 days. Individuals underwent histamine challenge one hour before and three hours after allergen challenge. Bronchoalveolar lavage (BAL) fluid was obtained at bronchoscopy 24 hours after allergen challenge.

Results – There was no difference in baseline lung function, EAR, LAR, or in airway responsiveness to histamine before and after allergen between placebo and LY293111. By contrast, treatment with LY293111 significantly reduced the number of neutrophils in BAL fluid expressed as both absolute cell numbers and percentage cell differential counts: absolute cell counts, median (range) 0.04 (0.02–0.15) × 10⁶ after LY293111, 0.09 (0.02–0.43) × 10⁶ after placebo; percentage differential cell counts 0.35 (0.1–2.0) after LY293111, 0.80 (0.1–3.6) after placebo (p<0.05). Eosinophils, macrophages, and lymphocytes in BAL fluid did not differ between treatments. There was a significant reduction in the concentration of myeloperoxidase (MPO) with both placebo (16 (6.6) ng/ml) and LY293111 (3.5 (1.8) ng/ml) and of LTB₄ (placebo 4.6 (1.2) pg/ml, LY293111 2.2 (0.2) pg/ml). Concentrations of LTC₄, and interleukin 8 were reduced, although not significantly, whereas concentrations of interleukin 6, GM-CSF, and TNF-α were unchanged by LY293111.

Conclusions – These results demonstrate an influence of LTB₄ on neutrophil influx and activation in the airway following allergen challenge. Despite this anti-inflammatory effect, there was no measured physiological benefit and this questions the functional role of the neutrophil in the pathophysiology of allergen induced asthma.

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Keywords: leukotriene B₄, neutrophil, asthma, myeloperoxidase.

Airway inflammation in asthma is associated with the release of multiple inflammatory mediators including several lipid derived mediators.¹ The leukotrienes (LTs) are a family of molecules that evolve from arachidonic acid metabolism via the 5-lipoxygenase pathway. The cysteinyl leukotrienes C₄, D₄, and E₄ are potent bronchoconstrictors, affect airway responsiveness, increase vascular permeability, and increase mucus production.² By contrast, LTB₄ is a potent chemoattractant and activator of neutrophils without significant effect on airway smooth muscle.³ Inhaled LTB₄ has no significant effect on airway function when given by inhalation to patients with asthma,⁴ although it does increase the number of neutrophils in bronchoalveolar lavage (BAL) fluid.⁵

The late asthmatic response (LAR) to inhaled allergen is associated with airway inflammation and increased airway hyper-responsiveness.⁶ The cellular response is characterised by increased migration of eosinophils to the airway.¹ Allergen induced airway changes are likely to be influenced by complex inflammatory events involving cell–cell interactions through cytokine networks as levels of interleukins IL-1, IL-2, IL-5, IL-6, IL-8, tumour necrosis factor α (TNF-α), and granulocyte-macrophage colony stimulating factor (GM-CSF) are increased in BAL fluid from asthmatic subjects 18 hours after allergen challenge.⁸ The presence of increased numbers of neutrophils in the BAL fluid during the LAR suggests that neutrophils may contribute to this process.⁹ Furthermore, the neutrophil may...
have a role in acute exacerbations of asthma as a significant airway neutrophilia predominates in sudden onset fatal asthma, suggesting that one or more neutrophil chemoattractants have been released. \(^{10}\) LTB\(_4\) may be important as a neutrophil chemoattractant in asthma as increased levels of this mediator are found in BAL fluid from asthmatic patients. \(^{11}\) LTB\(_4\) may also regulate eosinophil numbers and function in asthma as it appears to be involved in eosinophil recruitment during allergen challenge. \(^{12}\)

If a mediator is involved in the pathogenesis of asthma, it should mimic a component of the asthmatic response, it should be detectable in a biological fluid associated with the asthmatic response, and a selective mediator antagonist or synthetase inhibitor should inhibit a component of the asthmatic response. \(^1\) Although LTB\(_4\) fulfils the first two criteria, its potential role in asthmatic inflammation has not yet been defined in the absence of available potent inhibitors. LY293111 sodium is a potent receptor antagonist of LTB\(_4\) in vitro and inhibits neutrophil chemotactic activity, superoxide generation, and CD18/CD11b adhesion molecule expression. \(^{13}\) Preliminary human studies in vivo have shown that LY293111 abrogates the inflammatory response to intradermal challenge with LTB\(_4\). \(^{14}\) To evaluate the potential role of LTB\(_4\) in asthma we have used allergen challenge as a model of allergic inflammation and have tested whether the specific antagonist LY293111 sodium has any effect on the airway response to allergen challenge, either in terms of lung function or of the inflammatory response measured by BAL. In particular, we wished to evaluate the role of LTB\(_4\) in the airway recruitment and activation of eosinophils and neutrophils following the late asthmatic response to allergen. Furthermore, to explore the involvement of LTB\(_4\) in the complex airway events induced by allergen we examined the effect of antagonism of LTB\(_4\) on the release of several cytokines and inflammatory mediators from the cell population in BAL fluid obtained 24 hours after allergen challenge.

### Methods

**PROTOCOL**

The study protocol was approved by the ethics committee of the Royal Brompton Hospital NHS Trust and informed written consent was obtained from all subjects. A randomised double blind placebo controlled crossover study design was used. Twelve men aged 24–31 years were studied (table 1). All had clinical features of asthma including nocturnal cough, wheeze, and exercise induced symptoms. All had evidence of airway hyperresponsiveness, with a provocative concentration of histamine (PC\(_{20}\)) of less than 4 mg/ml. Each subject had a cutaneous response to at least one common aeroallergen of more than 6 mm. None of the subjects had previously taken inhaled or oral steroids, and the only medication used by any of the subjects was salbutamol on an as needed basis.

All subjects underwent a screening visit involving allergen challenge and were required to show a late asthmatic response (LAR) before study entry. At least 28 but not more than 84 days following this they were randomised to receive one of two oral treatments – 112 mg LY293111 three times daily or matched placebo in random order for seven days. At the end of treatment subjects were admitted to the Clinical Studies Unit for repeat allergen challenge and fibreoptic bronchoscopy plus BAL at 24 hours after the allergen. Histamine challenge was performed one hour before and three hours after each allergen challenge. The measured end points were lung function response to allergen, changes in airway responsiveness, biochemical and cellular changes in the BAL fluid. A washout period of 28 days elapsed before subjects crossed over to the second treatment period. On completion of both treatments, subjects reattended for a final visit one week later to confirm that asthma symptoms were stable. Routine biochemistry and haematology were checked at screening, before and after both treatment periods, and at follow up.

The compliance of each individual was assessed by assay of plasma levels of LY293111 before and after each treatment period.

### ALLERGEN AND PC\(_{20}\) CHALLENGE

All challenge tests were performed using a nebuliser attached to a breath activated dosimeter (M3B, MEFAR, Bovezzo, Italy). The nebuliser delivered particles with an aerodynamic mass median diameter of 3.5–4 \(\mu\)m at an output of 9 \(\mu\)l per breath. The nebuliser was set to nebulise for one second with a pause time of six seconds at a pressure of 22 pounds per square inch.

Airway responsiveness was measured by each subject inhaling doubling concentrations of histamine from 0.03 mg/ml to 32 mg/ml until the FEV\(_1\) fell by 20%. The provocative concentration (PC\(_{20}\)) was derived by linear interpolation of log dose–response curves.

Freeze dried allergen extracts (Aquagen SQ, Allerayde, Nottingham, UK) were used for allergen challenge. At screening known dilutions of the allergen were made to give final concentrations of 200, 1000, 2500, 5000, 12500, 25000, and 50000 IU/ml. The initial dose of allergen was 200 IU/ml and FEV\(_1\) was measured five and 10 minutes after allergen dose. Serially increasing doses of allergen were inhaled and the cumulative dose resulting in a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>FEV(_1), % predicted</th>
<th>Allergen</th>
<th>PC(_{20}), histamine (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>112</td>
<td>Grass pollen</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>91</td>
<td>House dust</td>
<td>0.6</td>
</tr>
<tr>
<td>3</td>
<td>31</td>
<td>103</td>
<td>House dust</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>80</td>
<td>Cat</td>
<td>0.9</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>101</td>
<td>Cat</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>85</td>
<td>Grass pollen</td>
<td>0.2</td>
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<tr>
<td>7</td>
<td>25</td>
<td>99</td>
<td>Cat</td>
<td>1.1</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>89</td>
<td>House dust</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>108</td>
<td>House dust</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>84</td>
<td>House dust</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>38</td>
<td>88</td>
<td>Grass pollen</td>
<td>2.7</td>
</tr>
<tr>
<td>12</td>
<td>29</td>
<td>101</td>
<td>Grass pollen</td>
<td>1.3</td>
</tr>
<tr>
<td>Mean</td>
<td>28.2</td>
<td>95 (3.0)</td>
<td></td>
<td>0.8 (0.28)*</td>
</tr>
</tbody>
</table>

FEV\(_1\)= forced expiratory volume in one second; PC\(_{20}\)= provocative concentration of histamine causing a 20% fall in FEV\(_1\); * geometric standard error of the mean.
15% reduction in FEV₁ within 10 minutes was recorded as constituting an adequate allergen challenge for the early asthmatic response (EAR). The FEV₁ was measured every 15 minutes for the first hour and hourly thereafter. The LAR was defined as a fall in FEV₁ of greater than 15% between four and 10 hours. For subsequent allergen challenges the cumulative dose used at screening was given as a single concentration at the end of each treatment period.

All bronchodilators were withheld for eight hours before the inhalation challenge. At 21–24 hours following allergen challenge, 30 minutes before bronchoscopy, subjects were treated with nebulised salbutamol 2.5 mg and, after bronchoscopy, were rescued with nebulised budesonide 2 mg and, if required, with further bronchodilator. All subjects monitored peak flows for 24 hours at home following discharge.

**BRONCHOSCOPY AND BRONCHOALVEOLAR LAVAGE (BAL)**

All subjects underwent bronchoscopic examination 21–24 hours after allergen challenge following an overnight fast. The fiberoptic bronchoscope (Olympus, Keymed Ltd, Southend, UK) was introduced via the nose following local anaesthesia induced by lignocaine 4%, after sedation with midazolam 5 mg intravenously. The bronchoscope was wedged in the right middle lobe. After instillation of 240 ml of warmed sterile 0.9% saline BAL fluid was retrieved and the total volume was recorded.

Pooled BAL fluid was stored on ice, centrifuged at 300 g, and the supernatant collected and frozen at −70°C after recording the volume. A total cell count and cell viability count, using trypan blue exclusion, were taken and the pellet was resuspended at a concentration of 10⁶ cells/ml. Cytospins were made using 100 μL of cell suspension.

**CELL DIFFERENTIAL COUNTS**

From the cytopsins Giemsa stained slides were prepared for differential cell counts and were examined under light microscopy. The percentages of eosinophils, neutrophils, alveolar macrophages, lymphocytes, and epithelial cells were recorded. A minimum of 1500 cells was counted.

**DATA ANALYSIS**

Airway data were expressed as means (SE) and, for airway responsiveness, PC₂₀ data are expressed as the geometric means (SE). The extent of the LAR was assessed by the maximal fall in the FEV₁ and expressed as the percentage change from the baseline value after diluent.

All PC₂₀ histamine values were log transformed for analysis.

The effect of allergen on airway responsiveness to histamine was calculated using the formula:

\[ \text{log}_{10}\text{PC}_{20} \text{ hist} (\text{pre-allergen}) - \text{log}_{10}\text{PC}_{20} \text{ hist (3 hours)} \]

and was expressed as the change in doubling dose. All airway data were statistically analysed using the Student’s paired t test.

Absolute cell numbers, differential cell counts, and measurements of assays obtained from BAL fluid after each treatment were compared using Wilcoxon’s non-parametric test. Absolute cell numbers and differential cell counts are expressed as median (range) values. The volume of BAL and results of assays are expressed as means (SE).

**Results**

Treatment with LY293111 was well tolerated in all subjects and there were no clinically significant adverse effects during either period.
The randomisation of treatment was balanced and there was no order or carryover effect.

AIRWAY FUNCTION
At the end of both treatment periods all allergen challenges resulted in an EAR apart from one individual who had only a 10% drop in FEV₁ following the placebo limb. The mean maximal fall in FEV₁ during EAR after placebo was 32.5 (4.3)% and after LY293111 was 22.9 (4.3)%. During the late response to allergen 21 of the 24 challenges resulted in an LAR; two patients after the placebo limb had a maximal fall in FEV₁ of 10% and 14%, respectively, and one other patient on the LY293111 limb had a maximal fall in FEV₁ of 13%. The mean maximal fall in FEV₁ for the LAR after placebo was 30.5 (4.5)% and after LY293111 was 31.3 (3.7)% (table 2, fig 1).

Neither baseline spirometric tests nor baseline airway responsiveness were affected by either treatment. Airway responsiveness to histamine at three hours after allergen challenge was increased to a similar extent after both treatment periods – by 0.9 (0.3) doubling doses after placebo and by 1.1 (0.4) doubling doses after LY293111 (both p<0.05; table 3).

Table 3 Airway responsiveness pre- and post-allergen challenge

<table>
<thead>
<tr>
<th>Patient</th>
<th>Placebo</th>
<th>LY293111</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PC₂₀ Pre</td>
<td>PC₂₀ Post</td>
</tr>
<tr>
<td>1</td>
<td>2.33</td>
<td>0.53</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>0.18</td>
<td>0.64</td>
</tr>
<tr>
<td>4</td>
<td>1.03</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>0.16</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>0.06</td>
<td>0.1</td>
</tr>
<tr>
<td>7</td>
<td>0.31</td>
<td>0.38</td>
</tr>
<tr>
<td>8</td>
<td>0.37</td>
<td>0.25</td>
</tr>
<tr>
<td>9</td>
<td>3.4</td>
<td>2.1</td>
</tr>
<tr>
<td>10</td>
<td>2.45</td>
<td>1.25</td>
</tr>
<tr>
<td>11</td>
<td>0.6</td>
<td>0.16</td>
</tr>
<tr>
<td>Mean</td>
<td>0.68 (1.38)</td>
<td>0.38 (1.4)</td>
</tr>
</tbody>
</table>

PC₂₀ = provocative concentration of histamine causing a 20% fall in FEV₁.

INFLAMMATORY MEDIATORS
Analysis of BAL fluid showed differences between placebo and LY293111 (table 4). The concentrations of several mediators were reduced in the BAL fluid following the allergen challenge after dosing with LY293111. In particular, there was a significant reduction in the concentrations of LTC₄ (2.2 (0.2) pg/ml after LY293111, 4.6 (1.2) pg/ml after placebo; p<0.05) and MPO (3.5 (1.8) ng/ml after LY293111, 6.6 (1.6) ng/ml after placebo; p<0.05). There was a trend for concentrations of LTB₄ to be lower after LY293111 (760 (192) pg/ml) than after placebo (1141 (239) pg/ml). There was no difference in the concentrations of PGD₂, PGF₂α, PGE₂, PGL₂, and TXB₂. Of the cytokines assayed, there was a non-significant trend for IL-8 to be reduced after LY293111 (114 (7.9) pg/ml)
Table 5 Bronchoalveolar Lavage Absolute and Differential Cell counts

<table>
<thead>
<tr>
<th>Cell types</th>
<th>LY293111</th>
<th>Placebo</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute cell counts (x 10^4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.04 (0.02-0.15)</td>
<td>0.09 (0.02-0.43)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.41 (0-6.80)</td>
<td>0.28 (0.08-1.68)</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophages</td>
<td>11.0 (2.7-20.7)</td>
<td>12.1 (2.7-17.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.42 (0-1.67)</td>
<td>0.49 (0.18-1.06)</td>
<td>NS</td>
</tr>
<tr>
<td>Epithelial</td>
<td>0 (0-0.13)</td>
<td>0 (0-0.10)</td>
<td>NS</td>
</tr>
</tbody>
</table>

% cell differential count:

| Neutrophils      | 0.35 (0-1.2) | 0.8 (0.1-3.6) | <0.05 |
| Eosinophils      | 2.5 (0-27)   | 3.0 (0.5-10)  | NS    |
| Macrophages      | 92 (70-97)   | 90 (84-94)    | NS    |
| Lymphocytes      | 4.0 (0-11)   | 4 (2-6.5)     | NS    |
| Epithelial       | 0 (0-0.5)    | 0 (0-0.5)     | NS    |

Values are median (range).

Compared with placebo (168 (36) pg/ml), IL-6, GM-CSF, and TNF-α were no different between treatments.

INFLAMMATORY CELLS

The volume of BAL fluid retrieved after each treatment was similar: 134 (9.0) ml after LY293111 and 128 (6.1) ml after placebo.

There was no difference in absolute cell numbers: median (range) 11.9 (4.4-25.2) x 10^6 following LY293111, 13.6 (3.2-19.2) x 10^6 cells following placebo. Treatment with LY293111 significantly reduced the number of neutrophils in BAL fluid expressed as both absolute cell numbers and percentages from cell differential counts minus absolute cell counts: median (range) 0.04 (0.02-0.15) x 10^6 after LY293111, 0.09 (0.02-0.43) x 10^6 after placebo; percentage differential cell counts 0.35 (0.1-2.0) after LY293111, 0.80 (0.1-3.6) after placebo, p<0.05 (table 5, fig 2). There was no difference in either absolute or differential cell counts of macrophages, lymphocytes, or eosinophils in BAL fluid between treatments (table 5).

COMPLIANCE

All of the subjects were compliant, demonstrating satisfactory plasma levels during treatment with LY293111 (plasma concentration 380 (10) ng/ml). During placebo plasma levels of drug were below the quantification limit of the assay (<16 ng/ml).

Discussion

This is the first study of the effect of an LTB4 receptor antagonist in asthma. Treatment with LY293111 for seven days significantly decreased the numbers of neutrophils and the levels of MPO obtained from BAL fluid 24 hours after allergen challenge in 12 mild asthmatic subjects with a demonstrable LAR. Furthermore, active treatment significantly reduced concentrations of LTB4 in the BAL fluid and also decreased IL-8, a cytokine associated with neutrophil inflammation, albeit to a lesser degree which did not reach statistical significance. LY293111 failed to affect numbers of eosinophils, macrophages, or lymphocytes in BAL fluid. The other cytokines (IL-6, GM-CSF, TNF-α) and inflammatory mediators (PGD2, PGE2, PGI2, TXB2) were unchanged by treatment, although LTC4 levels were reduced but not significantly. Finally, LY293111 did not alter either the LAR or allergen induced increase in airway responsiveness.

The LAR after inhaled allergen is characterised by increased migration of eosinophils to the airway and there is some evidence to support an increase in the number of neutrophils.10 We have shown that LY293111 caused a significant reduction in neutrophils, MPO (a marker of neutrophil activation), and LTB4 (a potent neutrophil chemoattractant which is synthesised in large numbers by neutrophils) without any effect on the LAR. This suggests that neutrophils are not important in the development of allergen induced airway obstruction. It is possible that the failure to demonstrate a clinical effect is due to lack of efficacy of the active drug. This is unlikely as other studies with this compound have confirmed that it is a potent and specific receptor antagonist producing complete blockade of LTB4 in vitro and the abolition of the inflammatory response to intradermal LTB4 in vivo.11 The latter study was the first clinical report of this compound to confirm that LY293111 is a potent LTB4 receptor antagonist in humans, thus validating its use in our study. We chose the same dose three times daily, the highest dose for which safety and pharmacokinetic data are available from phase I volunteer clinical trials (Lilly Research Laboratories, data on file). As the compound has an elimination half life of 7-12 hours, steady state levels were likely to have been achieved within 72 hours. Furthermore, all the subjects were compliant demonstrating satisfactory plasma levels on the day of allergen following treatment with LY293111.

The lack of clinical effect may be due to the patient population examined. All the subjects had an FEV1 in excess of 80% and a geometric mean PC20 of 0.5 mg/ml. The group were mild asthmatics who did not require inhaled steroids and therefore failure to demonstrate a clinical effect of LY293111 using allergen challenge as a model of asthma in this population may not be predictive for patients with more severe asthma. Furthermore, the levels of neutrophils, eosinophils, and inflammatory cytokines in the BAL fluid were lower than in other studies of patients with impaired lung function exhibiting either spontaneous or allergen induced symptoms.10-12 Irrespective of the fact that our study population were mild asthmatics, we have shown an effect on neutrophils which may be clinically relevant as neutrophils are present in the airways of patients during and after spontaneous asthma attacks18 and may be important in acute episodes in patients with severe disease as airway neutrophilia predominates in sudden onset fatal asthma.13 Both neutrophils and LTB4 have been implicated in animal and human studies of asthma.14,15 Inhalation of LTB4 in dogs induces both airway hyperresponsiveness and airway neutrophilia,16 whereas in the human LTB4 inhalation results in increased numbers of neutrophils in the BAL fluid but does not alter airway function or responsiveness. The
neutrophil is involved in the development of increased airway hyperresponsiveness in dogs after ozone inhalation and in the late asthmatic response in rabbits sensitised to *Alternaria tenuis*, but not in allergen induced responses in guinea pigs. Six hours after allergen challenge neutrophils are increased in the BAL fluid of asthmatic subjects who demonstrate an LAR. By contrast, 24 hours after allergen challenge in human asthma eosinophils are increased more than neutrophils in BAL fluid. This suggests that the neutrophil may be transiently recruited in the acute inflammatory response to allergen and that the eosinophil is involved in ongoing inflammation. Nevertheless, 24 hours after allergen challenge neutrophils have been shown to be present in BAL fluid of subjects with a fall in FEV₁ of >25% from baseline during an LAR. Thus, as seven of our subjects experienced such an extensive LAR it is likely that the effects observed on the neutrophil in our study are allergen related.

The role of LTB₄ in the inflammatory response to allergen challenge is unknown. Unlike the cysteinyl leukotrienes it does not have a powerful pressor effect on airway smooth muscle, but it may be important in recruiting eosinophils to the airway after allergen challenge. It is a potent neutrophil chemoattractant and is synthesised in large amounts by the neutrophil. Furthermore, LTB₄ activates not only neutrophils but also enhances mediator release from eosinophils. We have shown that antagonism of LTB₄ with LY293111 reduces airway neutrophil numbers and activation without an effect on airway eosinophils. Because the neutrophil is a rich source of LTB₄, the reduction in airway neutrophils is likely to account for the decrease in concentrations of LTB₄, which, in turn, may have contributed to less neutrophil chemotaxis and activation. Thus, antagonism of LTB₄ receptors expressed in abundance on neutrophils may have indirectly reduced LTB₄ synthesis and release.

LTB₄ receptors are also expressed on eosinophils and, when activated, may regulate eosinophil numbers and function in asthma as LTB₄ appears to be involved in eosinophil recruitment during allergen challenge. Furthermore, antigen induced airway eosinophila in guinea pigs is markedly decreased by an LTB₄ receptor antagonist. Thus, in our study we might reasonably have expected LY293111 to reduce eosinophils in a similar manner to neutrophils. The absence of such an effect suggests that LTB₄ does not influence allergen induced eosinophil activity in human asthma, in contrast to its effect in guinea pigs. A minor action of LTB₄ on eosinophils cannot be excluded as there was a small fall in BAL fluid levels of LTC₄, a mediator probably released by eosinophils, which did not quite reach statistical significance.

The BAL fluid populations of alveolar macrophages and lymphocytes after allergen challenge were unaffected by treatment. Compatible with this finding, there was no change in concentrations of GM-CSF and IL-6, cytokines predominantly released from monocytes and alveolar macrophages and, to a lesser extent, from lymphocytes. Also, levels of TNF-α, which is released in abundance from alveolar macrophages, did not differ between treatments. However, there was a tendency to reduced levels of IL-8 after LY293111. Although this effect was not statistically significant, it requires exploration.

IL-8 is a pro-inflammatory cytokine that is a potent chemoattractant for and activator of neutrophils with lesser effects on eosinophils. It is synthesised and released by several inflammatory cells within the airway including macrophages, activated lymphocytes, epithelial cells, and neutrophils. Increased levels of IL-8 have been found in the BAL fluid of asthmatic subjects 18 hours after allergen, although its cellular source was unclear. As LY293111 did not change the numbers of macrophages or lymphocytes in the BAL fluid, it is unlikely that an effect on the release of IL-8 from these cells could account for its partial decrease. Although speculative, it is possible that neutrophils are an important source of IL-8 after allergen and that a reduction in the neutrophil count by LY293111 led to a decrease in IL-8 levels in BAL fluid. Alternatively, IL-8 may have been generated by epithelial cells which, in our study, were almost undetectable in the BAL fluid. Irrespective of the source of the IL-8, we did not see changes in eosinophil numbers even though the IL-8 level fell with treatment. This suggests either that IL-8 is not critical for recruitment of eosinophils following allergen challenge or that the small changes demonstrated for this cytokine are insufficient to exhibit an effect on eosinophil trafficking within the airway.

The concentrations of prostanoids in BAL fluid were unchanged between treatment periods. This is consistent with the lack of clinical effect. PGD₂, PGE₂, and TXA₂ (TXB₂ is the stable metabolite) increase airway responsiveness, are potent bronchoconstrictors, and are likely to contribute to the LAR, whereas PGE₂ and PG₁ relax airway smooth muscle and may inhibit the LAR. PGD₂ is released by several inflammatory cells after allergen challenge. The failure to observe differences in prostanoids is not surprising as LY293111 affected neutrophils and neutrophil-derived mediators only without influencing other cells or mediators of the LAR.

We have suggested that LTB₄ influences allergen induced neutrophil activity in asthmatic airways. Without baseline data on BAL cell populations and mediator release we cannot be certain that our results represent allergen induced responses as the levels of eosinophils, neutrophils, and cytokines are lower than in other studies. It is therefore possible that the effect of LTB₄ receptor antagonism on neutrophils and airway inflammation would be observed even in unprovoked mild asthma. Nonetheless, we feel that our study relates to airway events stimulated by allergen as all the cytokines we measured are increased in the BAL fluid at 18 hours after allergen and several studies have confirmed that BAL fluid levels of eosinophils and neutrophils are increased.
after allergen.\textsuperscript{67,11} Regardless of this, challenge with sensitising agents other than allergen may be a more appropriate model to evaluate the clinical potential of LY293111 and other drugs of this class in asthma. In view of our results, it is likely that these agents would reduce the late asthmatic responses to challenge with ozone and toluene diisocyanate which are characterised predominantly by an airway neutrophilia.\textsuperscript{28,29} Furthermore, LY293111 may have therapeutic potential in other respiratory diseases where LTB\textsubscript{4} and neutrophils are major inflammatory mediators such as chronic bronchitis, bronchiectasis, and toluene diisocyanate-induced asthma. The role of LTB\textsubscript{4} and the neutrophil in asthma therefore remains uncertain but could be clarified by further evaluation of potent inhibitors of LTB\textsubscript{4} in challenge studies using sensitising agents or in clinical trials of patients with more severe disease.

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