

## Editorials

Leukotriene B<sub>4</sub> and asthma

P E Christie, N C Barnes

Lipid derived mediators generated from arachidonic acid metabolism have attracted considerable interest as mediators of asthma. Arachidonic acid, a 20-carbon polyunsaturated fatty acid, is released from membrane phospholipases by the action of phospholipases under the influence of a variety of stimuli.<sup>1</sup> The 5-lipoxygenase pathway generates the intermediate 5-HPETE which is reduced to 5-HETE or is converted to the unstable epoxide leukotriene A<sub>4</sub> (LTA<sub>4</sub>). LTA<sub>4</sub> is acted on by an epoxide hydrolase to form leukotriene B<sub>4</sub> (LTB<sub>4</sub>) or by a glutathione transferase to generate leukotriene C<sub>4</sub>.<sup>2</sup> LTC<sub>4</sub> is cleaved by gamma glutamyl transferase to generate leukotriene D<sub>4</sub> (LTD<sub>4</sub>) and then a dipeptidase to form leukotriene E<sub>4</sub> (LTE<sub>4</sub>). LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> comprise the cysteinyl leukotrienes previously recognised as slow-reacting substance of anaphylaxis (SRS-A).

Asthma is characterised by airways inflammation in which eosinophilic infiltration is prominent. There is a complex interaction between many mediators, cytokines, and cells. The role of cysteinyl leukotrienes (LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>) in modulating and regulating this inflammation has been extensively studied and supported by their properties as potent bronchoconstrictors, ability to increase airway responsiveness, vascular permeability and mucus production.<sup>3</sup> The recovery of cysteinyl leukotrienes from the bronchoalveolar lavage (BAL) fluid of asthmatic subjects<sup>4</sup> and increased urinary excretion of LTE<sub>4</sub> during asthmatic attacks further supports a role for these as important mediators in asthma.

There is now considerable evidence that cysteinyl leukotriene receptor antagonists have effects in asthma.<sup>6</sup> The role, if any, for LTB<sub>4</sub> in asthma is less clear.

Leukotriene biosynthesis occurs in a variety of cells (neutrophils, eosinophils, monocytes, macrophages, mast cells, basophils, and B lymphocytes).<sup>3</sup> Eosinophils preferentially generate LTC<sub>4</sub><sup>7</sup> while peripheral blood neutrophils, alveolar macrophages, and monocytes generate mainly LTB<sub>4</sub>.<sup>8</sup> LTB<sub>4</sub> is also generated from eosinophils.<sup>9</sup> The current evidence supports the cysteinyl leukotrienes as mediators in asthma and LTB<sub>4</sub> a mediator of greater significance in diseases where neutrophil inflammation predominates. However, the presence of neutrophils in some instances in asthma and the biological and physiological properties of LTB<sub>4</sub> mean that a role in asthma for LTB<sub>4</sub> remains a possibility.

LTB<sub>4</sub> is a potent pro-inflammatory mediator. Its activities include chemokinesis and chemotaxis of human neutrophils and eosinophils,<sup>8</sup> chemokinesis of monocytes, aggregation of neutrophils, and enhanced expression of complement receptors on granulocytes.<sup>10</sup> It induces release of lysosomal enzymes from neutrophils and augmentation of neutrophil adherence to endothelial cell

monolayers.<sup>11</sup> LTB<sub>4</sub> induced endothelial cell adhesions for neutrophils depend on increased CD11/CD18 expression on the neutrophil surface.<sup>12</sup> LTB<sub>4</sub> causes release of enzymes from neutrophils and augments interleukin 6 (IL-6) production in human monocytes; it may modulate the production of other cytokines and also has effects on T lymphocyte proliferation. In vivo intradermal injection of LTB<sub>4</sub> promotes neutrophil infiltration into human skin.<sup>13</sup> It activates not only neutrophils but also enhances mediator release from eosinophils.<sup>14</sup> LTB<sub>4</sub> is spasmogenic for smooth muscle; in the guinea pig this is indirectly through the stimulation of secondary cyclooxygenase synthesis<sup>15</sup> although tachyphylaxis occurs rapidly. It is thought to have its chemotactic effect on neutrophils via a specific high affinity receptor site and at higher concentrations it induces neutrophil enzyme release, possibly via a low affinity receptor.<sup>16</sup> LTB<sub>4</sub> receptors are also expressed on eosinophils and, when activated, may help regulate eosinophil numbers and function.<sup>10</sup>

Inhalation of ozone in dogs induces airway hyper-responsiveness and airway neutrophilia<sup>17</sup> as does inhalation of LTB<sub>4</sub>.<sup>18</sup> In the brown Norway rat allergen challenge resulted in an increased capacity of lung cells to synthesise LTB<sub>4</sub> from macrophages rather than eosinophils.<sup>19</sup> Antigen induced airway eosinophilia in guinea pigs is decreased by an LTB<sub>4</sub> receptor antagonist.<sup>20</sup> In man allergen challenge of atopic individuals causes an early increase in neutrophils but by 24 hours the eosinophil predominates.<sup>21</sup>

In asthmatic and normal individuals cysteinyl leukotrienes are the predominant leukotriene recovered from BAL fluid. However, 20-hydroxyl LTB<sub>4</sub> (an oxidation product of LTB<sub>4</sub>) has also been recovered in one study.<sup>22</sup> Evidence against a fundamental role for LTB<sub>4</sub> comes from studies of inhaled LTB<sub>4</sub> in asthmatics when airway function or responsiveness are not affected<sup>23</sup> despite the observation that intratracheal instillation of LTB<sub>4</sub> induces the recruitment of active neutrophils into BAL fluid in humans.<sup>24</sup>

Studies of LTB<sub>4</sub> mediator release further provide contradictory evidence for a role for LTB<sub>4</sub> in asthma. Increased production of LTB<sub>4</sub> by neutrophils occurs in patients with allergic rhinitis<sup>25</sup> and in asthmatic children or adults during exacerbations of asthma.<sup>26,27</sup> Alveolar macrophages from wheezy infants release larger amounts of LTB<sub>4</sub> under resting conditions than do alveolar macrophages from control subjects.<sup>28</sup> However, Restrick *et al*<sup>29</sup> found a decreased synthesis of LTB<sub>4</sub> from alveolar macrophages obtained by BAL in asthmatic patients. LTB<sub>4</sub> may play a contributory role in nocturnal asthma. More neutrophils, eosinophils, LTB<sub>4</sub>, and cysteinyl leukotrienes are recovered in BAL fluid at 0400 hours in subjects with nocturnal asthma than in those without nocturnal asthma.<sup>30</sup> LTB<sub>4</sub> levels correlate significantly with nocturnal falls in forced expiratory vol-

ume in one second (FEV<sub>1</sub>). Treatment with the 5-lipoxygenase inhibitor zileuton results in decreased recovery of LTB<sub>4</sub> and reduction in the recovery of eosinophils from BAL fluid and a trend to improvement in night time FEV<sub>1</sub>. After treatment with prednisolone pulmonary alveolar macrophage stimulated production of LTB<sub>4</sub> decreases with a reduction in neutrophil influx which correlates with an improvement in the nocturnal FEV<sub>1</sub>.<sup>31</sup> One important contribution of LTB<sub>4</sub> in asthma may be related to the considerable airway neutrophilia which predominates in sudden onset fatal asthma, suggesting that the neutrophil products released may be important in the underlying fatal pathology.<sup>32</sup>

The assessment of drugs that antagonise the effects of cysteinyl leukotrienes or prevent their synthesis have demonstrated beneficial effects on asthma induced by antigens, exercise, and cold air and in clinical asthma. The lack of any clear difference between cysteinyl leukotriene receptor antagonists and 5-lipoxygenase inhibitors would argue against an important role for LTB<sub>4</sub> in asthma.<sup>33</sup> To elucidate the role of LTB<sub>4</sub> in asthma further specific leukotriene B<sub>4</sub> receptor antagonists have now been synthesised.

In the paper by Evans *et al* in this issue of *Thorax* the authors report the first study of the effect of an LTB<sub>4</sub> receptor antagonist, LY293111, in asthma.<sup>34</sup> The role of LTB<sub>4</sub> in the airway recruitment and activation of eosinophils and neutrophils following the late asthmatic response to allergen was determined by evaluating the effect of antagonism of LTB<sub>4</sub> on lung function and the release of several cytokines and inflammatory mediators from the cell population in BAL fluid obtained 24 hours after allergen challenge. Treatment for seven days with LY293111 significantly decreased the numbers of neutrophils and the level of myeloperoxidase hours after allergen challenge in 12 mild asthmatic subjects with a demonstrable late asthmatic response (LAR). Active treatment reduced concentrations of LTB<sub>4</sub> in the BAL fluid and also decreased levels of IL-8, a cytokine associated with neutrophil inflammation. It failed to have any effect on the numbers of eosinophils, macrophages, or lymphocytes in the BAL fluid. The other cytokines (IL-6, GM-CSF, TNF $\alpha$ ) and inflammatory mediators (PGD<sub>2</sub>, PGF<sub>2 $\alpha$</sub> , PGI<sub>2</sub>, TXB<sub>2</sub>) were unchanged by treatment although LTC<sub>4</sub> was reduced, but not significantly. LY293111 did not alter either the size of the LAR or the allergen induced increase in bronchial hyperresponsiveness. From these findings the authors suggest an influence of LTB<sub>4</sub> on neutrophil influx and activation in the airways following allergen challenge. The absence of physiological effects of pretreatment with LY293111 on allergen induced airway responses questions the functional role of the neutrophil in the pathophysiology of allergen induced asthma. The absence of an effect on eosinophil numbers suggests that LTB<sub>4</sub> is not a major mediator of allergen induced eosinophil activity in human asthma, although a minor action of LTB<sub>4</sub> on eosinophils cannot be excluded due to a small fall in BAL fluid levels of LTC<sub>4</sub>, a mediator released by eosinophils. LY293111 did not change the numbers of macrophages or lymphocytes in the BAL fluid. IL-8 is synthesised and released by several inflammatory cells within the airways including macrophages, lymphocytes, epithelial cells, and neutrophils. Although IL-8 levels fell with treatment there was no change in eosinophil numbers suggesting that IL-8, which acts as a potent chemoattractant and activator of neutrophils with lesser effects on eosinophils, was not a major contributory cytokine to eosinophil recruitment. The effect of an LTB<sub>4</sub> antagonist on IL-8 levels suggests that leukotrienes may be involved with the modulation of cytokine production.

While such studies provide an important insight into the pathogenesis of asthma, further studies may elucidate a role for LTB<sub>4</sub> in the clinical treatment of asthma. In addition to the potential therapeutic benefits of the LTB<sub>4</sub> inhibitor LY293111 for patients with asthma, it would be of considerable interest to determine the effects that such agents might have on asthmatic responses to challenge with ozone and toluene diisocyanate (TDI) which are characterised by an airway neutrophilia.<sup>35</sup> The effects of an LTB<sub>4</sub> receptor antagonist in respiratory diseases where LTB<sub>4</sub> and neutrophils are major inflammatory mediators and cells – such as the adult respiratory distress syndrome,<sup>36,37</sup> chronic bronchitis,<sup>37</sup> bronchiectasis,<sup>38</sup> cryptogenic fibrosing alveolitis,<sup>39</sup> and cystic fibrosis<sup>40</sup> – would be interesting to evaluate. Treatment aimed at interfering with the production or action of LTB<sub>4</sub> may be beneficial in such respiratory diseases, although current evidence does not suggest an important role in asthma.

Correspondence to: Dr N C Barnes.

Department of Respiratory Medicine,  
London Chest Hospital,  
Bonner Road,  
London E2 9JX, UK

PANDORA E CHRISTIE  
NEIL C BARNES

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