Thorax

Editorials

Leukotriene B_4 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.[1] The 5-lipoxygenase pathway generates the intermediate 5-HPETE which is reduced to 5-HETE or is converted to the unstable epoxide leukotriene A_4 (LTA_4). LTA_4 is acted on by an epoxide hydrolase to form leukotriene B_4 (LTB_4) or by a glutathione transferase to generate leukotriene C_4.[2] LTC_4 is cleaved by gamma glutamyl transferase to generate leukotriene D_4 (LTD_4) and then a dipeptidase to form leukotriene E_4 (LTE_4). LTC_4, LTD_4, and LTE_4 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_4, LTD_4, and LTE_4) 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.[3] The recovery of cysteinyl leukotrienes from the bronchoalveolar lavage (BAL) fluid of asthmatic subjects[4] and increased urinary excretion of LTE_4 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.[6] The role, if any, for LTB_4 in asthma is less clear.

Leukotriene biosynthesis occurs in a variety of cells (neutrophils, eosinophils, monocytes, macrophages, mast cells, basophils, and B lymphocytes).[3] Eosinophils preferentially generate LTC_4[7] while peripheral blood neutrophils, alveolar macrophages, and monocytes generate mainly LTB_4.[8] LTB_4 is also generated from eosinophils.[9] The current evidence supports the cysteinyl leukotrienes as mediators in asthma and LTB_4 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_4 mean that a role in asthma for LTB_4 remains a possibility.

LTB_4 is a potent pro-inflammatory mediator. Its activities include chemokinesis and chemotaxis of human neutrophils and eosinophils,[8] chemokinesis of monocytes, aggregation of neutrophils, and enhanced expression of complement receptors on granulocytes.[10] It induces release of lysosomal enzymes from neutrophils and augmentation of neutrophil adherence to endothelial cell monolayers.[11] LTB_4 induced endothelial cell adhesions for neutrophils depend on increased CD11/CD18 expression on the neutrophil surface.[12] LTB_4 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_4 promotes neutrophil infiltration into human skin.[13] It activates not only neutrophils but also enhances mediator release from eosinophils.[14] LTB_4 is spasmogenic for smooth muscle; in the guinea pig this is indirectly through the stimulation of secondary cyclooxygenase synthesis[15] 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.[16] LTB_4 receptors are also expressed on eosinophils and, when activated, may help regulate eosinophil numbers and function.[10]

Inhalation of ozone in dogs induces airway hyperresponsiveness and airway neutrophilia[17] as does inhalation of LTB_4.[18] In the brown Norway rat allergen challenge resulted in an increased capacity of lung cells to synthesise LTB_4 from macrophages rather than eosinophils.[19] Antigen induced airway eosinophilia in guinea pigs is decreased by an LTB_4 receptor antagonist.[20] In man allergen challenge of atopic individuals causes an early increase in neutrophils but by 24 hours the eosinophil predominates.[21]

In asthmatic and normal individuals cysteinyl leukotrienes are the predominant leukotriene recovered from BAL fluid. However, 20-hydroxyl LTB_4 (an oxidation product of LTB_4) has also been recovered in one study.[22] Evidence against a fundamental role for LTB_4 comes from studies of inhaled LTB_4 in asthmatics when airway function or responsiveness are not affected[23] despite the observation that intratracheal instillation of LTB_4 induces the recruitment of active neutrophils into BAL fluid in humans.[24]

Studies of LTB_4 mediator release further provide contradictory evidence for a role for LTB_4 in asthma. Increased production of LTB_4 by neutrophils occurs in patients with allergic rhinitis[25] and in asthmatic children or adults during exacerbations of asthma.[26,27] Alveolar macrophages from wheezy infants release larger amounts of LTB_4 under resting conditions than do alveolar macrophages from control subjects.[28] However, Restrick *et al*[29] found a decreased synthesis of LTB_4 from alveolar macrophages obtained by BAL in asthmatic patients. LTB_4 may play a contributory role in nocturnal asthma. More neutrophils, eosinophils, LTB_4, and cysteinyl leukotrienes are recovered in BAL fluid at 0400 hours in subjects with nocturnal asthma than in those without nocturnal asthma.[30] LTB_4 levels correlate significantly with nocturnal falls in forced expiratory vol-

ume in one second (FEV_1). Treatment with the 5-lipoxygenase inhibitor zileuton results in decreased recovery of LTB_4 and reduction in the recovery of eosinophils from BAL fluid and a trend to improvement in night time FEV_1. After treatment with prednisolone pulmonary alveolar macrophage stimulated production of LTB_4 decreases with a reduction in neutrophil influx which correlates with an improvement in the nocturnal FEV_1.[31] One important contribution of LTB_4 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.[32]

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_4 in asthma.[33] To elucidate the role of LTB_4 in asthma further specific leukotriene B_4 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_4 receptor antagonist, LY293111, in asthma.[34] The role of LTB_4 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_4 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_4 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, TNFα) and inflammatory mediators (PGD_2, PGF_{2a}, PGI_2, TXB_2) were unchanged by treatment although LTC_4 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_4 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_4 is not a major mediator of allergen induced eosinophil activity in human asthma, although a minor action of LTB_4 on eosinophils cannot be excluded due to a small fall in BAL fluid levels of LTC_4, 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_4 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_4 in the clinical treatment of asthma. In addition to the potential therapeutic benefits of the LTB_4 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.[35] The effects of an LTB_4 receptor antagonist in respiratory diseases where LTB_4 and neutrophils are major inflammatory mediators and cells – such as the adult respiratory distress syndrome,[36,37] chronic bronchitis,[37] bronchiectasis,[38] cryptogenic fibrosing alveolitis,[39] and cystic fibrosis[40] – would be interesting to evaluate. Treatment aimed at interfering with the production or action of LTB_4 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

1 Kaiser E, Chiba P, Zaky K. Phospholipases in biology and medicine. *Clin Biochem* 1990;**23**:349–70.
2 Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis and biological effects. *Science* 1987;**237**:1171–6.
3 Drazen JM. Leukotrienes. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*. London: Blackwells, 1995:838–50.
4 Wenzel SE, Larsen GL, Johnston K, Voelkel NF, Westcott JY. Elevated levels of leukotriene C_4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990; **142**:112–9.
5 Taylor GW, Black P, Turner N, Taylor I, Maltby NH, Fuller R Wetal. Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1989;**139**:584–8.
6 Taylor I. Cysteinyl leukotrienes in asthma: current state of therapeutic evaluation. *Thorax* 1995;**50**:1005–10.
7 Shaw RJ, Walsh GM, Cromwell O, Moqbel R, Spry CJ, Kay AB. Activated human eosinophils generate SRS–A leukotrienes following IgG-dependent stimulation. *Nature* 1985;**316**:150–2.
8 Bray MA. The pharmacology and pathophysiology of leukotriene B_4. *Br Med Bull* 1983;**39**:249–54.
9 Henderson WR, Harley JB, Fauci AS. Arachidonic acid metabolism in normal and hypereosinophilic syndrome human eosinophils: generation of leukotrienes B_4, C_4 and D and 15-lipoxygenase products. *Immunology* 1984;**51**:679–86.
10 Nagy L, Lee TH, Goetzl EJ, Pickett W, Kay AB. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin Exp Immunol* 1982;**47**: 541–7.
11 Hoover RL, Karnovsky MJ, Austen KF, Corey EJ, Lewis RA. Leukotriene B_4 action on endothelium mediates augmented neutrophil/endothelial adhesion. *Proc Natl Acad Sci USA* 1984;**81**:2191–3.
12 Tonnesen MG. Neutrophil-endothelial cell interactions: mechanism of neutrophil adherence to vascular endothelium. *J Invest Dermatol* 1989; **93**:53–8S.
13 Camp RD, Coutts AA, Greaves MW, Kay AB, Walport MJ. Responses of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. *Br J Pharmacol* 1983;**80**:497–502.
14 Ford-Hutchinson AW. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* 1980;**286**:264–5.
15 Piper PJ, Samhou MN. Stimulation of arachidonic acid metabolism and generation of thromboxane A_2 by leukotrienes B_4, C_4 and D_4 in guinea pigs lung in vitro. *Br J Pharmacol* 1982;77:267–75.
16 Goldman DW, Goetzl EJ. Specific binding of leukotriene B_4 to receptors on human polymorphonuclear leukocytes. *J Immunol* 1982;**129**:1600–4.
17 Holtzman MJ, Fabbri LM, O'Bryne PM, Gold WM, Aizawa H, Walters H, *et al*. Importance of airway inflammation for hyperresponsiveness induced by ozone. *Am Rev Respir Dis* 1983;**127**:686–90.
18 O'Byrne PM, Leikauf GD, Aizawa H, Beithel RA, Ueki IF, Holtzmann MJ, *et al*. Leukotriene B_4 induces airway hyperresponsiveness in dogs. *J Appl Physiol* 1985;**59**:1941–6.
19 Yu W, Xu L, Martin JG, Powell WS. Cellular infiltration and eicosanoid synthesis in brown Norway rat lungs after allergen challenge. *Am J Respir Cell Mol Biol* 1995;**13**:477–86.
20 Richards IM, Griffin RL, Morris J, Wishka DG, Dunn CJ. Effect of the selective leukotriene B_4 antagonist U-75302 on antigen-induced bronchopulmonary eosinophilia in sensitized guinea pigs. *Am Rev Respir Dis* 1989;**140**:1712–6.
21 Metzger WJ, Zavala D, Richerson HB. Local allergen challenge and bronchoalveolar lavage of allergic asthmatic lungs. *Am Rev Respir Dis* 1987;**135**: 433–40.
22 Lam S, Chan H, Le Riche JC, Chan-Yeung M, Salan H. Release of leukotrienes in patients with bronchial asthma. *J Allergy Clin Immunol* 1988;**81**:711–7.
23 Black PN, Fuller RW, Taylor GW, Barnes PJ, Dollery CT. Effect of inhaled leukotriene B_4 alone and in combination with prostaglandin D_2

on bronchial responsiveness to histamine in normal subjects. *Thorax* 1989; **44**:491–5.
24 Martin TR, Pistorese BP, Chi EY, Goodman RB, Matthay MA. Effects of leukotriene B_4 in the human lung. Recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest* 1989;**84**:1609–19.
25 Cheria-Sammari S, Aloui R, Gormand F, Chabannes B, Gallet H, Grosclaude M, *et al.* Leukotriene B_4 production by blood neutrophils in allergic rhinitis: effects of cetirizine. *Clin Exp Allergy* 1995;**25**:729–36.
26 Seggev JS, Wiessner JH, Thornton WH Jr, Edes TE. Comparison of serum and plasma leukotriene B_4 levels in normal and asthmatic subjects. *Ann Allergy Asthma Immunol* 1995;**75**:365–8.
27 Shindo K, Miyakawa K, Fukumura M. Plasma levels of leukotriene B_4 in asthmatic patients. *Int J Tissue React* 1993;**15**:181–4.
28 Azevedo I, de–Blioc J, Scheinmann P, Vargaftig BB, Bachelet M. Enhanced arachidonic acid metabolism in alveolar macrophages from wheezy infants. Modulation by dexamethasone. *Am J Respir Crit Care Med* 1995;**152**: 1208–14.
29 Restrick LJ, Sampson AP, Piper PJ, Costello JF. Reduction in leukotriene B_4 generation by bronchoalveolar lavage cells in asthma. *Thorax* 1995;**50**: 67–73.
30 Wenzel SE, Trudeau JB, Kaminsky DA, Cohn J, Martin RJ, Westcott JY. Effect of 5–lipoxygenase inhibition on bronchoconstriction and airway inflammation in nocturnal asthma. *Am J Respir Crit Care Med* 1995;**152**: 897–905.
31 Wenzel SE, Trudeau JB, Westcott JY, Beam WR, Martin RJ. Single oral dose of prednisolone decreases leukotriene B_4 production by alveolar macrophages from patients with nocturnal asthma but not control subjects: relationship to changes in cellular influx and FEV_1. *J Allergy Clin Immunol* 1994;**94**:870–81.
32 Sur S, Crotty TB. Sudden onset fatal asthma – a distinct entity with few eosinophils and relatively more neutrophils in the airway mucosa. *Am Rev Respir Dis* 1993;**148**:713–9.
33 Barnes NC, Alexander AG, Kuitert LM. New medications for asthma. In: Busse WW, Holgate ST, eds. *Asthma and rhinitis*. London: Blackwells, 1995: 1337–48.
34 Evans PJ, Barnes PJ, Spaethe SM, van Alstyne EL Mitchell MI, O'Connor BJ. Effect of a leukotriene B_4 receptor antagonist, LY293111, on allergen induced responses in asthma. *Thorax* 1996;**51**:1178–84.
35 Fabbri LM, Boscehetto P, Zocca E, Milain G, Pirirotto F, Plebani M, *et al.* Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate. *Am Rev Respir Dis* 1987;**136**:36–42.
36 Stephenson AH, Lonigro AJ, Hyers TM, Webster RO, Fowler AA. Increased concentrations of leukotrienes in bronchoalveolar lavage fluid of patients with ARDS or at risk for ARDS. *Am Rev Respir Dis* 1988;**138**:714–9.
37 Steinberg KP, Milberg JA, Martin TR, Maunder RJ, Cochrill BA, Hudson LD. Evolution of bronchoalveolar cell populations in the adult respiratory distress syndrome. *Am J Respir Crit Care Med* 1994;**150**:113–22.
38 Zakrzewski JT, Barnes NC, Costello JF, Piper PJ. Lipid mediators in cystic fibrosis and chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1987;**136**:779–82.
39 Wardlaw AJ, Hey H, Cromwell O, Collins JU, Kay AB. Leukotrienes LTC_4 and LTB_4 in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. *J Allergy Clin Immunol* 1989;**84**:19–26.
40 Zakrzewski JT, Barnes NC, Piper PJ, Costello JF. Detection of sputum eicosanoids in cystic fibrosis and in normal saliva by bioassay and radio-immunoassay. *Br J Clin Pharmacol* 1987;**23**:19–27.