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Potential role of leukotrienes in other disease states

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In view of its historical importance as a context for investigations involving leukotrienes (LTs), it is not surprising that asthma is the disease in which the role of LTs and the utility of anti-LT agents have been most extensively studied. However, the biological actions of LTs and other 5-lipoxygenase (5-LO) metabolites go beyond effects on smooth muscle contraction, mucus secretion and vascular permeability. They also modulate such fundamental processes as immune responsiveness, cell proliferation, apoptosis and transcriptional activation. Such pleiotropic actions suggest possible roles for these mediators in a variety of other disease states (see Fig. 1). Indeed, LTs have been implicated in a spectrum of conditions ranging from ischaemic tissue injury to inflammatory bowel disease and pulmonary hypertension. Their involvement in certain inflammatory and immunological conditions has already been discussed in accompanying articles.

This review will focus on the potential role of LTs in a select group of pulmonary disorders: namely, acute respiratory distress syndrome (ARDS), idiopathic pulmonary fibrosis and lung cancer. In addition, a homeostatic role for these mediators in antimicrobial host defence will be discussed.

ARDS

Increased permeability of the alveolar capillary membrane is a common response of the lungs following a number of insults. These include both systemic (such as sepsis and severe pancreatitis) as well as inhalational (such as aspiration of gastric contents and toxic gas inhalation) insults. The alveoli become flooded with protein-rich oedema fluid and this causes microatalectasis, reduced pulmonary compliance and impaired gas exchange. When this process is severe enough to meet criteria for respiratory failure, the term ARDS is applied. This syndrome accounts for significant morbidity, mortality and consumption of health care resources.

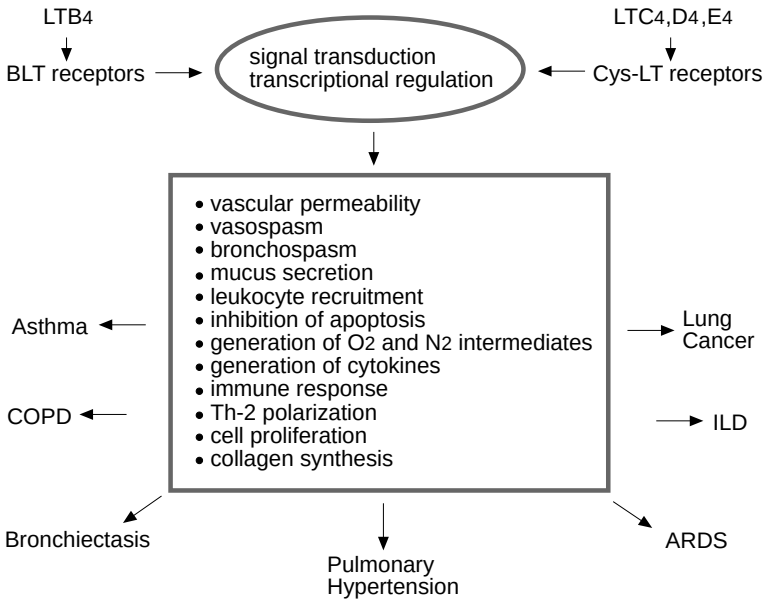

Fig. 1. Spectrum of LT actions relevant to various lung diseases. Abbreviations: COPD, chronic obstructive pulmonary disease; ARDS, acute respiratory distress syndrome; ILD, interstitial lung diseases.

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A consistent feature of ARDS is the accumulation of large numbers of neutrophils in the airspaces. These cells contribute to lung injury by the elaboration of toxic substances such as proteases and oxygen radicals. The

known actions of LTs are quite salient to these pathogenetic features of acute lung injury, since neutrophil recruitment/activation and increased microvascular permeability could be attributed, at least in part, to LTB_4 and Cys-LTs (LTs C_4, D_4 and E_4), respectively.

Increased levels of both LTB_4 and Cys-LTs have been documented in bronchoalveolar lavage fluid (BALF), blood and urine of patients with ARDS [1–3]: appropriate control groups in these studies included mechanically ventilated non-ARDS patients and mechanically ventilated patients with cardiogenic pulmonary oedema. In addition, LT levels were higher in ARDS patients than in patients with predisposing conditions placing them at risk for ARDS, but in whom ARDS did not develop, and were also higher in these at risk patients than in healthy subjects. Interestingly, the magnitude of LTE_4 excretion in ARDS patients was far greater than that observed following antigen challenge of asthmatics [3].

Overproduction of LTs has been observed in animal models of ARDS, including oxygen toxicity and endotoxaemia. Moreover, improvements in neutrophil alveolitis, pulmonary oedema, lung mechanics, haemodynamics, oxygenation and mortality have been reported with the use of 5-LO inhibitors [4], LTB_4 receptor antagonists [5] and Cys-LT receptor antagonists [6]. In view of the evidence that LTs are produced in the setting of ARDS and may participate in its pathogenesis, these animal data provide additional justification for investigating the potential of anti-LT therapy in this syndrome.

Idiopathic pulmonary fibrosis

The interstitial lung diseases comprise a large group of parenchymal disorders characterized by inflammation, derangement of normal lung architecture and fibrosis. Clinically, these disorders are characterized by a restrictive ventilatory defect with impaired gas exchange, diffuse radiographic infiltrates and dyspnoea. These conditions may be attributable to a known exposure (e.g. asbestosis) or systemic disease process (e.g. scleroderma), or may be idiopathic in nature [e.g. sarcoidosis and idiopathic pulmonary fibrosis (IPF)]. IPF, the most common of these fibrotic lung diseases, is thought to represent an abnormal response to some inciting injurious event. The key elements in its pathogenesis include: epithelial injury, an accumulation of inflammatory and immune effector cells, a Th2-type of cellular immune response, elaboration by activated inflammatory cells of oxygen radicals, cytokines, and fibroblast growth factors, and an altered fibroblast phenotype characterized by unchecked proliferation and excess synthesis and deposition by fibroblasts of extracellular matrix proteins such as collagen. It is apparent that these features are highly reminiscent of those implicated in the initiation and perpetuation of airway disease in asthma.

Standard treatment of IPF with corticosteroids has been disappointing, prompting a need for therapeutic alternatives. The actions of LTs are consistent with a possible role in the pathogenesis of interstitial lung diseases such as IPF. Their ability to promote accumulation of inflammatory cells, Th2 immune responses and generation of mediators such as oxygen radicals and cytokines are well known. However, LTs have also been shown to promote the release of fibroblast growth factors. Finally, LTs exert direct effects on fibroblasts, stimulating fibroblast chemotaxis, proliferation and collagen synthesis [7,8].

LTB_4 levels in BALF have been reported to be greater in patients with IPF than in healthy volunteers [9]. More recently, we have demonstrated markedly greater levels of both LTB_4 and Cys-LTs in homogenates of IPF lung than in nonfibrotic lung, and have identified the alveolar macrophage (AM) as a major cellular source of the LTs [10]. Homogenate LT levels correlated significantly with the histological extent of fibrosis, consistent with the notion that LTs participate in not only the inflammatory but also the fibrotic phases of this disorder.

In asbestosis, a disease that shares many pathobiologic features with IPF, increased LTB_4 content has likewise been demonstrated in both BALF and AM conditioned medium [11]. The *in situ* stimulus for LT synthesis in the lungs of patients with IPF or other fibrotic diseases is unknown, but substances known to be both elevated in pulmonary fibrosis and capable of activating 5-LO metabolism include immune complexes, cytokines, endothelin and transforming growth factor-β.

These human data, of course, fall short of establishing a causal relationship between LT overproduction and fibrosis. Data consistent with a pathogenetic role for LTs in the evolution of fibrotic lung disease have been obtained using the rodent model of intratracheal instillation of the chemotherapeutic drug bleomycin. More than a decade ago, the first-generation (and not entirely specific) lipoxygenase inhibitor nordihydroguaiaretic acid was shown to markedly attenuate bleomycin-induced fibrosis and, concomitantly, the release of AM-derived fibroblast growth factor activity [12]. More recently, dietary γ-linolenic acid was reported to suppress bleomycin-induced fibrosis in parallel with bleomycin-induced elevations of lung LTB_4 content [13]. We have obtained preliminary data indicating that bleomycin-induced fibrosis is significantly abrogated in mice rendered LT-deficient by a targeted disruption of

the 5-LO gene. These knockout mice also show a dramatic reduction, as compared with wild-type mice, in the accumulation of macrophages, neutrophils, eosinophils and lymphocytes observed after bleomycin administration. Furthermore, when macrophages were isolated 7 days after bleomycin instillation and cultured, only cells from the 5-LO knockout animals constitutively expressed interferon-γ at the mRNA and protein levels. These results suggest that the protection from fibrosis observed in the LT-deficient animals could, among other mechanisms, also reflect a shift to a Th-1 type of immune response with upregulated expression of the antifibrotic Th-1 cytokine interferon-γ. A clinical trial with the 5-LO inhibitor zileuton in IPF is currently underway at our institution.

As the pathobiology of fibrosis in the pulmonary parenchyma is likely to be very similar to that in the subepithelial airway tissue, these findings may be highly relevant to airway remodelling in asthma. Indeed, cysteinyl-LT receptor antagonists have been shown to ameliorate the influx of inflammatory cells, the skewing towards a Th-2 immune response, and the increased smooth muscle mass and airway fibrosis observed in animal models of allergen-induced asthma.

Lung cancer

Cigarette smoking is the greatest risk factor for the development of lung cancer, but asbestos exposure and pre-existing chronic inflammation and scarring also contribute. Neoplasms arising in the lung can cause local manifestations, such as haemoptysis and bronchial obstruction with postobstructive pneumonia, but their morbidity also reflects dissemination to other sites. As lung cancer is often clinically silent and usually not detected until it has already metastasized, only a small minority is amenable to surgical resection for cure. Moreover, the efficacy of nonsurgical treatment modalities remains disappointing. As a result, lung cancer is the leading cause of death among neoplasms and the third leading cause of mortality from all causes in the USA.

As with other neoplasms, recent efforts to better understand the biology of lung cancer have focused attention on autocrine growth factors and the control of apoptosis. In colon cancer, there is now compelling evidence that metabolism of arachidonic acid via the cyclooxygenase pathway to prostanoids is upregulated in cancer cells and that these mediators inhibit apoptosis of these cells, thereby contributing to tumour growth. Cyclooxygenase inhibitors have thus emerged as a promising approach for the prevention and treatment of colon cancer. Although the knowledge base is more limited, recent information suggests the possibility that 5-LO metabolites might play a comparable role in lung cancer.

In vitro, 5-LO products have been shown to stimulate the proliferation of and inhibit apoptosis of a number of cell types. A similar growth-promoting effect of the 5-LO metabolite 5-hydroxy-eicosatetraenoic acid on lung cancer cell lines has recently been reported [14]. These actions may relate to the ability of 5-LO metabolites to promote expression of various oncogenes. In addition, primary lung cancer cell lines express both 5-LO and 5-LO activating protein (FLAP) and produce 5-LO metabolites in response to *in vitro* stimulation with the growth factors insulin-like growth factor-1 and gastrin-releasing peptide [14]. Three pharmacologically unrelated inhibitors of LT synthesis were capable of inhibiting growth of these cells *in vitro*. When lung cancer cells were injected subcutaneously into athymic mice, tumour size was significantly reduced and the number of apoptotic cells in the tumours was significantly increased by the chronic administration of the lipoxygenase inhibitor nordihydroguaiaretic acid [14]. Specific inhibitors of 5-LO and of FLAP were also shown to substantially reduce the number of lung tumours induced by the administration of a tobacco-specific carcinogen [15].

It is interesting to note that the relative risk of lung cancer is markedly elevated in the setting of certain interstitial lung diseases, including IPF [16], and is also modestly elevated in patients with chronic asthma [17]. These may be examples of 'scar carcinoma'. In conditions such as these, and perhaps in smokers as well, it is attractive to speculate that overproduction of 5-LO metabolites might provide a mechanistic link for the association of chronic inflammation, fibrosis and malignancy of the lung. Clinical trials with anti-LT agents will be required to test that hypothesis.

Antimicrobial defence

The increasing incidence of immunosuppression and the emergence of antibiotic-resistant microbes underscore the need to supplement antibiotic development by gaining a greater understanding of innate host defence mechanisms. It is hoped that by augmenting such endogenous mechanisms, the outcome of host–microbe interactions can be positively influenced. The roles of cytokines and chemokines in the host response to infection have received considerable attention. However, little was known about the possible role of LTs in host defence. Several years ago, we hypothesized that LTs were integral components of the host response to infection. Because in industrialized nations, pneumonia is associated with greater morbidity, mortality and economic cost than any other infection, we have focused

our studies primarily on the lung. Evidence supporting a role for LTs in antimicrobial defence is reviewed below.

1 LTs are produced at sites of infection. We have demonstrated increased levels of both LTB_4 and cysteinyl-LTs in homogenates of lungs of mice intratracheally inoculated with bacteria [18]. Elevated LTB_4 levels in BALF of patients with pneumonia has also been reported [19].
2 We found that 5-LO knockout mice had impaired bacterial clearance and impaired survival in a model of bacterial pneumonia, establishing an important role for endogenously produced LTs in pulmonary antibacterial defence [18].
3 Interestingly, the knockout mice may also model clinically relevant circumstances, since we and others have demonstrated that leukocytes from a number of patient populations known to be highly susceptible to infection, manifest an impairment in LT synthetic capacity [20]. This has been reported for HIV infection, diabetes mellitus, malnutrition, vitamin D deficiency and the newborn age group.
4 LT-deficient AMs and neutrophils (either obtained from knockout mice, or obtained from normal animals but treated with LT synthesis inhibitors or receptor antagonists) exhibit reduced bacterial phagocytosis and killing. These functional defects were overcome by the addition of nM doses of exogenous 5-LO metabolites. Although only LTB_4 was important for phagocytosis by neutrophils and for killing by either cell type, both LTB_4 and cysteinyl-LTs enhanced phagocytosis by AMs [21].
5 Neutrophils from patients with end-stage HIV infection exhibited a defect in fungicidal activity that paralleled their reduction in FLAP expression and in LT synthetic capacity. When granulocyte-colony stimulating factor was administered systemically to these individuals, concomitant increases were observed in neutrophil FLAP expression, LT synthesis and fungicidal activity: the enhanced fungicidal activity was blocked when a FLAP inhibitor was included in the neutrophil-fungus cocultures [22].

These results are interesting in light of the fact that colony-stimulating factors are the immunostimulants that are the furthest along in clinical development. Since they upregulate LT synthesis and since LTs share most of their biological actions, it is possible that LTs actually mediate much of the effects of these cytokines. We are interested in the possibility that direct administration of LTB_4 to the lung by inhalation may have distinct advantages over systemic cytokine administration as an immunostimulant strategy.

How can the above data supporting a role for LTs in host defence be reconciled with the fact that no increased incidence of infections has been observed despite the substantial worldwide experience with anti-LT agents in asthma? It must first be acknowledged that asthmatics do not have a high intrinsic susceptibility to bacterial respiratory infections. Second, most patients treated with this class of agents have received cysteinyl-LT receptor antagonists, and it is likely that cysteinyl-LTs are less important than LTB_4 in host defence. Third, asthmatics are LT overproducers at baseline, and treatment with anti-LT agents, which do not completely suppress LT synthesis or actions *in vivo*, would be expected to merely bring their LT production down into the normal range, rather than actually rendering them LT deficient in the way that a genetic knockout or *in vitro* treatment with anti-LT agents does.

Conclusion

A highly plausible rationale exists for implicating LTs in the pathogenesis of a variety of lung diseases other than asthma. With the development of anti-LT agents with acceptable potency, specificity and safety, it is anticipated that their application will be extended beyond asthma to the diseases discussed in this and companion papers. For many of these diseases, certainly including COPD, ARDS, IPF and lung cancer, a case can be made for the important involvement of not only cysteinyl-LTs, but also of LTB_4 and other 5-LO products. It is hoped that decisions regarding targets for pharmaceutical development are made with appropriate consideration of this fact. Of course, these opportunities for anti-LT therapy will need to be balanced against the possible risks of increased susceptibility to infection (Fig. 2). These risks would be anticipated to be of particular

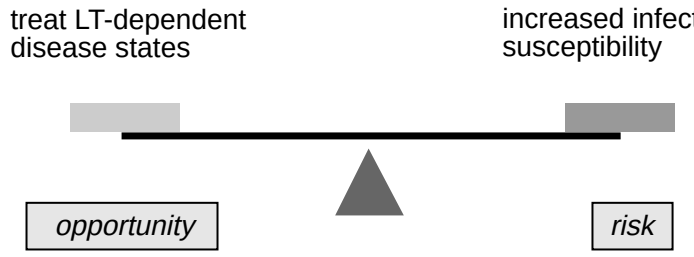

Fig. 2. Anti-LT therapy: balancing therapeutic opportunities against the risk of impaired antimicrobial defence.

concern if anti-LT agents were applied to diseases with high intrinsic susceptibility to infection (e.g. COPD, ARDS, IPF and lung cancer) or to patients with concomitant immunosuppression.

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