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-lipoxigenase (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 microatelectasis, 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.

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

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**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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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 LTB4 and Cys-LTs (LTs C4, D4 and E4), respectively.

Increased levels of both LTB4 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 LTE4 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], LTB4 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].

LTB4 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 LTB4 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 LTB4 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 production 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 LTB4 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, eosino-
phils and lymphocytes observed after bleomycin admin-
istration. Furthermore, when macrophages were
isolated 7 days after bleomycin instillation and cultured,
only cells from the 5-LO knockout animals constitut-
evously 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,
cysteinyLT 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 modal-
ities 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 can-
cer 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 subcuta-
neously 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 lipooxygenase 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 condi-
tions 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 LTB₄ and cysteinyllLTs in homogenates of lungs of mice intratracheally inoculated with bacteria [18]. Elevated LTB₄ 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]. 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.

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 LTB₄ was important for phagocytosis by neutrophils and for killing by either cell type, both LTB₄ and cysteinyllLTs 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 LTB₄ 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 cysteinyllLT receptor antagonists, and it is likely that cysteinyllLTs are less important than LTB₄ 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 cysteinyllLTs, but also of LTB₄ 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

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<th>Treat LT-dependent disease states</th>
<th>Increased infection susceptibility</th>
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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.

References


