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Introduction: The anti-inflammatory role of cysteinyl leukotriene receptor antagonists in asthma

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Considerable progress has been made toward defining the cellular elements and the key mediators involved in the inflammatory processes now recognized as the central pathogenic features of asthma. Multiple inflammatory cell types are capable of producing cysteinyl leukotrienes (CysLTs) and CysLT₁ receptors. Although few would have considered that inhibiting a single class of mediators would produce substantial therapeutic benefits, the inhibition or antagonism of the CysLTs has produced widespread cellular and mediator effects that ameliorate asthma's inflammatory response. Furthermore, the CysLTs may play a pivotal role in the development of airway remodeling in persistent asthma.

Airway remodeling remains an exciting area of investigation and, consequently, numerous paradigms have been proposed to connect this feature to the various severities and forms of asthma. Unfortunately, there are no clinically expeditious tools for directly measuring remodeling in individual patients at successive stages or states of asthma. Consequently, it is vital that biomarkers of inflammation be developed that can be obtained clinically to advance our understanding of both inflammation and remodeling as well as the extent to which the 2 processes are linked.

Abbreviations used

5-LO:	5-lipoxygenase
BAL:	Bronchoalveolar lavage
CysLT:	Cysteinyl leukotrienes
EMTU:	Epithelial-mesenchymal trophic unit
FeNO:	Fraction of exhaled nitric oxide
GCS:	Glucocorticosteroids
GM-CSF:	Granulocyte-macrophage colony-stimulating factor
ICS:	Inhaled glucocorticosteroids
IL:	Interleukin
LTRA:	CysLT receptor antagonist
NO:	Nitric oxide
SMC:	Smooth muscle cell

In December 2001, a roundtable symposium of invited experts was assembled in Key Biscayne, Florida, to review and discuss both basic science and clinical data related to the CysLT's inflammatory effects and the extent to which CysLT receptor antagonists (LTRAs) exert anti-inflammatory actions and reduce asthma symptoms. The essential contents of each presentation and the subsequent discussions were captured and included with each paper. To pull the concepts together and to explore the critical issues that relate to bronchial asthma, a more lengthy discussion was held at the end of each day's presentations.

The first of the 2-day meetings focused on mobile and structural cells involved in inflammation, and the mediator molecules they produce that affect the airway's structural or functional elements. The concepts of inflammation and remodeling were used to describe the core pathogenic processes in the asthmatic airway.

In asthma, there is a complex interplay among numerous inflammatory cells. Understanding the principal inflammatory processes involved will not only clarify

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asthma's pathogenesis but will refine therapeutic options as well. Qutayba Hamid, MD, PhD, FRCPATH (Director of the McGill Asthma Center and the Respiratory Axis at McGill University Health Centre, Montreal, Canada), Marc C. Liu, MD (Associate Professor in the Division of Pulmonary and Critical Care Medicine and the Division of Allergy and Clinical Immunology at Johns Hopkins University School of Medicine, in Baltimore, Maryland), and Redwan Moqbel, PhD, FRCPATH (Professor and Director of the Pulmonary Research Group at the University of Alberta, Edmonton, Canada), examined the cellular mechanisms involved in asthma's inflammatory processes and the influence of therapeutic agents, especially leukotriene modifiers, on these processes. Dr Hamid has studied the role of cytokines in asthma and allergic inflammation as well as the immunology of asthma. Dr Liu's research interests encompass the mechanisms of allergic inflammation and cell recruitment, the effects of prednisone on cellular responses, and the release of cytokines and mediators after segmental allergen challenge. Dr Moqbel is a recognized authority on the immunology of human eosinophils and their potential role in airway damage and asthma progression.

Dr Liu described several inflammatory cell types important in the pathogenesis of asthma: T-cells, mast cells, basophils, macrophages, and eosinophils, all of which are activated or increased in number in patients with asthma. Dr Hamid pointed out that these cells secrete cytokines, chemokines, and other mediators that perpetuate and augment the inflammatory response. For instance, the cytokines derived from the helper T cell T_H2 —interleukin (IL)-3, IL-4, IL-9, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF)—are all upregulated in patients with asthma and have been implicated with asthma pathology. Furthermore, Dr Moqbel indicated that the CysLTs are mediators of inflammation produced by the 5-lipoxygenase (5-LO) arachidonic acid metabolic pathway, and are now recognized as critical mediators of inflammation and modulators of cytokine function. Indeed, the CysLTs have been linked, via multiple mechanisms, to asthma's cardinal pathophysiologic features, among them mucus hypersecretion, increased microvascular permeability, ciliary activity impairment, inflammatory cell recruitment (including eosinophils), and edema.

Clinical studies of agents that target aspects of the inflammatory response in asthma have produced encouraging results. For example, omalizumab, an anti-IgE antibody, and zafirlukast, an LTRA, have been shown to inhibit the early- and late-phase responses to allergen challenge. Important pathophysiologic elements, such as activated T-cells and eosinophils, secrete cytotoxic and proinflammatory mediators including CysLTs that can cause mucosal damage in chronic asthma. Eosinophils obtained from asthmatic subjects appear to produce more LTC_4 than those from normal persons, and LTRAs, including montelukast, zafirlukast, and pranlukast, dampen the biologic activity of LTC_4 and downregulate eosinophil activity.

Is there a link between airway inflammation and remodeling in asthma? Stephen T. Holgate, MD, DSc (Medical Research Council Professor of Immunopharmacology and Director of Respiratory Cell and Molecular Biology Research at the University of Southampton, UK), Marc Peters-Golden, MD (Professor of Internal Medicine at the University of Michigan, Ann Arbor, Michigan), Reynold A. Panettieri, Jr, MD (Robert C. Mayock and David A. Copper Associate Professor of Medicine at the University of Pennsylvania, Adjunct Associate Professor at the Wistar Institute, and Director of the Comprehensive Asthma Program of the University of Pennsylvania Health System, Philadelphia, Pennsylvania), and William R. Henderson, Jr, MD (Professor of Medicine, Head of the Allergy Section, and Director of the Allergy and Immunology Training Program at the University of Washington in Seattle, Washington), explored the role of CysLTs in airway inflammation and remodeling in asthma, and presented a new paradigm for asthma pathogenesis. Dr Holgate has focused his research efforts on the cellular and molecular mechanisms of asthma, with a special emphasis on applications to diagnosis and treatment. Dr Marc Peters-Golden's research interest lies in the regulation of eicosanoid synthesis and the actions and role of eicosanoids in asthma, pulmonary fibrosis, and host defense. Dr Panettieri's interests are in cytokine modulation of smooth muscle function, leukotrienes, and asthma. Dr Henderson has performed innovative research into mast cell and eosinophil biology and function, of the interaction of these 2 cell types in inflammation, and of the formation and metabolism of the potent 5-LO products, particularly the leukotrienes.

Dr Peters-Golden noted that an accumulating body of evidence suggests that CysLTs promote airway inflammation and modulate airway smooth muscle cell (SMC) function, which are antecedent changes for airway hypertrophy and hyperplasia. Dr Holgate pointed out that atopy, although the strongest risk factor for asthma, does not inevitably lead to asthma. Although almost 50% of the population exhibits atopy, asthma actually develops in less than 10% of these persons. Thus, local factors—among them pollutant exposure, diet, and respiratory tract infection—probably play an important role in asthma development in predisposed persons. On his part, Dr Henderson noted that airway remodeling, once considered secondary to long-standing inflammation, has now been detected in early asthma, parallel with, or even obligatory for, the establishment of persistent inflammation. To account for the recent dramatic increase in asthma prevalence, environmental changes may permit the expression of preexisting asthma susceptibilities. Specifically, a new paradigm, the epithelial-mesenchymal trophic unit (EMTU), was introduced in which exaggerated parallel inflammation and remodeling of the bronchial epithelium are a consequence of abnormal tissue injury and repair processes. In asthma, the epithelium displays evidence of stress (activation of transcription factors, presence of activator proteins and heat shock

proteins), and the epithelium is an important source of elements, such as chemokines and growth factor, which create an ideal local environment for sustaining cycles of inflammation. As a result of a stressor, the EMTU activates the pathologic remodeling and smooth muscle proliferation that is characteristic of chronic asthma. Certain inflammatory cells are suggested to produce mediators of remodeling in asthma, including histamine, prostanoids, and CysLTs. They not only induce asthmatic SMC contractions but also promote SMC proliferation. Dr Panetier provided preliminary data suggesting a central role for CysLTs (and other 5-LO metabolites) in airway remodeling and fibrosis, as well as data hinting that CysLT receptor antagonists may inhibit LTD₄-induced airway endothelial cell proliferation. For instance, CysLT₁ receptor blockade with montelukast has blunted airway eosinophil accumulation, hyperplasia of smooth muscle and goblet cells, mucus plugging, and subepithelial fibrosis in ovalbumin-sensitized/challenged mice.

Asthma is a multifactorial disease, and CysLTs appear to enhance the inflammatory action of a variety of mediators. Thus, LTRAs may produce benefits that exceed those that would be expected from an agent targeting a single mediator. Glucocorticosteroids (GCS), the most common treatment for asthma, dampen the inflammatory action of a large array of mediators as well. The actions of GCS and LTRAs may not coincide, however. Marc Peters-Golden, MD and Anthony P. Sampson, PhD (Senior Lecturer and Deputy Director of the Respiratory Cell and Molecular Biology Division at Southampton University School of Medicine in Southampton, UK), examined the effect of CysLTs on key mediators of inflammation. They also explored whether GCS reduce CysLT levels in the airway. From a clinical perspective, understanding this link is important in determining the clinical value of combining GCS and LTRA. Dr Sampson's principal research interest is in the field of leukotrienes, especially as to why leukotrienes are overproduced in the inflamed airways of patients with asthma and allied diseases.

Dr Peters-Golden noted that the efficacy of leukotriene modifiers may relate to the extensive interaction between CysLTs and other asthma mediators. Asthma is characterized by the preferential production of cytokines derived from T_H2 lymphocytes. He provided evidence that CysLTs promote the generation of T_H2 cytokines, as well as other mediators that contribute to asthmatic inflammation, such as tumor necrosis factor α , and nitric oxide (NO). Conversely, many of these mediators, including IL-4, IL-5, and GM-CSF, augment CysLT production. Furthermore, the expression of the CysLT₁ receptor can be upregulated by certain cytokines, such as IL-4.

The GCS dampen numerous inflammatory pathways and yet, their effects are unpredictable in many patients with asthma. Dr Sampson reviewed data showing that GCS display a bewildering array of anti-inflammatory actions that suppress the expression of most cytokines and other molecules involved in the inflammatory process, with the notable exception of the leukotriene

pathway, thus raising the question of whether GCS reduce CysLT levels. Evidence suggests that the overall effect of GCS on leukotriene syntheses is neutral. In short-term studies, inhaled and oral GCS do not reduce leukotriene levels in the bronchoalveolar lavage (BAL) fluid or the urine of normal or asthmatic subjects. Long-term studies (>1 year), however, do indicate some inhibition of the leukotriene pathway in asthmatic patients treated with GCS. Furthermore, GCS appear capable of suppressing leukotriene synthesis in alveolar macrophages but not in neutrophils and monocytes. Dr Sampson suggests that the neutral effect of GCS on the leukotriene synthesis accounts for the additive anti-inflammatory effects seen when GCS is combined with leukotriene modifier therapy.

So many individualized definitions exist throughout the literature that, when "inflammation" describes asthma, and "anti-inflammatory" describes the mechanism of an asthma therapy, the words are not used consistently or clearly. In asthma, the term "remodeling" is used frequently, and its meaning is neither clear nor consistent. Similarly, if a drug is referred to as "disease-modifying," the meaning for that also needs to be clear. After these presentations on the first day, a general discussion ensued during which each participant could elucidate what he considered these terms to mean. This was not meant to be an academic exercise but was, rather, an important step toward achieving a collective understanding of what should be meant by these words and what the implications are for understanding the natural history and treatment of asthma.

The second day's presentations concentrated on the clinical use of available biomarkers of inflammation that could be used to objectively categorize asthma severity, and to ascertain which would be sensitive to "improvements" resulting from asthma therapy.

A growing realization that the importance of inflammation as a central pathophysiologic feature of asthma has spurred the need to identify useful biomarkers of inflammation. Emilio Pizzichini, MD (Professor of Respiriology and Head of the Asthma and Airway Inflammation Centre at the Universidade Federal de Santa Catarina in Florianopolis, Brazil), Hans Bisgaard, MD (Professor of Paediatrics in the Department of Paediatrics at Copenhagen University Hospital, Denmark), and Anthony P. Sampson, PhD, explored the utility and validity of various biomarkers of inflammation suitable for use in the asthma setting and the effect of CysLTs on these measures. Dr Emilio Pizzichini has focused on the epidemiology, pathophysiology, and treatment of asthma and chronic obstructive pulmonary disease, and Dr Hans Bisgaard has focused on pediatric asthma management, including inhaled drug delivery, lung function measurements, effects of inhaled steroids, and LTRAs in asthma.

Dr Sampson suggested that the biomarkers of inflammation can be obtained using a variety of methods varying in invasiveness. Among those described are blood and urine, BAL and sputum induction, and exhaled NO. Measurement of inflammatory biomarkers in urine may

be useful in assessing whole body LT production. For instance, a reversible and significant rise in urinary LTE_4 has been detected during acute severe exacerbations in asthmatic patients. Generally, urinary LTE_4 levels showed a correlation with persistent, severe asthma but no association with objective measurements of pulmonary function, bronchial responsiveness, or anti-inflammatory therapy. Measurements of CysLTs in the blood of patients with asthma are too variable to be a meaningful marker of inflammation, however.

Dr Pizzichini noted that, because of its safety and validity, the use of sputum induction by the inhalation of hypertonic saline to obtain airway secretions was one of the most important advances for assessing airway inflammation in subjects with asthma. Induced sputum is typically rich in neutrophils, whereas BAL is rich in macrophages and lymphocytes, suggesting a complementary role for these methods. Nonetheless, subjects with uncontrolled asthma present with sputum eosinophilia, a sign of active inflammation, and are responsive to GCS therapy. Furthermore, the concentration of CysLTs is significantly higher in the sputum of subjects with persistent asthma, and CysLT levels increase in these subjects after allergen challenge. The LTRA has been shown to decrease mean sputum eosinophil levels in asthmatic patients.

Dr Bisgaard reviewed data showing that the respiratory epithelium is an important source of NO and that the fraction of exhaled NO (FeNO) is increased substantially in asthmatic subjects when compared with normal controls, suggesting a role for exhaled NO as a biomarker of inflammation. The FeNO correlates with asthma symptoms, airway responsiveness, blood eosinophil levels, and sputum eosinophil levels; FeNO levels increase during asthma exacerbation. The LTRAs have been shown to reduce FeNO levels in children and adults with asthma and to block the increase in FeNO levels typically observed after GCS reduction. Dr Bisgaard surmised that FeNO may become a convenient method for detecting and monitoring asthma and that FeNO elevations in asymptomatic subjects may be an early marker of emerging asthma.

Because of the abundance of inflammatory processes involved in asthma, no single biomarker will assess the overall level of airway inflammation accurately. Instead, novel immunologic or genetic biomarkers, such as variant forms of the genes that encode for the LT pathway, may help identify subgroups of asthmatic patients in which specific inflammatory anomalies can be therapeutically targeted.

Dr Steven P. Peters' (Director of Research and Interim Director of the Division of Critical Care, Pulmonary,

Allergic, and Immunologic Diseases at Jefferson Medical College of Thomas Jefferson University in Philadelphia, Pennsylvania) principal research interests include the mechanisms of subepithelial fibrosis in asthma, eosinophil apoptosis, and the control of IgE-mediated pulmonary inflammation in humans. Dr Peters explored the role of LTRAs in asthma therapy, with particular attention to the heterogeneity of patient responses to medication, the failure of symptoms to correlate with commonly used endpoints, and the importance of medication delivery to distal airways.

The LTRAs such as montelukast have been shown not only to reduce markers of inflammation but also to improve measures of pulmonary function and reduce asthma symptoms. Despite these benefits, reservations that focus on two issues persist about LTRAs: variability of patient response and efficacy less than that of inhaled glucocorticosteroids (ICS) in some asthma endpoints. Dr Peters provided data showing that heterogeneity of patient response is actually a feature of asthma and asthma medications generally and not of LTRAs specifically. Thus, a major challenge in asthma management is to match asthmatic patients with a medication to which they will respond. Typically, physicians are more comfortable dealing with objective endpoints such as pulmonary function, whereas patients are more concerned with asthma symptoms and exacerbations. In fact, pulmonary measures, such as forced expiratory volume in one second, correlate poorly with respiratory symptoms in asthma, indicating that pulmonary function tests alone may draw an incomplete picture of controlled airways. LTD_4 inhalation in patients with asthma can exacerbate symptoms (wheeze and chest tightness) and pulmonary function, affecting both the large and small airways. The author proposes that some asthma symptoms result from dysfunction in both the large airways and the small airways of the distal lung, possibly secondary to the actions of CysLTs. These endpoints, which are not typically captured in clinical trials, may be especially amenable to treatment with LTRAs.

After this final formal presentation, the group discussed each participant's perspective on the value of biomarkers and which are most apt to facilitate clinical decisions of whether additional controllers are needed to treat the disease and what necessary role they may play in the diagnosis of asthma.

We hope that you will find this symposium as exciting as we did as participants and part of a panel of distinguished scientists aiming to elucidate some of the more challenging issues concerning inflammation and remodeling in asthma.