

Cysteinyl leukotriene interactions with other mediators and with glucocorticosteroids during airway inflammation

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Unexpected aspects of the antiasthmatic efficacy of leukotriene modifiers and glucocorticosteroids have been observed. For both classes, the observed effects may be partially explainable on the basis of underrecognized interactions involving leukotrienes. This review examines the interactions between leukotrienes and other mediators of asthma. It details the effects of glucocorticosteroids on leukotriene synthesis and on leukocyte populations in asthmatic airways. Unexpected controller effects of the leukotriene modifiers may reflect the fact that leukotrienes and other mediators of asthma, such as T_H2 cytokines, positively influence each other's generation. The ability of the leukotriene modifiers to disrupt such extensive interactions means that other relevant mediators are targeted indirectly by leukotriene blockade. Among asthma therapies, the glucocorticosteroids have numerous anti-inflammatory activities, but their effects may be unpredictable. Many processes involved in inflammation appear to escape modulation by glucocorticosteroids, including leukotriene synthesis, and leukotriene generation is among them. Understanding whether glucocorticosteroids reduce cysteinyl leukotriene levels in the airway is important in determining the clinical value of combining glucocorticosteroid therapy with leukotriene modifier therapy. (J Allergy Clin Immunol 2003;111:S37-48.)

Key words: Cysteinyl leukotrienes, glucocorticosteroids, leukocytes, asthmatic airway

Among asthma therapies, the most frequently prescribed are the glucocorticosteroids (GCS), which have a variety of anti-inflammatory activities. The GCS influence the regulation of numerous compounds involved in the inflammatory process, including a wide range of cytokines, adhesion molecules, and inducible enzymes. Nevertheless, GCS may have unpredictable effects on the inflammatory processes, and it is increasingly recognized that they should not be seen as "pan-anti-inflammatory" drugs, as many processes involved in inflammation escape modulation by GCS. Understanding the effects of GCS on cysteinyl leukotriene (CysLT) levels in the airway is important in determining the

Abbreviations used

BAL:	Bronchoalveolar lavage
BDP:	Beclomethasone dipropionate
COX:	Cyclooxygenase (PGH synthase)
CysLT:	Cysteinyl leukotriene
fMLP:	<i>N</i> -formyl-L-methionyl-L-leucyl-phenylalanine
5-LO:	5-Lipoxygenase
FLAP:	5-Lipoxygenase activating protein
IFN:	Interferon
IgE:	Immunoglobulin E
IL:	Interleukin
GCS:	Glucocorticosteroids
GM-CSF:	Granulocyte macrophage colony-stimulating factor
GRE:	Glucocorticosteroid response element
NO:	Nitric oxide
SDIA:	Steroid-dependent intractable asthma
TGF:	Transforming growth factor
TNF:	Tumor necrosis factor

potential clinical value of combining GCS therapy with leukotriene modifier therapy.

Because it is a truism that asthma is a multimediator disease, it is easy to be skeptical about the potential utility of any pharmacologic agent that targets only a single mediator in this disease. Although specific inhibitors of CysLT synthesis or action (leukotriene modifiers) are no panacea in asthma therapy, it can be argued that the extent of their efficacy is actually their most surprising feature. Certainly, their efficacy may be a function of the many actions of CysLTs that contribute to asthma biology. It also may reflect the fact that these agents are absorbed systemically and are thereby able to reach the small airways and that patient compliance with this class of oral drugs is good. One can argue, however, that another explanation for the utility of leukotriene modifiers relates to the extensive interactions that exist between CysLTs and other mediators of asthma. The ability of leukotriene modifiers to disrupt such interactions means that a host of other relevant mediators are targeted indirectly with their use.

This review examines the bidirectional interactions between CysLTs and other asthma mediators. It also will detail the effects of GCS on leukotriene synthesis *in vivo* and *in vitro* (including their effects on expression of the 5-lipoxygenase [5-LO] pathway enzyme) and how they affect leukocyte populations in asthmatic airways.

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EFFECTS OF CYSTEINYL LEUKOTRIENES ON OTHER MEDIATORS

Asthma and other atopic diseases are characterized by polarization of the immune response, with preferential production of a profile of cytokines derived from T_H2 lymphocytes, including interleukin (IL)-4, IL-5, and granulocyte macrophage colony-stimulating factor (GM-CSF) rather than from T_H1 lymphocytes, including IL-2, IL-12, and interferon- γ (IFN- γ). Such polarization is thought to drive an immune inflammatory response that is characterized by immunoglobulin E (IgE) production and eosinophilic inflammation, which are features central to asthma. Evidence suggests that CysLTs selectively promote the generation of T_H2 cytokines. Antigen-induced production by peripheral blood mononuclear cells of IL-4, IL-5, and GM-CSF but not of IL-2 was inhibited *in vitro* by the CysLT receptor antagonist pranlukast.¹ In a murine antigen-induced asthma model, *in vivo* administration of montelukast reduced lung levels of IL-4 and IL-5, whereas levels of IFN- γ increased.² Pulmonary eosinophilia induced in guinea pigs by the aerosolization of LTD₄ was abrogated by the administration of an anti-IL-5 antibody, implying that this T_H2 cytokine was not only produced but was a critical mediator of the CysLT effect.³ In a preliminary study, the increase in eotaxin expression in bronchial biopsy specimens obtained from subjects after antigen challenge was abrogated by pretreatment with zafirlukast.⁴ Recent data demonstrated that lung leukocytes from 5-LO gene-deleted mice produced greater levels of the T_H1 cytokine IFN- γ than did cells from wild-type animals.⁵ Information derived from a variety of experimental approaches, therefore, strongly indicates that CysLTs drive the generation of T_H2 cytokines and that leukotriene modifiers are able to reduce their generation. This ability of CysLTs to amplify T_H2 cytokine production represents an indirect mechanism by which they promote eosinophilic inflammation that supplements their direct ability to promote eosinophilic recruitment and survival. Because the abrogation of leukotriene synthesis or action either fails to inhibit or actually stimulates the production of T_H1 cytokines, leukotriene modifiers have the potential to promote polarization of the immune response toward a T_H1 phenotype.

The CysLTs also increase, and leukotriene modifiers inhibit, the production or expression of a variety of other mediators that may contribute to asthmatic inflammation. These include other cytokines such as tumor necrosis factor (TNF)- α and IL-6, endothelin, adhesion molecules, nitric oxide (NO), proteases, and reactive oxygen intermediates.⁶⁻¹² Moreover, CysLTs have increased the expression of histamine receptors on macrophages and smooth muscle cells, providing a mechanism for amplifying responses to this mast cell-derived mediator.¹³

EFFECTS OF OTHER MEDIATORS ON CYSTEINYL LEUKOTRIENES

Just as CysLTs amplify the production of a variety of other mediators relevant to asthmatic inflammation,

other mediators likewise enhance the capacity for CysLT generation. This phenomenon has been reported in all of the major CysLT-synthesizing cell types: mast cells, eosinophils, and macrophages. It has been observed for mediators including IL-4, IL-5, GM-CSF, eotaxin, endothelin, and transforming growth factor (TGF)- β .¹⁴⁻¹⁸ This phenomenon reflects a number of distinct molecular mechanisms, including increased transcription or translation of proteins involved in CysLT synthesis (including cytosolic phospholipase A₂, 5-LO, 5-LO activating protein [FLAP], and LTC₄ synthase), phosphorylation-dependent activation of some of these proteins, and alterations in their intracellular compartmentalization. Generation of CysLTs may actually be required for the biological actions of some other mediators. For example, pulmonary eosinophilic inflammation in eotaxin-treated IL-5 transgenic mice was abrogated by inhibitors of CysLT synthesis or action.¹⁹ Also, the ability of intrapulmonary administration of IL-13 to evoke pulmonary eosinophilia and bronchial hyperreactivity, as observed in wild-type mice, was impaired when performed in 5-LO knockout mice.²⁰ Not all inflammatory mediators amplify leukotriene synthesis; however, NO has been reported to inhibit the enzymatic action of 5-LO.²¹ This may help to explain why NO has been seen to exert anti-inflammatory as well as proinflammatory actions. Finally, expression of the CysLT₁ receptor, which mediates many of the relevant actions of these lipids, can also be upregulated by IL-4 and IL-13.²² It seems probable that some of the broad beneficial effects of leukotriene receptor antagonists (LTRAs) reflect their ability to interrupt the extensive bidirectional amplifying interactions between CysLTs and other mediators (and their receptors) (Fig 1).

INFLUENCE OF GLUCOCORTICOSTEROIDS ON CYSTEINYL LEUKOTRIENE SYNTHESIS: UNPREDICTABLE EFFECTS AT KEY SYNTHETIC STEPS

The GCS have a wide variety of anti-inflammatory activities, which are mediated by the interaction of GCS bound to their cytosolic receptors with GCS response elements (GRE) in gene promoters. About 20% of human gene promoters contain GRE sequences, amounting to some 10,000 to 15,000 genes, the transcription of which may be altered by GCS. In addition, even in the absence of GRE, gene transcription may be modulated by GCS through their indirect effects on the binding of transcription factors, such as NF- κ B, and on other cell-signaling processes, including posttranscriptional events. Gene products suppressed by GCS include a wide range of cytokines (IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-11, IL-12, IL-13, TNF- α , GM-CSF), adhesion molecules (intracellular adhesion molecule [ICAM]-1, vascular cell adhesion molecule [VCAM]-1), and inducible enzymes including NO synthase (iNOS) and cyclooxygenase-2 (COX-2). Transcription of other genes may be enhanced, such as lipocortin-1 and the β -2 adrenergic receptor.²³

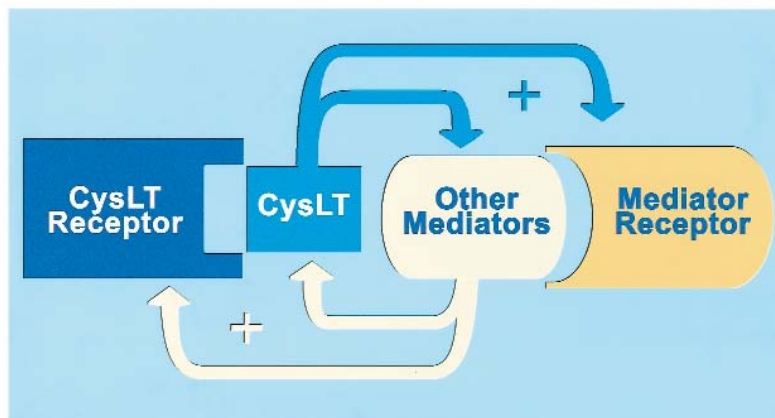


FIG 1. Interplay between CysLTs/CysLT receptors and other mediators/mediator receptors. CysLTs amplify the generation of other classes of mediators as well as the expression of receptors for other mediator classes. Likewise, other mediators amplify CysLT generation as well as CysLT receptor expression. By inhibiting either the synthesis or the actions of CysLTs, leukotriene modifiers disrupt these interactions and thereby indirectly target the actions of other interacting mediators.

Nevertheless, many inflammatory processes escape modulation by GCS, whereas GCS have paradoxical effects on other processes. Notable among these is the leukotriene pathway. Understanding whether GCS reduce CysLT levels in the airway is important in determining the clinical value of combining leukotriene modifiers with GCS therapy. Several clinical trials indicate an additive effect of the two drug classes on pulmonary function,²⁴ even in patients receiving high-dose inhaled or oral GCS, suggesting that leukotriene synthesis in asthmatic persons is resistant to GCS suppression.

The traditional view is that lipocortin-1 induced by GCS inhibits phospholipase A₂ activity, consequently reducing the synthesis of all arachidonate products, including leukotrienes and prostanoids (Fig 2). More recently, it has become apparent that glucocorticosteroids have variable effects on leukotriene synthesis in different cell types and act at key synthetic steps in the 5-LO pathway in ways that appear to be counterintuitive.

EFFECT OF GLUCOCORTICOSTEROIDS ON LEUKOTRIENE SYNTHESIS IN VIVO

There are two studies of relatively short-term GCS effects on leukotriene levels in vivo in normal subjects. In the first study, oral prednisone (60 mg/d) for 8 days failed to alter urinary levels of LTE₄ or prostanoid metabolites in 8 normal subjects.²⁵ Cells obtained by bronchoalveolar lavage (BAL) of these subjects showed reduced ex vivo synthesis of LTB₄ and prostanoids, however, when stimulated in vitro. In the second study, treatment of 5 to 6 normal subjects with inhaled budesonide (1600 µg/d for 7 days) or oral prednisolone (30 mg/d for 3 days), or oral dexamethasone (8 mg/d for 2 days) failed to alter urinary LTE₄ excretion.²⁶ Curiously, ex vivo synthesis of thromboxane A₂ by zymosan-stimulated peripheral blood mononuclear cells from these subjects was reduced after the GCS treatment.

Two short-term studies showed a similar lack of effect of GCS on leukotriene synthesis in asthmatic subjects. In a placebo-controlled trial in 10 asthmatic patients with mild atopy, treatment with fluticasone propionate (1000 µg/d) for 2 weeks impaired the early and late responses to inhaled allergen challenge and ameliorated baseline and allergen-induced bronchial hyperresponsiveness but had no effect on urinary LTE₄.²⁷ These results contrasted strongly with those of an analogous study in ovalbumin-sensitized rats, in which biliary excretion of *N*-acetyl-LTE₄ was 80% inhibited by GCS treatment.²⁸ In a study in 14 adults with asthma, up to 9 days of treatment with oral prednisone (30 mg/d) had no effect on BAL levels of LTC₄ and LTE₄ or urinary levels of LTE₄, either at baseline or after allergen challenge.²⁹

Few studies have examined the effect of GCS treatment periods longer than 2 weeks, but longer treatment may be required to affect leukotriene synthesis by modifying the populations of leukotriene-synthesizing cells in the airway. In one study, 22 asthmatic patients receiving terbutaline were randomly assigned to receive either beclomethasone dipropionate (BDP) (800 µg/d), or ipratropium (160 µg/d) or placebo for 2.5 years.³⁰ Compared with the pooled ipratropium and placebo-treated patients, the 9 BDP-treated patients had significantly improved pulmonary function, reversibility, and bronchial hyperreactivity. BAL fluid levels of LTC₄ were reduced from 16 to 9 pg/mL. BAL cell differential counts were not different. Further studies between 1 and 12 months in duration are required to clarify this finding.

EFFECTS OF GLUCOCORTICOSTEROIDS ON LEUKOTRIENE SYNTHESIS BY LEUKOCYTES

A number of studies have investigated the impact of GCS on leukotriene synthesis in a variety of leukocyte subtypes after stimulation by arachidonic acid, calcium ionophore, or receptor-dependent stimuli (anti-IgE, the

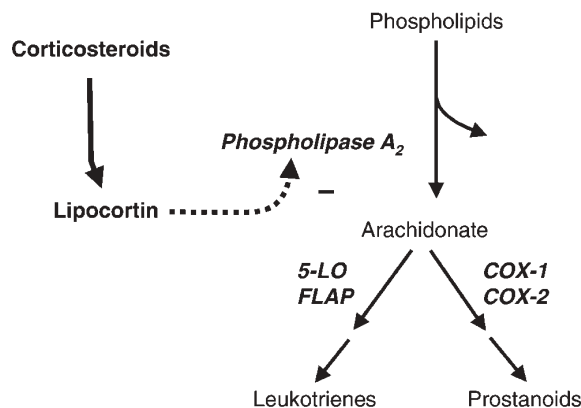


FIG 2. Traditional view of corticosteroid induction of lipocortin-1 leading to inhibition of prostanooid and leukotriene synthesis through suppression of phospholipase A_2 . This view is challenged by evidence of negative and positive effects of GCS at multiple sites in the 5-lipoxygenated pathway, with little or no impact on leukotriene levels in vitro in normal or asthmatic subjects.

bacterial *N*-formyl peptide *N*-formyl-L-methionyl-L-leucyl-phenylalanine [fMLP], zymosan). In most studies, leukocytes were treated with GCS for several hours in vitro, but some ex vivo studies involved leukocytes freshly isolated from subjects treated with inhaled or oral GCS. In both cases, the artificial environment in which cells were cultured and the requirement for in vitro stimulation may reduce the clinical relevance of the findings.

In alveolar macrophages isolated from rats, treatment with methylprednisolone for 16 hours in vitro reduced ^{14}C -arachidonic acid release associated with induction of lipocortin-1 and impaired synthesis of LTB_4 and LTC_4 .³¹ A study of alveolar macrophages from 3 human subjects showed that in vitro treatment with dexamethasone ($1\text{ }\mu\text{mol/L}$) for 16 hours reduced the stimulated release of LTB_4 by 35% to 85%.³² A similar study of methylprednisolone treatment of alveolar macrophages from asthmatic, atopic, and normal subjects for 16 hours showed a lower degree of inhibition of the 5-LO pathway than of the COX pathway, with no differences in response between the subject groups.³³

The sensitivity of the leukotriene pathway in alveolar macrophages to GCS inhibition contrasts with a relative lack of sensitivity in mast cells and blood leukocytes in most studies. The ex vivo effects of GCS therapy on alveolar macrophages and blood leukocytes were examined in 21 asthmatic patients and 6 patients with interstitial lung disease.³⁴ After stimulation with A23187, fMLP, or zymosan, alveolar macrophages from 9 GCS-treated patients showed a reduced capacity for synthesis of PGE_2 , TXB_2 , and LTB_4 . Inhibition of LTB_4 synthesis was modest in comparison with the prostanoids. Importantly, GCS treatment had no effect on stimulated synthesis of any lipid mediator in blood leukocytes when assessed either in whole blood or in purified granulocyte and mononuclear cell preparations. Consistent with this result, lipocortin-1 was induced by GCS in alveolar macrophages but not in blood leukocytes.³⁴

Resistance to GCS suppression of leukotriene synthesis in blood leukocytes has been demonstrated. Human blood monocytes cultured with budesonide (10^{-9} to 10^{-5} mol/L) for 20 hours displayed an unaltered capacity for LTB_4 synthesis.³⁵ Treatment of human blood neutrophils with dexamethasone ($1\text{ }\mu\text{mol/L}$) for 16 hours followed by stimulation with the ionophore A23187, fMLP, zymosan, or a phorbol ester similarly showed unaffected LTB_4 synthesis, chemotaxis, and granule enzyme release, although spontaneous adherence was reduced by 83%.³⁶

Basophils and mast cells are potentially important sources of LTC_4 in the human lung. Treatment of basophils with dexamethasone for 24 hours has been reported to inhibit LTC_4 release markedly.³⁷ In these early experiments without immunomagnetic purification techniques, however, basophil purity was variable and low (12% to 90%) by modern standards, and confirmation in highly purified basophils is desirable. The same researchers found no effect of dexamethasone on LTC_4 synthesis in mast cells isolated from human lung by enzymatic dispersion. Again, this negative result could be confirmed in immunomagnetically purified lung mast cells.

EFFECT OF GLUCOCORTICOSTEROIDS ON 5-LO PATHWAY ENZYME EXPRESSION IN LEUKOCYTES

The relative lack of effect of GCS on leukotriene release in neutrophils and monocytes has been explored in several studies examining the impact of GCS on cellular expression of 5-LO pathway enzymes. Leukotriene synthesis is initiated by the donation of free arachidonic acid from FLAP to 5-LO followed by conversion to LTB_4 and LTC_4 by LTA_4 hydrolase and LTC_4 synthase, respectively.

In human blood neutrophils, dexamethasone increased the expression of FLAP mRNA and protein, and this effect was synergistic with GM-CSF.³⁸ In the human monocytic cell line THP-1, treatment with dexamethasone (10^{-9} to 10^{-6} mol/L) induced FLAP mRNA and protein, but FLAP induction did not enhance LTC_4 synthesis. Induction of COX-2 by a phorbol ester phorbol myristate acetate (PMA) was inhibited.³⁹ In contrast, similar studies in THP-1 monocytic cells and in human peripheral blood monocytes showed that dexamethasone induced mRNA and protein expression for both FLAP and 5-LO but not for LTA_4 hydrolase.⁴⁰ It has been confirmed that dexamethasone induces 5-LO and FLAP expression and leukotriene synthesis in human blood monocytes and that the effect is additive to that induced by cytokine-rich supernatants from activated lymphocytes (Riddick et al, unpublished data).

The effect of IL-5 and dexamethasone on leukotriene synthesis and 5-LO pathway enzyme expression was investigated in immunomagnetically purified human blood eosinophils.¹⁴ With the use of immunohistochemistry, Western blotting, confocal immunofluorescent microscopy, and leukotriene immunoassays, IL-5 was shown to transcriptionally upregulate FLAP expression and to translocate 5-LO to the nucleus. These changes were accompa-

nied by an increased capacity for ionophore A23187-stimulated LTC₄ synthesis. Intriguingly, culture with dexamethasone (1 μ mol/L) for 6 hours also increased FLAP expression (Fig 3), and the response to dexamethasone was additive to that of IL-5. Dexamethasone did not upregulate LTC₄ synthetic capacity, however, suggesting that the effect of IL-5 is dependent on upstream changes, perhaps at the level of phospholipase A₂.

EFFECT OF GLUCOCORTICOSTEROIDS ON LEUKOCYTE POPULATIONS IN THE ASTHMATIC AIRWAY

A comprehensive survey of the effects of GCS on inflammatory cell populations in the airway is beyond the scope of this review, but it is clear that GCS do suppress airway populations of those leukocyte subtypes closely implicated in CysLT synthesis (Table I). The rise in bronchial biopsy counts of eosinophils that occurs after inhaled allergen challenge of atopic persons with asthma is markedly suppressed by pretreatment with beclomethasone.⁴¹ In a longer-term study, treatment of 10 atopic persons with asthma with 2000 μ g/d of beclomethasone for 2 weeks followed by 4 weeks at 1000 μ g/d produced marked improvement in pulmonary function (FEV₁, morning peak expiratory flow), asthma symptoms, bronchial reactivity, and use of albuterol (salbutamol).⁴² These improvements were contemporaneous with significant reductions in bronchial biopsy counts of mast cells and eosinophils within the epithelium and submucosa and in submucosal T cells.

In contrast, several studies indicate that GCS use is associated with enhanced neutrophilia in the airway and the blood, and this is linked to evidence that neutrophil apoptosis is suppressed by GCS. A study of long-term GCS use in asthmatic patients showed that those with steroid-dependent intractable asthma (SDIA) had a higher proportion of neutrophils in BAL than those with tractable asthma and that pulmonary function was particularly poor in those patients with SDIA with neutrophil differentials greater than 10%.⁴³ The European Network for Understanding Mechanisms of Severe Asthma (ENFUMOSA) study similarly suggested that neutrophilia was more prominent in steroid-dependent uncontrolled asthma.⁴⁴ Nevertheless, it is unclear whether the tendency to neutrophilia is a primary factor leading to a higher requirement for GCS therapy or if it is secondary to the use of GCS. Despite the neutrophilia in the patients with SDIA, these patients had fewer BAL mast cells, basophils, and lymphocytes and lower BAL cell release of LTC₄ and histamine.⁴³

CONCLUSIONS

Extensive interactions exist between CysLTs and other mediators. The CysLTs can increase the expression of T_H2 cytokines and a variety of other mediators implicated in asthma. They also can increase the expression of receptors for other mediators such as histamine. At the

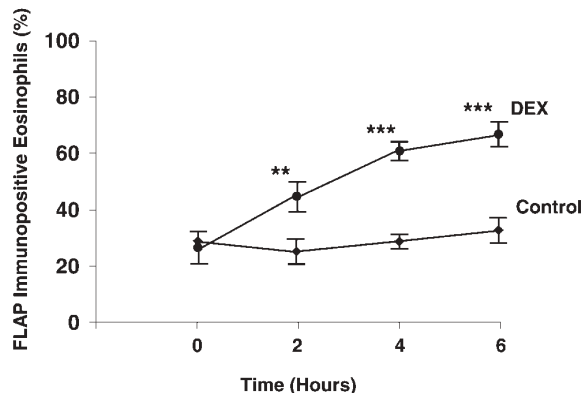


FIG 3. Effect of dexamethasone on FLAP immunostaining in normal eosinophils. Normal blood eosinophils were cultured for up to 6 hours in the presence (diamonds) and absence (circles) of dexamethasone (1 μ mol/L) before immunocytochemistry of cyto-centrifuge preparations for FLAP. Dexamethasone (DEX) significantly increased the proportion of eosinophils immunostaining for FLAP. Values are mean $\pm$ SEM. ** P < .005; *** P < .0001. From Cowburn et al. J Immunol 1999;163:456-65.

TABLE I. Effects of glucocorticoid therapy on leukocyte counts within the asthmatic lung and in vitro on both the expression of 5-LO/FLAP and on stimulated leukotriene synthesis

Cells	No. in Lung	5-LO/FLAP Expression	Leukotriene synthesis
Alveolar macrophages	0	?	↓
Mast cells	↓	?	0
Neutrophils	↑	↑	0
Eosinophils	↓	↑	0
Basophils	↓	?	↓
Monocytes	↓	↑	0 or ↑

Arrows indicate an increase or decrease, zero indicates no effect, and question marks indicate not investigated or conflicting data.

same time, a variety of cytokines and other mediators can amplify CysLT generation as well as CysLT receptor expression. Such reciprocal interactions result in a self-amplifying proinflammatory circuit.

The effects of GCS on leukotriene generation and expression of leukotriene pathway enzymes are exceedingly complex. Different outcomes can be observed, depending on the cell type and whether the GCS is administered in vivo or in vitro. Because GCS may inhibit phospholipase A₂ activity but increase expression of 5-LO or FLAP, their ultimate effect in a given patient may depend on which steps in the pathway are rate-limiting as well as the extent to which each can be modulated by these agents. The GCS also inhibit COX-2, and because PGE₂ inhibits leukotriene synthesis, GCS may indirectly amplify leukotriene synthesis by removing this prostanoid break on leukotriene generation.

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QUESTIONS AND DISCUSSION

William Henderson: Marc, How do lipoxins fit into this complex interaction and blend the two pathways together?

Marc Peters-Golden: Lipoxins are metabolites in which arachidonic acid is lipoxygenated at the 15 carbon, by 15-lipoxygenase, and then at the 5 carbon, by a 5-LO. This 5-,15-double-lipoxygenation product is a lipoxin. When synthetic analogs of lipoxins are administered to animals in various models of inflammation, their effects are quite impressively anti-inflammatory. Their endogenous role is less well established, however. Simply speaking, the idea that anti-inflammatory 5-LO product (namely, lipoxins) exists goes against a substantial body of information. What do you think?

William Henderson: We did early work with human and horse eosinophils, and eosinophils do form large amounts of lipoxins. After 5-LO products, they are second. We looked long and hard to find biological activity for lipoxins because reports suggested that they had potent chemotactic activity. However, we were unable to reproduce that with the ones we had synthesized.

Stephen Holgate: I think this integrated model that you presented with the leukotrienes as a passenger of multiple feeding points is a very attractive one that probably needs to be thought through a bit further. It's as if the leukotrienes are a mediator that alters the set point when tissues or cells will respond to lots of different stimuli and the gain with which they will respond. That's probably the way we are thinking about steroids: They affect the set point of multiple different cells, and that's probably why they are so effective. Any one cell is not going to determine the equation, but rather the whole gamut of cells. I think this concept that inflammation has mediated pathways to which multiple inputs can alter the gain is very attractive. It reminded me of earlier work that used inhibitors for histamine, leukotrienes, and prostaglandins, and for each combination of them. You could never get an additive inhibition of allergen-induced or exercise-induced asthma but an unexplainable interaction for which you could not make the numbers add up properly.

Mark Liu: Tony, basophils make a lot of IL-4 and IL-13 on a per-cell basis, probably more than lymphocytes. The dose-response of IL-4 and IL-5 to corticosteroids is much more sensitive than is the response of leukotriene synthesis to corticosteroids. Hence, there is dissociation between the corticosteroid effects on leukotriene production and other cytokine production in the basophils.

Anthony Sampson: Corticosteroids do appear to be inhibitors of leukotriene production by basophils of low purity, but studies of GCS effects on 5-LO pathway enzyme expression in highly purified basophils are required to quantify this fully. Dissociation between the effects of GCS on the leukotriene pathway and on cytokines such as IL-4 and IL-13 in basophils would strengthen the argument that the leukotriene pathway

generally is unresponsive to steroids, hence the case for combining GCS and leukotriene modifier therapy. Of course, this does presuppose that basophils are important sources of CysLTs in the asthmatic lung, of which I am skeptical.

Marc Peters-Golden: From our studies that you cited in alveolar macrophages using the same protocol with both rat and human alveolar macrophages, corticosteroids inhibited leukotrienes in human macrophages by only 20% (inhibition of prostaglandins was much more) and by approximately 85% in rat alveolar macrophages. Corticosteroids appear to have an important species difference in alveolar macrophages.

Anthony Sampson: The only cell that seems to show an almost complete inhibition of leukotriene synthesis is the human basophil, and we do not understand why.

Stephen Peters: We treated human volunteers with oral steroids for several days, labeled their alveolar macrophages, and then stimulated them ex vivo. The protocol had a few hours when the macrophages were not exposed to corticosteroids. Whatever inhibition we got was lost very quickly after removing macrophages from the body and re-exposing them to GCS. The insensitivity is really quite fragmented.

Marc Peters-Golden: Are you suggesting that steroids do not inhibit phospholipase effects?

Anthony Sampson: I don't know exactly what steroids do to phospholipase activity in the long term. By blocking cytokine synthesis, GCS may reduce tyrosine kinase-dependent phosphorylation of cPLA₂, leading to reduced enzymatic activity. Also, fluticasone has been shown to reduce cPLA₂ mobilization to the nuclear envelope and inhibit LTC₄ synthesis in stimulated eosinophils. GCS may also have differential transcriptional effects on cPLA₂ isozymes, whereas secretory PLA₂ has also been suggested to play an important role in leukotriene synthesis in eosinophils. Overall, effects seem to be very time- and cell-dependent.¹

Marc Peters-Golden: Many people have observed steroid inhibition of both cytosolic PLA₂ and secretory PLA₂ expression. So, I assume that there is an inhibitory effect, and I thought that the neutral effect of steroids on leukotriene production may be a reflection of the fact that there may be less arachidonic acid, but more 5-LO and FLAP to process it, and those cancel each other out.

Anthony Sampson: I think that's probably right.

Stephen Holgate: It strikes me as an area in need of better, more comprehensive studies in which one looks at more than one steroid-dependent end point in a larger number of people. This is such a crucial topic. It's very confusing as it stands, and it sounds as if an integrated approach needs to be taken, looking at the number of different targets in a larger population of people to determine what the bottom line is.

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DISCUSSION OF INFLAMMATION AND REMODELING: STEPHEN HOLGATE, CHAIRMAN

Stephen Holgate: I want to capture a component of what is important if we are trying to understand the role of leukotrienes as mediators beyond just smooth muscle contraction and vascular permeability to inflammation and remodeling.

First of all, when we use the word inflammation, can we think about cells, beyond just mobile cells? Think about some of the structural cells that we've been hearing about that we normally do not associate with inflammation.

Second, think about compartments, because we can only measure things in certain compartments at the present time. However, the clinical expression of asthma often does not reflect what we're actually measuring in those compartments. Urinary leukotrienes are a good example. Another is that the lower part of the lung provides 95% of the surface area, but we cannot get near these parts.

Third, what are the interactions between cells in inflammation and altered structure? Recruitment as a part of inflammation is a very attractive concept we need to be thinking about because inflammation is a normal body response, but in a disease such as chronic asthma, it gets out of hand, and many other events persist.

Finally, inflammation in asthma often has been defined on the basis of its response to corticosteroids. The anti-inflammatory effects of a corticosteroid have been set as the benchmark against which all other drugs must be measured. This is a very restrictive way of defining cell-cell interactions that contribute to inflammation that we have been thinking about. What terminology would you prefer to use to capture the concept of what inflammation really is? While inflammation is a difficult thing to define, try to loosen your vision of inflammation and look at the broader context of what these particular interactions are all about and how different drugs may interfere with them.

Marc Peters-Golden: Historical notions of what the word inflammation means bind us. The most useful thing would be to throw out the term inflammation because as we try to discuss a given aspect of inflammation, we're constrained. Some would say that inflammation is confined to a consideration of mobile cells, but asthma involves altered phenotypes of structural cells, too. Does that mean that, by definition, when we talk about the altered phenotype of a structural cell, we're not talking about inflammation? We paint ourselves into a corner that isn't at all useful. What inflammation means depends on what we want it to mean in a particular context. I suggest that we dismiss the term and not use it because as we try to discuss a given aspect of inflammation, we're constrained. Some would say that inflammation is confined to a consideration of mobile cells.

When we talk about altered cellular phenotypes, we have a whole variety of individually altered cells, and we

talk about them interacting with each other. Leave the term inflammation out of it and avoid needless arguments. Some people simply think of inflammation as something that can be inhibited by steroids and, if steroids do not inhibit it, then it is not inflammation. That's just not a useful paradigm.

Stephen Holgate: You are right. This is a critical issue. It reminds me of how cancer researchers thought that all cancer was caused by mutagenic changes within cells that, as a consequence, turned cancerous and caused the tumor. This resulted in all therapies being directed at these mechanisms. Now the cancer literature shows that malignant transformation and subsequent tumor growth is as much about the environment where the tumor cells are found as problems with the cell cycle itself. More emphasis is being placed on what makes the blood vessels grow to maintain tumor growth, why a tumor metastasizes to one area and not another, and what it is about the microenvironment, in some organs and not others, that enables the cancer to thrive and grow. In some very elegant studies, they are targeting the "soil" rather than the "seed," if I could use these terms, as a therapeutic area for intervening in cancer. Angiogenesis is one good example, but it's not the only example.

In asthma, we have gotten completely hooked on the "seed." We totally focus on T_H2 cells, eosinophils, or mast cells, but without thinking about the context within which these cells contribute to the disease process. A picture of an asthma death shows some inflammatory cells in alveoli, but we do not generally regard asthma as a progressive disease involving the alveoli. We must ask serious questions about why that is, because the inhaled environment does penetrate to the lung periphery, and all the mobile cells supporting asthma are also potentially present at this site.

Marc Peters-Golden: All the compartments matter, and I would not discount the airway surface, the subepithelial compartment, or the vascular compartment. All are important. We must recognize that different compartments are going to be better or worse for telling us about different components of the disease.

Redwan Moqbel: The definition that we teach medical students is that inflammation is the response of living tissue to injury. In asthma, where is the injury, and where did the injury actually start? Is the epithelial damage the start of the injury that leads to inflammation, or is it the other way around, that the T cell induces the processes that result in epithelial damage?

Once the airways and the lung begin to face those changes, there are horrendous mechanical pressures on the airways that force a change in its morphology. You have that constant constriction that is pulling the airways inward, which induces a number of changes that have been shown in vitro. The forces actually induce fibrogenic responses. Which is the chicken and which is the egg? We have yet to identify where the actual injury starts if the inflammation is a response to injury. Is it an event that takes place very early in life? Is inflammation something that begins simple and becomes more complex with time?

The whole combination of different cell types in asthma is like a society. It is the cross-talk between so many different cell types that ultimately results in either homeostasis or a diseased condition if the balance is lost. If we assume that we're going to be able to find one key cell and one key mediator to explain it all, we are on the wrong track. Therefore, we have to accept the complexity and the networking that takes place in the airways and try to understand the complexities.

I think the body compartments respond in totality. Whether it is from the brain or other organs, other compartments become involved. Because the circulatory system messengers, like hormones, take messages everywhere, the disease can move from large airways to alveolar spaces.

We're moving away from where we think the asthma is, and gradually we're going to realize that it actually goes beyond this. This is a jigsaw puzzle disease in which the pieces of the puzzle uniquely fit in only one place. Unique to a biological jigsaw puzzle is that there is redundancy in the system. There is a built-in capacity of the pieces (cells) to respond differently if there is the biological need for them to do something that they are not usually involved in—homeostatic control. Therefore, we may never find the ultimate drug to treat every particular aspect of the disease. With the genetic code, we may be able to identify the success of particular drugs. Treatment may be a combination treatment that is going to bring genomics, proteinomics, and therapy together.

Stephen Holgate: I think that this same debate is going on for other chronic inflammatory diseases, for example, rheumatoid arthritis and inflammatory bowel disease. Hans, give us another angle on this.

Hans Bisgaard: I am coming from a slightly different angle than the rest of you. I am a clinical pediatrician doing clinical research. In clinical research, and particularly in pediatrics, the name of the game in asthma research is heterogeneity. When I hear these presentations of very exquisite data, you always seem to define asthma as one phenotype of asthma. It is becoming increasingly clear that there are many subtypes of asthma within age groups and between age groups, and I think we have to try to use models to help us define subgroups of asthma within a certain age range. We are seeing good data suggesting that asthma has a different pathology at different times during life. In infancy, the eosinophil just isn't the major player. It's not primarily an eosinophilic-driven inflammation in the early years of life. Later in life, the eosinophils become more dominant. Since the pathophysiology changes through life, heterogeneity of the disease must be considered before we really understand inflammation and remodeling.

Stephen Holgate: That's a very nice way of stating that we do not know enough about the natural history of the different phenotypes of asthma diseases from birth to late in life. And I think that the work of Dr Fernando Martinez and others, who have tracked clinical phenotypes through childhood, is therefore quite relevant. Obviously, this becomes incredibly important therapeutically.

Hans, a few years ago it was very popular to talk about infantile wheezing as wheezy bronchitis. But because that involved an antibiotic prescription quite often, pediatricians and general practitioners were asked to think about this in terms of asthma and use that terminology. So in fact, in some ways, we have gone backward in that all wheezing in childhood is called asthma by the family doctor now and gets a steroid or whatever therapy. And, of course, that's really blunted intellectual thinking in the subcompartments that you were describing a few moments ago about these different characteristic features. What do you think we should be trying to do to improve the situation, because part of the reason we are here today is to try to think ahead a bit about what needs to be done to help redefine and reclassify some of the process that we've just been referring to?

Hans Bisgaard: I think we're just beginning to realize that there are different pathologies throughout life. There was the recent publication from Sally Wenzel's group in 15-month-old babies with clinically defined asthma that appeared to be predominantly driven by macrophages and very few eosinophils. In our best studies, we can control about half of the exacerbations, leaving the other half uncontrolled. A different pathophysiology explains why steroids are less effective in this age group than in older children. But this is probably just one example of patient heterogeneity. Lumping all patients together is bound to result in treatment failure.

Stephen Peters: I think your terms "inflammation" and "remodeling" are sufficiently imprecise and ambiguous. They're useful for us to speak to our primary care colleagues but not to other specialists. Until we operationally understand them better, we can still talk specifically about subepithelial fibrosis or eosinophil granulation or epithelial phenotype changes.

I was struck by how much we really don't know about the integrative pharmacology of the leukotrienes, such as, what cells make them? How can we affect them by various drugs? By integrating in vivo and more complex in vitro models, there is a potentially fascinating and exciting area that the pathophysiology of the CysLT₂ receptor will open up for us.

I'm struck the most by the disconnect between the relatively modest clinical effects of the LTRAs in adult patients with asthma and the dramatic effects that Bill Henderson saw in his mouse model. If these are important remodeling and airway changes that are very important early on, then an early intervention study needs to test the hypothesis in humans.

Marc Peters-Golden: Of course, we must keep in mind that we are comparing a relatively simple animal model with a complex human disease. However, there are several possible explanations for different effects of antileukotrienes in the two circumstances. First of all, montelukast may be able to do things in a mouse that it can't do in a human. This is because species differences determine which leukotrienes are made. For example, in human idiopathic pulmonary fibrosis, we found higher levels than normal of LTB₄ and LTC₄ in lung

homogenates, and LTB_4 was 10 times higher than LTC_4 . In the bleomycin-treated mouse model, it is the opposite—10 times more LTC_4 in the lung than LTB_4 . These differences mirror exactly what alveolar macrophages from the two species make. Human alveolar macrophages make 10 times more LTB_4 than LTC_4 . Murine alveolar macrophages make 10 times more LTC_4 than LTB_4 . That is rarely talked about. Eosinophils or mast cells produce the same relative amounts across species, but macrophages, if they are important, have very different profiles according to species, and this difference may be important in certain models we use.

Second, in some instances we are comparing the effects of a CysLT_1 receptor antagonist with the phenotype observed in 5-LO knockout mice. It is certainly possible that 5-LO products other than CysLTs may be important, or that CysLT receptors other than CysLT_1 may contribute to the pathologic responses under investigation. For all these reasons, the current generation of CysLT_1 receptor antagonists may not represent the ultimate potential of what antileukotriene therapy may offer.

Reynold Panettieri: I think species differences are very important when you look at contraction as readout of smooth muscle function. We need to use very high doses of LTD_4 to get bronchoconstriction in a mouse, whereas in humans, LTD_4 is the most potent bronchoconstrictor known. The differences may be in the receptor level and signaling postreceptor, which we know nothing about.

Bill Henderson: Corticosteroids do not appear to be asthma disease modifiers, as indicated by the observations that if they are stopped, the asthmatic patient returns to their baseline lung function and indices of airway inflammation. An important goal is to determine whether CysLT_1 receptor antagonists may be disease modifying by preventing and/or reversing such airway remodeling changes as fibrosis in patients with asthma.

Stephen Holgate: Is it that we cannot get close enough to the origins of the disease? Although we can measure things that happen at a moment in time in the course of the disease, we don't really understand what it is that initiates the process. Why do some atopic people develop inflammation in their lower airways and others do not? It's a very fundamental question when approximately half the population is atopic in the westernized world.

Another aspect of asthma that is striking is how little we understand about the underlying mechanisms of bronchial hyperresponsiveness. We actually do not know why the airways become hyperresponsive, and why some people's BHR is responsive to corticosteroids and others' is not. BHR is regarded as a physiological feature at the core of asthma, and, therefore, we need more focus on what different components of inflammation do and do not correlate with BHR.

Anthony Sampson: First, I agree that the terms "inflammation" and particularly "remodeling" are imprecise and inadequate to describe the various phenotypes that you see in asthma. We can define inflammation any way we want, but it's how we measure the components that is

important. What worries me a lot is how to measure remodeling processes in a relatively noninvasive way. Remodeling of airway structure is unlikely to generate soluble mediators or cellular traffic that can be monitored at downstream sites. If remodeling cannot be readily and reliably measured, then there's a risk of it becoming a nonproductive hypothesis.

Second, the original definition of asthma, I think it was by Galen, included redness, heat, swelling, and pain. Three of those are vascular in origin, but my impression is that there is relatively little work going on within the asthma field into cell trafficking at endothelium, vascular remodeling, and angiogenesis and so on.

Finally, it's clear that there will not be a single ultimate cause or grand unified theory of asthma. Maybe 30 to 40 mechanisms will be involved, and the relative importance of these will vary between individuals. You may ultimately have 30 or 40 drugs, antibodies, or other therapeutic approaches, and a limited cocktail of them will be applied, based on which ones work best for the individual subject. Asthma therapy will be a complex process with a lot more choices than now.

Stephen Holgate: It is quite interesting that geneticists are suggesting that asthma is a disease of a restricted number of genes. The way it is inherited can be accounted for on the basis of 12 to 15 major genetic pathways, which is not a huge amount.

I think if one used a hierarchy, then there might be something in all of this. I was very struck that some rheumatoid patients have x-rays showing that the anti-TNF treatment produced healing of erosions. It's very dramatic. Now, who would have ever have guessed a few years ago that reducing one cytokine could do that?

Emilio Pizzichini: I am also a clinician and see patients who usually complain of wheezing, dyspnea, cough, and phlegm. You need to treat them. Some will improve and some will not. Inflammation is the cause of these symptoms, but the definition of inflammation depends on the instruments one uses. We have instruments (sputum examination) that can measure most of the components of airway inflammation in asthma and other airways diseases. However, up to now, this instrument does not help us entirely discriminate the role of cells and their proinflammatory effects in the whole context of the inflammatory process. Also, we do not yet have reliable markers of structure-related changes.

We have used induced sputum in clinical practice in uncontrolled asthma and exacerbations of asthma. The profile of inflammation is not always the same. More frequently, they are eosinophilic, but sometimes they are not. In patients with eosinophilic inflammation, the use of steroids will improve their condition. However, up to 40% of the patients present with an inflammatory response that is noneosinophilic. In this situation, steroids appear to be less effective or ineffective. We do not know what happens if we were to follow the inflammatory response in these patients for 2 to 5 years. In these patients, perhaps a key environmental factor might play a role, such as viral or bacterial infections.

Mark Liu: I think that there is little doubt that chronic inflammation can lead to a lot of changes. And when I look at a disease like asthma, I think of remodeling as something that may underlie hyperresponsiveness or another physiologic phenomenon that we can't quite explain because inflammation or anti-inflammatory therapy doesn't explain it all. But it's certainly not a disease such as chronic obstructive pulmonary disease (COPD) or emphysema that progressively deteriorates and clearly has morphologic sequelae to it.

I also look at the issues related to the heterogeneity of the disease from people who die of severe attacks of asthma without a lot of inflammation, to people who have a progressive disease along the lines of COPD. But asthma is not a disease of mortality, and people are not progressively deteriorating despite the ongoing inflammation and structural changes. I like the idea that inflammation and remodeling are parallel pathways but have a lot of intersection points.

I think it's important to answer the questions of whether or not the developing lung responds differently to anti-inflammatory therapy such that it would fix the "soil" or modify the "soil" so that the "seed" of allergy does not have a place to grow in the lower airway. Local factors may be critical to localizing the generalized atopic diathesis.

In addition, the responses of the nervous system cannot be ignored. They are rapid, with clear plasticity, and affect airway function. The integrated effect of mediators, cytokines, nerves, and blood vessel is extremely complex.

The small airway is where it is, and large airway changes are not a big deal. A lot of disease in the "silent" small airways can appear to be subclinical until a certain level is reached when the principal clinical symptoms of asthma are manifested. What goes on in large airways may well reflect what goes on in the more distal small airways because there is not necessarily a disconnection between those two aspects. I think that you make decisions based on what you can measure and make inferences about those areas that we'll never be able to practically study.

Finally, inflammation is what we can treat and I do think that the data does support leukotriene modifiers as being anti-inflammatory, especially in the classic sense of cell recruitment, vascular permeability, and other responses of vascularized tissue to injury.

Stephen Holgate: Very good. I think this concept of thinking about the disease as an integrated response is quite an interesting one because it can be thought of at several different levels. It can be integrated between inflammatory cells and mediators. It can be integrated in a relationship of inflammation to the structural elements of the airway wall. And it can be integrated in terms of the whole body's response to these interactions, and the complexity of all of this is now just beginning to be realized.

Stepping out of the rather restricted box we were in a year or two ago into this broader domain is quite constructive. It helps us think about the disease differently and possibly closer to the patient's perception of the disease. Rey, would you like to add some pearls?

Reynold Panettieri: We need a broad definition of inflammation, and I think it is the following: trafficking leukocytes interacting with structural cells that render the secretion of soluble mediators or cell-to-cell interactions that have physiological consequences. Interactively, the soluble mediators feed back and affect the trafficking leukocytes.

The contents of each compartment can be measured, but at no time can you measure comprehensively all the events that are going to have the comprehensive answer. Remodeling is simply persistent structural abnormalities, but we need the definition to include something that is clinically or physiologically relevant. For example, if you show a subbasement membrane of fibrosis but the FEV₁ was not changed (which is in the vast majority of the literature to date on ICS) and it doesn't have clinical or physiologic relevance, the fibrosis is a scar and it's nothing more and nothing less. Mark Liu has mentioned that most people don't progress in disease severity, but the ones that do progress are very sick. Is it a sick asthmatic who developed this disease at age 5 that suddenly loses lung function beginning later in life and lasting until death, or does the disease actually progress with incremental changes over a long time period?

We don't know the natural history of asthma, and until we know it, any interaction that's going to affect the persistent structural abnormalities of physiologic relevance is not going to be determined.

Qutayba Hamid: Rey, I agree with your definition of inflammation. But what is it that makes inflammation persistent? In some patients, the cells continue to be activated and inflammation persists; in other patients, the activation resolves.

The other thing that we didn't cover today and which really has attracted a lot of attention during the last 2 years is the relationship between inflammation and infections, viral or bacterial infection. There is growing literature of the regulation of the inflammatory response by infectious material, including products of bacteria.

Part of the literature still indicates that LTRAs have no effect on inflammation. Everybody I've asked thinks that LTRAs have an effect on inflammation. I think that it is the heterogeneity of the disease that explains such differences.

Stephen Holgate: The concept of heterogeneity is something that must come through from this discussion. We are not talking about a single disease but a spectrum of disease processes with different inputs, some of which are going to be more sensitive than others to specific interventions. This is a very mature and sensible way of thinking about a complex disorder like asthma.

Qutayba Hamid: Everyone defines remodeling differently. We don't need to come to a universal agreement. Some define it only by epithelial fibrosis while others include all the structural changes. Others say it is a change of the phenotype of the cell without even structural changes. All definitions are relative to the nature of study. Any report needs to describe what is meant and what is being measured. We don't need to have a universal definition.

Stephen Holgate: A relatively new concept is that some of these pathophysiological events may happen very early in life and has shaken-up the debate somewhat. It is very important to focus studies on what structural events occur during the very first years of life and what inflammatory functions can be measured clinically. The course of asthma seems to be preset from an early age.

Qutayba Hamid: In addition, we need to know why people make the assumption that a child may grow out of their asthma. Clearly understanding why inflammation persists up to a lifetime in some patients and does not in others is very important, and perhaps viruses or bacteria play a unique role.

Mark Liu: However, although there may be progression of asthma in some people, we need to consider that it might have something to do with how we are treating them or something related to our therapy. While trying to help with therapy, we may be altering a pathway that then leads to more harm than good.

Stephen Holgate: As scientists, I think we need to start measuring what effects drugs or interventions have on the components of what we now are generalizing as inflammation. I would prefer we use a concept such as antileukotriene, which has one set of properties, or an

anti-mast cell drug that has another set of properties rather than say, "One group of drugs is anti-inflammatory, and another is not." This has important implications for linking the concept of influencing an aspect of airway inflammation in different ways to the use of the term "controller" drug.

Corticosteroids have effects on structural cells as well as on circulating cells. Recent studies indicate that inhaled corticosteroids reduce BHR in COPD, and yet there is no discernable effect on airway inflammation. The term anti-inflammatory has been a convenient parlance that has not worked very well at improving our understanding of how the different agents work. Rather, let us describe what we see or measure until we can get some molecules and identify the cause of disease. The conclusion is really about what's clinically measurable. If we have to openly admit that we just don't have the instruments yet, then we need to go out and try and find them. It's no good just saying we can't measure these things, therefore, let's ignore them. With new methods of imaging and new biomarkers of tissue turnover using various methods that were presented (exhaled air, sputum, BAL, or whatever), it may be possible to understand a series of processes that do reflect the natural history of asthma.