CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor*

Balancing the Effect of Leukotrienes in Asthma

Catherine Godson, Ph.D.

Inflammation and its timely resolution represent a vital physiologic response to infection, injury, and "sterile provocation" by endogenous stimuli. There is a growing appreciation that the initiation, progression, and resolution of inflammation and the return of tissue to its original state are the result of dynamic processes. The mediators and mechanisms that drive acute inflammation are well defined and are the targets of numerous antiinflammatory drugs. But effective resolution of inflammation rests not only on a dissipation of the signals that drive it but also on the activation of specific cell types and the generation of soluble factors, including cytokines, peptides, and specific lipid mediators. When inflammation does not resolve, low-level, insidious, continuing inflammation gives rise to numerous pathologic states.

Chemical instigators of inflammation, including prostaglandins and leukotrienes, enhance vascular permeability and the recruitment of leukocyte subsets. As the inflammatory response evolves, a shift in the biosynthesis of lipid mediators results in the generation of agents that limit the further trafficking and accumulation of leukocytes by modulating the expression of endothelial-cell adhesion molecules, promoting apoptosis of leukocytes at the inflammatory focus, and effecting their subsequent efferocytosis (i.e., removal of dying or dead cells by macrophages). Clearance of dead cells and associated debris is a key process in the resolution of inflammation, stimulating regeneration and repair. The endogenous lipids that promote such responses are termed "specialized proresolving mediators," or SPMs, and include eicosanoid-derived lipoxins and omega-3–derived resolvins, protectins, and maresins. Typically, these agents act as agonists of distinct receptors expressed on immune cells and on diverse types of parenchymal and stromal cells. Importantly, the generation of SPMs has been shown in self-limiting inflammation in humans, coincident with the onset of resolution. Furthermore, the addition of an exogenous cocktail of SPMs at the peak of experimentally induced inflammation in humans accelerated its resolution.[1] In several models of resolution of pathogen-driven inflammation, SPMs have been associated with enhanced host defense. Such disease-modifying responses are especially noteworthy given the immune suppression that may be associated with conventional antiinflammatory drugs.[2]

Several high-affinity receptors for SPMs are also targets of proinflammatory mediators, highlighting the critical balance between the generation of proinflammatory signals and SPMs. Distortion of this equilibrium (which involves suppressed levels of SPMs) has been implicated in several human diseases, including atherosclerosis, chronic kidney disease, and severe asthma. For example, lower levels of lipoxins have been detected in bronchoalveolar lavage fluid from persons with severe asthma than are found in unaffected persons and in persons with asthma that is not severe.[3] Synthetic mimetics of SPMs have been generated by several groups and have shown efficacy in models of acute and chronic inflammation, supporting the value of a therapeutic paradigm focused on the resolution of inappropriate inflammation and fibrosis.[4]

The entire family of maresins (macrophage mediators in resolving inflammation) is generated from docosahexaenoic acid, the concerted actions of 12-lipoxygenase, and subsequent reactions with glutathione S-transferases (either glutathione S-transferase 4 or leukotriene C_4 synthase, which is also known as glutathione S-transferase 2).

Evidence points to a role for sulfido-conjugated maresins in the repair and regeneration critical to the restoration of homeostasis in inflamed tissue and to the enhancement of host responses to infection. These chemical mediators and their biologic activity have been conserved across evolution: maresin conjugates in tissue regeneration (MCTRs) mediate tissue regeneration in planaria (flatworms with an extraordinary regenerative response to surgical injury) and can attenuate infection-induced delay in tissue regeneration. The addition of MCTRs in models of reperfusion injury is protective of tissue, and MCTRs have been detected in human cells.[5]

Asthma is a common disease of chronic lung inflammation. In persons with asthma, arachidonic acid is converted to cysteinyl leukotrienes by 5-lipoxygenase and leukotriene C_4 synthase, thereby increasing vascular permeability and smooth-muscle contraction (Fig. 1). Responses to cysteinyl leukotrienes in asthma are pharmacologically targeted through antagonism of their receptors (as is the case with the drug montelukast) or through the inhibition of 5-lipoxygenase (as with the drug zileuton), but there is marked heterogeneity in response to pharmacologic blockade. This heterogeneity turns a spotlight on MCTR1, MCTR2, and MCTR3, members of the MCTR family that are functional antagonists of leukotriene-induced vascular permeability. These maresin conjugates disrupt the binding of leukotriene D_4 to recombinant receptors, and responses to MCTRs are partially blocked by an antagonist of cysteinyl leukotriene receptors. Given the well-established role of cysteinyl leukotrienes in asthma, it was logical for Levy et al. to investigate the expression of MCTRs in the human lung in an effort to determine whether they drive homeostatic reconciliation in inflammatory disease, as recently reported.[6]

Metabololipidomic analyses of healthy human lung tissue revealed the presence of MCTR1, MCTR2, and MCTR3 in addition to the classic cysteinyl leukotrienes. In healthy lung tissue, the ratio of SPM sulfido-conjugates — MCTR, PCTR (a peptide conjugate of protectin), and RCTR (a peptide conjugate of resolvin) — to cysteinyl leukotrienes was approximately 10:3. In contrast, in samples from diseased lung tissue, the ratio was reversed, at approximately 1:10. Analysis of human lung tissue in ex vivo culture showed the presence of MCTR1 and its conversion to MCTR2 and MCTR3. The authors modeled airway contraction in asthma by exposing slices of human lung tissue to leukotriene D_4 in vitro. MCTR3 significantly attenuated airway contraction in response to leukotriene D_4, and a combination of MCTR1, MCTR2, and MCTR3 attenuated resistance to the airflow brought about by exposure to the leukotriene D_4 (Fig. 2), an effect that was partially blocked by montelukast.

Levy et al. performed a metabololipidomic analysis of tissue from mouse lungs at baseline, at the peak of inflammation (caused by ovalbumin-induced airway allergy), and during resolu-

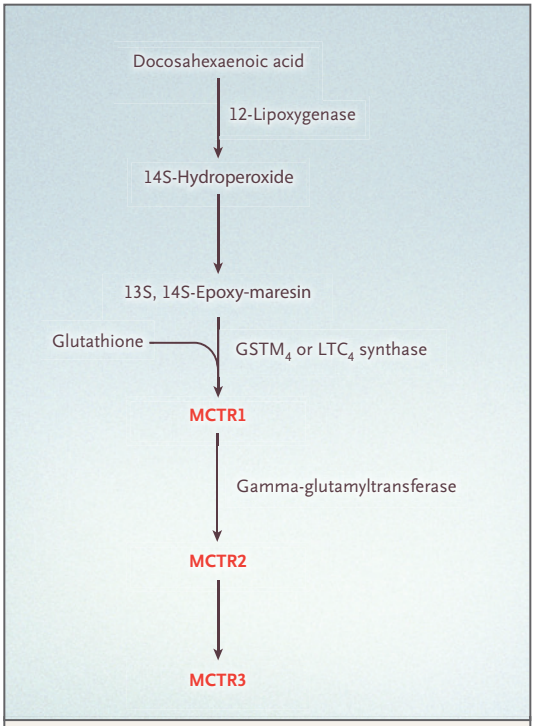
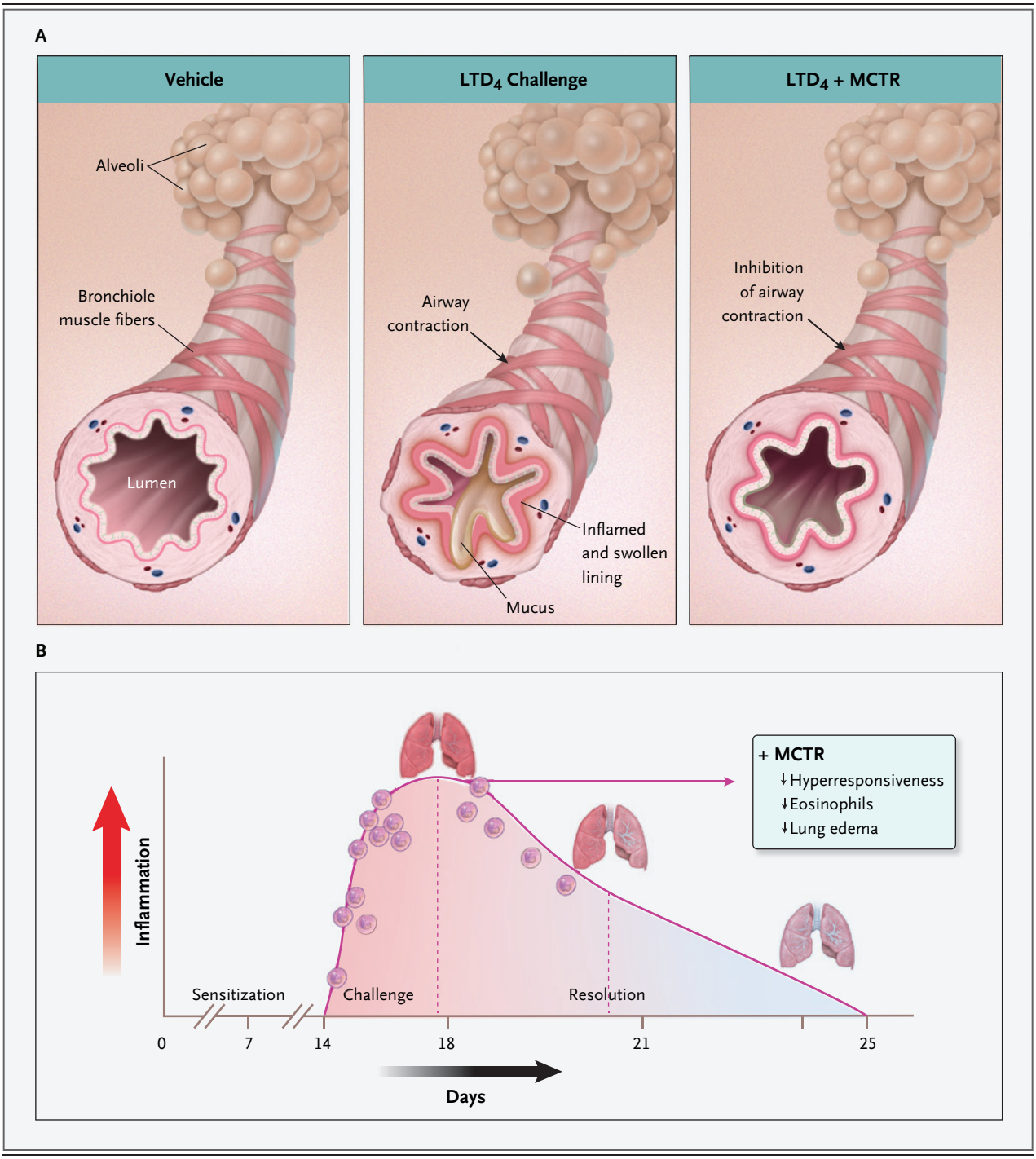


Figure 1. MCTR Biosynthesis.

Maresins are generated within macrophages, and their peptide conjugates are generated through the action of glutathione S-transferase $\mu 4$ ($GSTM_4$) or leukotriene C_4 [LTC_4] synthase. $GSTM_4$ has a higher affinity for 13S,14S-epoxy-maresin, whereas LTC_4 synthase has a higher affinity for LTA_4. Maresin conjugates in tissue regeneration (MCTR1s) can be further converted by gamma-glutamyltransferase to MCTR2, which can be converted by a dipeptidase to MCTR3. The results of a study recently reported by Levy et al. showed that MCTR1, MCTR2, and MCTR3 are present in a mouse model of asthma and in isolated human lung tissue.[6]



tion. MCTRs were detectable in the resolution phase, as were the sulfido-conjugated mediators protectin and resolvin, although the latter were less abundant than MCTRs.

The authors also tested the effect of intervention with MCTRs at the peak of inflammation. Methacholine-stimulated hyperreactivity was significantly decreased in mice who received MCTRs. This response was transient and dissipated 72 hours after administration of the last dose. Treatment was associated with decreased albumin levels in bronchoalveolar lavage fluid, a

Figure 2 (facing page). MCTRs and Response to Cysteinyl Leukotrienes in the Lung.
Stimulation of human lung tissue with LTC_4, ex vivo, induced airway contraction and reduced airflow. These responses were inhibited by MCTRs (Panel A). In a mouse model of allergic lung inflammation, distinct lipid profiles were detected basally, at the peak of inflammation and the initiation of resolution (Panel B). At the peak of inflammation (defined by maximal accumulation of eosinophils and cytokines), cysteinyl leukotrienes and MCTRs were detected (together with sulfido-conjugated protectins and resolvins). As resolution of inflammation proceeded, cysteinyl leukotriene levels declined and MCTR levels increased. The addition of exogenous MCTRs at the peak of inflammation protected against airway hyperreactivity, epithelial mucous metaplasia, eosinophilia, increased levels of albumin in the bronchoalveolar lavage fluid, an elevated cell count in bronchoalveolar lavage fluid, and elevated serum levels of IgE. Panel B adapted with permission from Levy et al.[6]

finding that is consistent with enhanced integrity of the airway as a barrier to pathogens and with decreased serum levels of IgE. In a model of airway inflammation induced by house-dust mites, in which house-dust-mite extract was used in direct sensitization of the airway, intranasal administration of MCTR3 during challenge was associated with fewer leukocytes and eosinophils in bronchoalveolar lavage fluid.

In the context of lung biology, the key findings of this work are that the activities of cysteinyl leukotrienes, established mediators of inflammation (and the target of drugs for the treatment of asthma), may be modulated by endogenously generated cysteinyl lipid mediators typified by the MCTR family, promoting the resolution of inflammation and attenuating hyperreactivity and compromise of the mucosal barrier. The balance between (or relative predominance of) these different classes of mediator reflects substrate availability and the varied interactions between glutathione S-transferases. Levy et al. showed that montelukast partially blocks responses to MCTRs, which may explain some of the heterogeneity in the clinical response to this drug and other antagonists of cysteinyl leukotriene receptors. Given the enhanced sensitivity of persons with asthma to cysteinyl leukotrienes and the efficacy of enzymes in stimulating their generation (or, alternatively, the generation of MCTRs), it will be interesting to see whether there exist associations between polymorphisms in the genes that encode these enzymes and risk of asthma or its responsiveness to treatment.

Responses to cysteinyl leukotrienes are not restricted to the lung. Cysteinyl leukotriene receptors are expressed in numerous cells and tissues and have been proposed as therapeutic targets in cardiovascular disease, ischemia-related reperfusion injury, and fibrosis. To date, the identification and characterization of responses to MCTR indicate enhanced host defense, regeneration, and repair across phyla. It would therefore seem prudent to avoid blocking responses to these endogenous regulators of inflammation. More generally, the work of Levy et al. adds to the growing evidence of a balance between inflammation and the counterregulatory signals that promote its resolution under physiologic conditions. It highlights, as an experimental therapeutic strategy, agonism of resolution as an alternative to antagonism of inflammation.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

From the Diabetes Complications Research Centre, Conway Institute and School of Medicine, University College Dublin, Dublin, Ireland.

1. Motwani MP, Colas RA, George MJ, et al. Pro-resolving mediators promote resolution in a human skin model of UV-killed Escherichia coli-driven acute inflammation. JCI Insight 2018;3:94463.
2. Tabas I, Glass CK. Anti-inflammatory therapy in chronic disease: challenges and opportunities. Science 2013;339:166-72.
3. Ricklefs I, Barkas I, Duvall MG, et al. ALX receptor ligands define a biochemical endotype for severe asthma. JCI Insight 2018;3:120932.
4. Brennan EP, Mohan M, McClelland A, et al. Lipoxins regulate the early growth response-1 network and reverse diabetic kidney disease. J Am Soc Nephrol 2018;29:1437-48.
5. Dalli J, Chiang N, Serhan CN. Identification of 14-series sulfido-conjugated mediators that promote resolution of infection and organ protection. Proc Natl Acad Sci U S A 2014;111:E4753-E4761.
6. Levy BD, Abdulnour RE, Tavares A, et al. Cysteinyl maresins regulate the prophlogistic lung actions of cysteinyl leukotrienes. J Allergy Clin Immunol 2020;145:335-44.

DOI: 10.1056/NEJMcibr2000118