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The Chemical Elucidation of Slow-Reacting Substance: Bronchospasm and Beyond

Marc Peters-Golden

In 1860, H.H. Salter first noted the importance of bronchial smooth muscle contraction in asthma (1). Eighty years later, Kellaway et al. (2) recognized a distinctive bioactivity whose release following Ag challenge of sensitized lung elicited a contractile response that was slower in onset and more protracted than that elicited by histamine, the only known spasmogen of the time; they named this material “slow-reacting substance” (SRS). In 1960, W. Brocklehurst recognized that whereas histamine existed preformed, SRS was instead generated de novo during the Ag–Ab reaction, suggesting an enzymatic mechanism (3). These foundational observations sparked the quest to elucidate the chemical structure of SRS and its mechanism of biosynthesis—a quest that would ultimately require two more decades.

Although research by a number of laboratories around the world had provided certain methodologic and compositional clues to the identity of SRS, the data were ambiguous and progress was limited by the small quantities of biological material available for analysis. During the 1960s and 1970s, Samuelsson et al. (4) at the Karolinska Institute had accumulated substantial expertise in lipid structural analysis and the biochemistry of arachidonic acid oxygenation via cyclooxygenase and lipoxygenase pathways (5). This expertise would prove instrumental in solving the SRS puzzle, as described in the featured 1979 *Pillars of Immunology* article (6). R.C. Murphy, a visiting scientist working with the Samuelsson group, used calcium ionophore-stimulated mouse mastocytoma cells to generate sufficient quantities of spasmogenic SRS for analysis. This material incorporated isotopically labeled arachidonic acid and cysteine, and had four double bonds. Its UV absorption spectrum was consistent with that of a conjugated triene chromophore. As SRS was formed by leukocytes, they coined the term “leukotriene” (LT) and designated this structure LTC₄. The cysteine-containing moiety was eventually determined to be the tripeptide glutathione, and SRS was subsequently revealed to represent a mixture of LTC₄ plus two bioactive degradation products, LTD₄ and LTE₄, formed from the sequential removal of glutamic acid and glycine,

respectively. As all three family members contained cysteine, these were collectively called cysteinyl LTs (cysLTs) (7). For this and other foundational work, Samuelsson was awarded the 1982 Nobel Prize for Physiology or Medicine.

Advances over the next two decades unraveled the biochemistry and cell biology of LT biosynthesis (8–12) and the pharmacologic characterization and cloning of the G protein-coupled CysLT1 receptor that mediates SRS contractile activity (13, 14). As prophesied by Brocklehurst in his Ph.D. thesis in 1958, the search for drugs inhibiting both the synthesis of (the 5-lipoxygenase inhibitor zileuton) and the receptor for (the CysLT1 antagonists zafirlukast, pranlukast, and montelukast) SRS came to fruition in the mid- to late-1990s (15). These agents not only represented the first example of an asthma therapy targeting a specific molecule (foreshadowing the development of targeted biologics such as anti-IL-5), but also the first new class of asthma therapy since corticosteroids, developed 40 years earlier. Together with studies of transgenic mice lacking specific enzymes or receptors (16–18), clinical studies and experience with these drugs have provided a wealth of information about the spectrum of biological actions of cysLTs (19). We can now reflect on the lessons learned and whether the promise envisioned by Brocklehurst, and enabled by the findings reported in this *Pillars of Immunology* article, has been realized.

Indeed, it has been established that clinical benefit derives from blocking the potent contractile activity that gave SRS its name (20). However, this was just the tip of the iceberg. From the mid-1970s to the 1980s, it became apparent that the hyper-responsiveness of asthmatic airways was in part attributable to eosinophilic, or what is now termed type 2, inflammation (21), which in turn could be attenuated by inhaled corticosteroids (22). An unexpected revelation from the new molecular and pharmacologic tools for understanding cysLT actions was that these mediators could also promote type 2 responses (reviewed in Refs. 19 and 23) by enhanced recruitment, survival, or functional activation of dendritic cells (24, 25), eosinophils (26, 27), mast cells (28, 29), T cells (30, 31), B cells (32), and, more recently, type 2 innate lymphoid cells (33). The promotion of these processes in part reflects cysLT regulation of cytokine and cytokine receptor expression. CysLTs can also contribute to airway remodeling by promoting epithelial cell TGF- β generation (34), smooth muscle cell proliferation (35), fibroblast collagen synthesis (36), and fibrocyte migration and proliferation (37). Thus, cysLTs have the ability to phenocopy important actions of cytokines (e.g., modulation of inflammation) while also eliciting actions of which cytokines are incapable (e.g., smooth muscle contraction). Consequently, anti-LT agents have the potential to combine the benefits of a bronchodilator with those of an

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Abbreviations used in this article: cysLT, cysteinyl LT; LT, leukotriene; SRS, slow-reacting substance.

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anti-inflammatory or “controller” agent—a profile of actions that otherwise requires treatment with inhaled corticosteroid plus bronchodilator; this combination remains unique among asthma treatments to this day. Moreover, although corticosteroids are often presumed to inhibit all aspects of inflammatory responses, it is notable that they have generally been found not to inhibit LT biosynthesis *in vivo* (38, 39); this may explain why in some patients an anti-LT drug can provide additive benefit to an inhaled corticosteroid. Indeed, oral anti-LT drugs are used today by millions of asthmatics and provide a unique profile of ease of use, efficacy, and safety.

On the other hand, the efficacy of anti-LT agents has not lived up to the high expectations that surrounded their development. This likely reflects limitations of existing drugs and of our ability to identify the patients who might benefit the most. First, zileuton inhibits *in vivo* LT synthesis by ~50% (40). Second, at least two receptors for cysLTs in addition to CysLT1 are now recognized, and although their biologic roles remain incompletely defined, neither of these is blocked by currently available antagonists (41). Finally, heterogeneity in responses to existing anti-LT agents is well recognized (42), and although especially high urinary or sputum cysLT levels might identify patients most likely to benefit [examples include those suffering from aspirin-exacerbated respiratory disease (43) and the obese (44)], no large clinical trials have endeavored to prospectively select patients stratified by this biomarker. It is possible that application of precision approaches might identify a subset of more robust responders, as is now considered routine for other targeted therapies.

It is also worth noting that the potential landscape of pathologic states in which cysLTs may participate now also extends beyond asthma. For example, data support potential roles in tissue fibrosis (45), atherosclerosis (46), multiple sclerosis (47), and cancer (48, 49). However, a note of caution also applies, as cysLTs have been implicated not only in pathologic inflammation but also in innate immune defense against microbes (50).

The chemical identification of SRS reported in this featured *Pillars of Immunology* article and the related Nobel Prize awarded three years after its publication cast cysLTs and other lipid mediators at center stage. In the decades since, however, protein mediators such as cytokines and chemokines have gained undisputed hegemony as the dominant players in immunologic and inflammatory responses. Indeed, possible roles of lipid mediators or of the interactions between lipid and protein mediators in these responses are often not even considered. It is hoped that highlighting the discovery reported in this *Pillars of Immunology* article and the drug development it catalyzed will remind scientists that “unconscious bias” against lipid mediators limits our ability to discover immunologic truth and translate it for the benefit of patients.

Disclosures

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