Commentary

The Scent of a Phagocyte: Advances on Leukotriene B_4 Receptors

By Charles N. Serhan* and Stephen M. Prescott‡

*From the *Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115; and ‡Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah 84112-5550*

To maintain its health, an organism must be able to repel infectious invaders. In animals, phagocytic cells—professional hunters—are essential to this process. This creates another level of challenge: how to get the phagocytic cells to the site(s) where they are needed without activating them prematurely. Several molecular events are required to orchestrate this properly. For example, endothelial cells must display appropriate adhesion proteins to supply the spatial specificity (1), and the phagocyte must be able to "smell" and respond to chemical stimuli that direct its activation and migration. Identification of the scents that attract the hunters and signals from phagocyte to phagocyte has progressed over the past two decades, and one of the first endogenous compounds to be chemically characterized was leukotriene B_4 (LTB_4).

In the first experiments that led to the discovery of leukotrienes, Borgeat et al. (2) reported a novel 5-lipoxygenase, and subsequent analysis of the products of this pathway led to the structural elucidation of three major compounds (3, 4), one of which was LTB_4. The biological function of LTB_4, which is generated by polymorphonuclear leukocytes (PMNs), was elucidated in experiments by Ford-Hutchinson et al. (5). At the time, it was a significant challenge to separate mono-, di-, and tri-hydroxy eicosatetraenoic acids (HETEs). This was problematic because several isomers of 5,12-di-HETE were present in the extracts from activated PMNs, and it was difficult to determine which of them carried biological activity. Ford-Hutchinson et al. tested fractions eluted from an HPLC separation and found a single major peak of chemoattractant activity. They also reported that this compound, which was subsequently shown to be LTB_4, was a potent stimulator of chemokinesis and aggregation. These findings led the field away from a prior focus on mono-HETE as primary mediator. A battery of papers that determined the complete structure of LTB_4 was published in this journal in the early 1980s. The first paper established the complete stereochemistry of LTB_4 as 5(S), 12(R)-dihydroxy-eicosa-6,8,10-(trans/trans/cis), 14(cis)-tetraenoic acid and showed that it was this isomer alone that accounted for the proinflammatory activity of LTB_4 (6). The second paper used materials prepared by total organic synthesis and confirmed that, of the several stereoisomers present in biological preparations, LTB_4 that was the relevant compound (7). This precise chemical characterization was important for many reasons, but one of the most profound insights was that the phagocytes were able to distinguish between very similar compounds and to respond to only the one with the "correct" stereochemistry. This implied that there was a specific receptor that could discriminate between stereoisomers. A series of papers in this issue addresses the regulation of expression of the LTB_4 receptor that was identified a few years earlier (Kato et al. [8]) and the role of this receptor in animal models of inflammation (Haribabu et al. [9] and Tager et al. [10]) and reports a second, lower affinity receptor in cells other than leukocytes (Yokomizu et al. [11]).

The extensive molecular characterization of cell surface receptors and the mechanisms by which they send signals into the cell are a remarkable recent advance in biology and medicine. But the ability of phagocytes and other cells of inflammation and immunity to respond selectively has been recognized for many years. As early as 1883, Metchnikoff presented his hypothesis that leukocytes recognized foreign microbes and destroyed them using a process of engulfment or phagocytosis, the term he introduced to describe their "eating character." Embodied in his observations was the concept that leukocytes chemotax in response to chemical signals, which we now know include LTB_4. We also know that the general structure of receptors and the subsequent events have been conserved and are the molecular underpinnings of our various senses. For example, the large family of receptors that detects odorants is a branch of the G protein–coupled receptor family, just as are the receptors for LTB_4 (12, 13). Such conservation and connections had been suspected. For example, Thomas (14) was intrigued by the exquisite ability to distinguish between different

Address correspondence to Charles N. Serhan, Brigham and Women's Hospital/Harvard Medical School, Center for Experimental Therapeutics and Reperfusion Injury, 20 Shattuck St., Thorn 724, Boston, MA 02115. Phone: 617-732-8822; Fax: 617-278-6957; E-mail: cnserhan@zeus.bwh.harvard.edu

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odorants and postulated that it was in some way related to the ability to distinguish self from nonself. He and his colleagues conducted experiments that showed that bloodhounds could distinguish between congenic mice that differed only at the H2 locus, and that the mice themselves seemed able to make the distinction (14). Thus, the ability to smell—either by nasal epithelia or phagocytes—influences our ability to mount appropriate immune and inflammatory responses.

The potential for therapeutic uses of inhibitors of leukotriene generation and/or their function(s) was apparent early based on the initial descriptions of their putative roles in inflammation and a wide range of human diseases and disorders (15). The subsequent discovery and cloning of the receptor for LTB_4 made an attack on this target possible. But too much antiinflammatory effect would put the organism at risk of not being able to fight infection or other challenges.

Using radiolabeled LTB_4 and traditional medicinal chemistry, a number of pharmacologically defined receptors were described, and receptor antagonists were prepared without having the leukotriene receptor in hand. Although antagonists were effective in vitro, there were few clear clinical indications for when to use them in vivo (16). The discovery and structural characterization of G protein–coupled receptors (GPCRs; for review see reference 17) opened the possibility of obtaining the elusive LTB_4 receptor. Shimizu's laboratory, after the initial description of specific binding sites for LTB_4 by Goetzl et al. (6), tried in what is reported to be more than a 10-year quest to identify and clone the LTB_4 receptor. In 1997, Yokomizo et al. (18) reported the identification of a GPCR that recognized LTB_4 in a stereoselective fashion and evoked chemotaxis when placed in a reporter cell line to demonstrate in vitro chemotaxis. As a wide range of closely related structures did not activate this receptor, it was named BLT1. The receptor had been described earlier but was misidentified as an atypical purinergic receptor by another group that had cloned it as a member of a set of orphan GPRCs. BLT1 belongs to the subfamily of GPRCs that includes chemokines and related chemotactic receptors and is distinct from the subfamily of prostaglandin receptors (19).

At this stage of the history of LTB_4, all of the evidence pointed to its likely role as a key mediator of inflammation, but the evidence in vivo was incomplete. The use of genetically altered mice was a leap forward in the evaluation of the 5-lipoxygenase pathway (20, 21). Results from these mice provided strong evidence that products of the pathway play an important role in inflammatory insults; deficient mice had blunted responses to topical arachidonic acid and increased resistance to platelet-activating factor (PAF)-induced shock. Also, zymosan-induced peritonitis was markedly reduced in mice deficient in their ability to generate leukotrienes, presumably LTB_4. Conversely, overexpression of the receptor in the leukocytes of BLTR-transgenic mice enhanced leukocyte responsiveness in acute dermal inflammation, their trafficking to remote organs, and their recruitment to a peritoneal challenge. These results also led to the conclusion that LTB_4 is a culprit in a variety of circumstances in which inappropriate or excessive activation leads to leukocyte-mediated injury (22). Of particular interest, overexpression of the receptor in vivo led to an unexpected upregulation of 5-lipoxygenase expression and leukotriene biosynthesis, emphasizing that receptor overexpression amplifies proinflammatory circuits in vivo. These findings reaffirmed the receptor as a logical target for therapy designed to suppress excessive inflammation. But related work in inflammation over the years had also led to the identification of additional signals for phagocytes—PAF, C5a, monocyte chemoattractant protein 1, and others—and whether loss of the scent for one of these would blunt inflammation was in question, as the redundancy seemed so extensive. Papers in the current series address this issue.

In the first paper, Kato et al. (8) report studies to define the regulation of BLT1, focusing on the transcriptional events that account for its selective expression in leukocytes. They determined the structure of the gene, which is simple as is typical with GPCR genes, and then identified the promoter region and transcription start site. They found that the region from the start upstream to -76, which contains a binding site for Sp1, was required for expression. Then, they showed that Sp1 binds to this region and activates transcription and that mutation of the site abolishes the response. This defined a key mechanism for expressing the gene but left the issue of why the expression is so selective essentially only in leukocytes of myeloid lineage. They went on to answer this question as well. The sequence around the Sp1 site is rich in CpG sites, and their experiments showed that there is methylation of such sites in nonmyeloid cells and that this prevents transcription of the BLT1 gene. Thus, this series of experiments provided a detailed mechanism for the selective expression of the BLT1 receptor. There is great interest in therapeutic approaches, particularly for cancer, that prevent methylation of regulatory sequences and activate the transcription of genes such as tumor suppressors. The work by Kato et al. (8) suggests that drugs that achieve this goal might also increase the spectrum of cells that express the BLT1 receptor, which could yield an unexpected set of responses.

In the course of their studies on expression of the BLT1 gene, Yokomizu et al. (11) discovered that an open reading frame for a second LTB_4 receptor, now denoted BLT2, is embedded in the promoter sequence, a first example of, as the investigators report, "a promoter and an open reading frame" in higher eukaryotes. BLT2 is highly similar to BLT1, with amino acid identity of $\sim$45%, but the protein is a low affinity receptor for LTB_4: the K_d value is 23 nM, compared with 1 nM for BLT1. BLT2, unlike BLT1, is expressed ubiquitously, and when expressed in CHO cells, the cells chemotax and mobilize calcium in a pertussis toxin–sensitive fashion in response to LTB_4. Thus, the signaling initiated by LTB_4 binding to the two receptors appears to be very similar except for their differences in affinity for the ligand. The role of BLT2 in responses associated with LTB_4 is not known but intriguing. For example, since

LTB_4 is a signaling molecule between leukocytes, promoting cell–cell communication, the presence of receptors of different affinity may somehow participate in innate immunity. Furthermore, this new receptor, BLT2, may be the key to understanding the role, if any, of LTB_4 in processes other than host defense. If it serves an independent function, BLT2 could be a target for pharmacological intervention if selective antagonists can be developed.

The focus of the other two papers in this series (9, 10) is the physiological and pathological roles of the BLT1, and both tackled the problem with the same approach, targeted gene disruption. In both reports, leukocytes from the knockout animals showed a loss of responsiveness to LTB_4 as expected when studied in vitro, but, importantly, the cells remained responsive to other proinflammatory agonists, a circumstance that would allow the test of the importance of LTB_4 sensing in vivo in models of inflammation. Both Haribabu et al. (9) and Tager et al. (10) found that peritoneal inflammation was suppressed in the deficient mice; interestingly, they described different time-dependent patterns: Haribabu et al. documented a loss of protection over 72 h, whereas Tager et al. observed increasing protection with time. In addition, Tager et al. found that a dramatic diminution in the number of eosinophils accounted for virtually all of the changes in cellular influx, while Haribabu et al. noted decrements in both PMNs and macrophages. The basis for these differences is not clear, but they may be the result of different provocations for the inflammation (zymosan versus thioglycollate). Tager et al. also documented that the adhesion of $BLT1^{-/-}$ leukocytes to endothelium, as expected, was diminished in response to LTB_4, findings that are in line with earlier observations with LTB_4 in vital microscopy using the hamster cheek pouch (23). Interestingly, Haribabu et al. (9) observed that female but not male knockout mice were highly resistant to PAF-induced anaphylaxis. It is essential to reemphasize that the cells in vitro responded to PAF and, thus, these experiments give strong support to the previous evidence that the effects of PAF in vivo largely depend on downstream generation of LTB_4 and its subsequent actions. The LTB_4 signaling cascade seems to be a common downstream pathway in these experimental settings and therefore an attractive target for pharmacological regulation in, acute inflammatory disorders.

What does the future hold, in view of these findings? It is clear that having a second receptor for LTB_4 as well as a high affinity receptor will permit the total organic synthesis and development of highly selective agonists and antagonists that should find their way to the clinic, perhaps initially in the treatment of acute, rapid onset events of pathogenesis such as adult respiratory distress syndrome or second organ reperfusion injury where neutrophil infiltration is a primary player that leads to organ failure (22). The availability of knockouts for the receptor will permit testing the selectivity of such receptor antagonists as well as exploration of other potential roles and mechanisms of action for LTB_4 itself. It will be interesting to see if these mice develop compensatory mechanisms along the lines of upregulation of the 5-lipoxygenase pathway, observed in mice overexpressing BLT1 (22), and how these receptor levels may be altered in disease models and during treatment with a specific leukotriene antagonist and/or selective cyclooxygenase-1,2 inhibitors (as in reference 24). Also, what is the basis for the unexpected gender-specific effects in PAF-induced shock? Could BLT2 be involved here, or are there other ways for PAF to work its mischief? Will manipulations of gene methylation with the intention of other therapeutic goals result in abnormal expression of BLT1 in tissues where it normally is suppressed? If so, what will the consequences be: Can a scent for inflammation smell not as sweet in a different context?

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