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THEMED SECTION: MEDIATORS AND RECEPTORS IN THE RESOLUTION OF INFLAMMATION

COMMENTARY

Lipid mediator interplay: resolvin D1 attenuates inflammation evoked by glutathione-conjugated lipid peroxidation products

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Non-enzymatic oxidation of cellular lipids, one of the characteristic features of inflammation, leads to formation of highly reactive and toxic α,β-unsaturated aldehydes, such as 4-hydroxy-*trans*-2-nonenal (HNE). Conjugation of HNE with reduced glutathione (GS-HNE) is widely believed to represent a form of detoxification. The study by Spite *et al.* in the current issue of the *British Journal of Pharmacology* shows that glutathiolation of HNE confers potent pro-inflammatory properties on this α,β-unsaturated aldehyde. They find that GS-HNE directly activates human neutrophil granulocytes *in vitro* and evokes peritonitis in mice. Pre-treatment with resolvin D1, which is derived from ω-3 fatty acids, markedly attenuated the peritoneal leukocyte accumulation and production of prostaglandins and leukotrienes induced by GS-HNE. Their findings have profound implications for the analysis of inflammation in describing the generation of a novel class of pro-inflammatory mediators, through glutathione-dependent metabolism of lipid-peroxidation products, and emphasize the therapeutic potential of resolvin D1 in inflammatory diseases.
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Abbreviations: GS-HNE, glutathionyl-4-hydroxy-*trans*-2-nonenal; HNE, 4-hydroxy-*trans*-2-nonenal; LXA_4, lipoxin A_4

Recruitment and activation of neutrophil granulocytes to sites of infection and injury play a central role in innate immunity. However, their many defence mechanisms that digest and destroy invading pathogens are also capable of inflicting injury to the host (Nathan, 2002). Dysregulated or excessive neutrophil accumulation and activation are hallmarks of most inflammatory diseases. Elimination of the initial stimulus, down-regulation of ongoing neutrophil trafficking and removal of neutrophils from inflamed areas will all facilitate resolution of the acute inflammatory response with minimal damage to the surrounding tissue (Nathan, 2002; Gilroy *et al.*, 2004). The ideal outcome of acute inflammation would be complete restoration of tissue homeostasis. If the life span of neutrophils is prolonged or their removal is impaired, chronic inflammation may ensue (Gilroy *et al.*, 2004). Over the past years, we have come to realize that both the initiation and resolution of the inflammatory response are tightly regulated. These processes are orchestrated mainly by inflammatory mediators, including chemokines, cytokines and, to an increasing degree, lipid mediators formed enzymatically and non-enzymatically. Many of the eicosanoids derived from arachidonic acid through the cycloxygenase and lipoxygenase pathways, including prostaglandins and leukotrienes, exert a wide range of pro-inflammatory actions. By contrast, lipoxin A_4 (LXA_4) and aspirin-triggered 15-epi-LXA_4

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derived from arachidonic acid via the lipoxygenase pathways have emerged as the first group of lipid mediators with potent anti-inflammatory and pro-resolution actions (Serhan *et al.*, 2008). There is evidence that pro-inflammatory eicosanoids promote a shift to LXA_4 during acute inflammatory exudate formation, thereby reprogramming the neutrophils in exudate to promote resolution (Levy *et al.*, 2001). A series of elegant studies using self-resolving inflammatory exudates and lipidomics have identified resolvins, protectins and maresins, all derived from essential ω-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, as potent initiators and regulators of inflammatory resolution (Serhan *et al.*, 2008; 2009).

Non-enzymatic oxidation of cellular lipids by reactive oxygen species results in generation of diverse adducts, including the isoprostanes and the oxygenated fragmentation products from polyunsaturated fatty acids, such as the α,β-unsaturated aldehyde, 4-hydroxy-*trans*-2 nonenal (HNE) (Benedetti *et al.*, 1980). Enhanced HNE formation is a characteristic feature of many inflammatory diseases, such as atherosclerosis, ischaemia/reperfusion injury and rheumatoid arthritis. HNE is freely diffusible, highly reactive with proteins and DNA, and exhibits a multitude of toxic and potentially deleterious effects. For instance, HNE modifies low-density lipoprotein, rendering it pro-atherogenic (Yla-Herttuala *et al.*, 1989) and induces neurotoxicity (Falletti *et al.*, 2007). Spontaneous or glutathione-S-transferase-catalysed conjugation of HNE with reduced glutathione to yield glutathionyl-HNE (GS-HNE) is thought to represent 25–70% of the total HNE metabolites depending on the cell type/tissue (Srivastava *et al.*, 2000). Consistent with this notion, glutathiolation of HNE was found to protect neurons against HNE toxicity (Falletti *et al.*, 2007).

The report by Spite *et al.* (2009) in this issue of the *British Journal of Pharmacology* challenges this view. This study clearly demonstrates that GS-HNE is a more potent trigger of inflammation than non-conjugated HNE. The observations suggest that the primary actions of GS-HNE (within the concentration range tested) are on neutrophils. Indeed, GS-HNE directly activated human neutrophils to generate superoxide. Thus, during inflammation, superoxide released from activated neutrophils may facilitate HNE formation, thereby initiating a vicious circle. GS-HNE also increased the surface expression of the β_2-integrin Mac-1 (CD11b/CD18) on isolated neutrophils, consistent with increased neutrophil influx into the peritoneal cavity in mice. The biphasic nature of leukocyte accumulation and rapid clearance of GS-HNE from the peritoneum would suggest that GS-HNE-induced generation of the known pro-inflammatory mediators leukotriene B_4, cysteinyl leukotrienes and/or prostaglandin E_2 was responsible for leukocyte recruitment during the second phase. Thus, GS-HNE is capable of orchestrating synthesis of secondary mediators that may serve to amplify the inflammatory response *in vivo*. It should be noted that leukocyte recruitment is likely to require GS-HNE actions on endothelial cells. The authors' observation that GS-HNE did not directly stimulate vascular permeability does not preclude the potential of GS-HNE for other direct actions (e.g. expression of adhesion molecules or release of chemokines) on endothelial cells. Alternatively, GS-HNE may activate endothelial cells through inducing release of other mediators from leukocytes. Clearly, additional studies investigating these possibilities are warranted.

The study of Spite *et al.* (2009) provides little insight into how GS-HNE activates neutrophils. Like cysteinyl leukotrienes, another glutathione-lipid conjugate, GS-HNE is unlikely to be cell-permeable. Furthermore, GS-HNE interactions with intracellular targets appear to be unlikely as most of GS-HNE is extruded from the cells (Srivastava *et al.*, 2000). Reduction of the aldehyde moiety to the alcohol, yielding glutathione-1,4,-dihydroxynonanol, the major metabolite of GS-HNE in urine, results in complete loss of bioactivity, indicating the importance of the aldehyde moiety for bioactivity. Considering the differences in the biological profile of cysteinyl leukotrienes and GS-HNE, cysteinyl leukotriene receptors are unlikely to function as receptors for GS-HNE, although this possibility cannot categorically be ruled out. The GS-HNE receptor(s) remains to be identified.

Another important finding of the study by Spite *et al.* (2009) is the marked reduction of peritoneal leukocyte accumulation by resolvin D1 (7*S*, 8*R*,17*S*-trihydroxy-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid). This finding support previous observations that resolvin D1 displays potent, multilevel, anti-inflammatory and pro-resolution actions in experimental models of peritonitis, colitis and peridontitis (Serhan *et al.*, 2008). Resolvin D1 suppresses neutrophil activation *in vitro* through yet unidentified receptors (Serhan *et al.*, 2008) and this could in part explain its actions *in vivo*, although this does not preclude potential actions on endothelial cells. Previous studies have demonstrated that LXA_4 and aspirin-triggered 15-epi-LXA_4 exert a range of actions to dampen inflammation and promote resolution, including inhibition of neutrophil adherence to the endothelium and transendothelial migration (Serhan *et al.*, 2008), redirecting neutrophils to apoptosis (El Kebir *et al.*, 2007) and facilitating their phagocytosis by scavenger macrophages (Godson *et al.*, 2000). As the same mechanisms are largely responsible for the reduction in the number of neutrophils in inflamed areas, it would be important to examine whether resolvin D1 could also exert similar actions. These issues notwithstanding, the results presented by Spite *et al.* (2009) imply a novel role for resolvin D1 in counteracting inflammation induced by glutathione-conjugated lipid peroxidation products.

The study by Spite *et al.* (2009) provides a solid starting point for future investigations into the biochemistry and pathophysiology of glutathione-conjugated lipid peroxidation products, and for exploring further the therapeutic potential of resolvin D1 in inflammatory diseases.

References

Benedetti A, Comporti M, Esterbauer H (1980). Identification of 4-hydroxynonenal as a cytotoxic product originating from peroxidation of liver microsomal lipids. *Biochim Biophys Acta* **620**: 281–296.

El Kebir D, József L, Pan W, Petasis NA, Serhan CN, Filep JG (2007). Aspirin-triggered lipoxins override the apoptosis-delaying action of serum amyloid A in human neutrophils: a novel mechanism for resolution of inflammation. *J Immunol* **179**: 616–622.

Falletti O, Cadet J, Favier A, Douki T (2007). Trapping of 4-hydroxynonenal by glutathione efficiently prevents formation of DNA adducts in human cells. *Free Radic Biol Med* **42**: 1258–1269.

Gilroy DW, Lawrence T, Perretti M, Rossi AG (2004). Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov* **3**: 401–416.

Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR (2000). Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J Immunol* **164**: 1663–1667.

Levy BD, Clisch CB, Schmidt B, Gronert K, Serhan CN (2001). Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* **2**: 612–619.

Nathan C (2002). Points of control of inflammation. *Nature* **420**: 846–852.

Serhan CN, Chiang N, Van Dyke TE (2008). Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* **8**: 349–361.

Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TE *et al.* (2009). Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* **206**: 15–23.

Spite M, Summers L, Porter TF, Srivastava S, Bhatnagar A, Serhan CN (2009). Resolvin D1 controls inflammation initiated by oxidative stress-derived glutathione-lipid conjugates. *Br J Pharmacol* **158**: 1062–1073.

Srivastava S, Dixit BL, Cai J, Sharma S, Hurst HE, Bhatnagar A *et al.* (2000). Metabolism of lipid peroxidation product, 4-hydroxynonenal (HNE) in rat erythrocytes: role of aldose reductase. *Free Radic Biol Med* **29**: 642–651.

Yla-Herttuala S, Palinski W, Rosenfeld ME, Parthasarathy S, Carew TE, Butler S *et al.* (1989). Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* **84**: 1086–1095.