Novel Lipid Mediators Promote Resolution of Acute Inflammation

Impact of Aspirin and Statins

Matthew Spite, Charles N. Serhan

Abstract: The resolution of acute inflammation is a process that allows for inflamed tissues to return to homeostasis. Resolution was held to be a passive process, a concept now overturned with new evidence demonstrating that resolution is actively orchestrated by distinct cellular events and endogenous chemical mediators. Among these, lipid mediators, such as the lipoxins, resolvins, protectins, and newly identified maresins, have emerged as a novel genus of potent and stereoselective players that counter-regulate excessive acute inflammation and stimulate molecular and cellular events that define resolution. Given that uncontrolled, chronic inflammation is associated with many cardiovascular pathologies, an appreciation of the endogenous pathways and mediators that control timely resolution can open new terrain for therapeutic approaches targeted at stimulating resolution of local inflammation, as well as correcting the impact of chronic inflammation in cardiovascular disorders. Here, we overview and update the biosynthesis and actions of proresolving lipid mediators, highlighting their diverse protective roles relevant to vascular systems and their relation to aspirin and statin therapies. (Circ Res. 2010;107:1170-1184.)

Key Words: resolution ■ lipid mediators ■ eicosanoids ■ omega-3 fatty acids ■ proresolving mediators
inflammation and potently stimulate this vital process. The identification of these new mechanisms and pathways challenged the preexisting paradigm that inflammation passively terminates. Within this context, a detailed appreciation of the endogenous pathways that actively turn off acute inflammation and stimulate resolution opens many new avenues for therapeutics and prevention that are aimed at controlling excessive inflammation without apparent immunosuppression.

**Inflammation and Its Natural Resolution**

The acute inflammatory response is a protective, physiological program that protects the host against invading pathogens. Local chemical mediators biosynthesized during acute inflammation give rise to the macroscopic events characterized by Celsus in the first century, namely, rubor (redness), tumor (swelling), calor (heat), and dolor (pain). Although these cardinal signs of inflammation were evident more than 2000 years ago, the cellular and molecular events that regulate the inflammatory response and its timely resolution are only recently beginning to be appreciated. Tissue edema is one of the earliest events of the acute inflammatory response that arises from increased permeability of microvasculature. Polymorphonuclear neutrophils (PMNs) are the first line of defense against microbial invasion, which contain potentially harmful stimuli via phagocytosis. PMNs traverse postcapillary venules at sites of inflammation, degrade pathogens within phagolysosomes, and undergo apoptosis. Next, mononuclear cells infiltrate, differentiate into macrophages, and clear apoptotic PMNs by phagocytosis in a noninflammatory manner termed effecorytosis. Ultimately, clearance of microbes and efflux of phagocytes allows for the tissue to return to homeostasis. Disruption of any of these specific checkpoints could potentially give rise to chronic inflammation, which is characterized primarily by excessive leukocyte infiltration and activation, delayed clearance, resultant tissue damage, and loss of function. Indeed, cardiovascular diseases such as atherosclerosis display many of these features.

Lipid mediators (LMs) biosynthesized from essential fatty acids play pivotal roles in distinct phases of the inflammatory response, with prostaglandin (PG)E2 and cysteinyl leukotrienes (cysLTs) promoting early vascular permeability and sclerosis display many of these features. During evolution of an inflammatory exudate, the profile of LM autacoids changes to biosynthesis of counter-regulatory mediators that limit further PMN congregation and stimulate resolution. Although the mechanisms that mediate progression from acute to chronic inflammation are not completely understood, chronic inflammation is widely viewed as an excess of proinflammatory mediators. In view of mounting evidence from our laboratory, and now many other groups, it is also plausible that disruptions in endogenous proresolving circuits could underlie some of the aberrant mechanisms that lead to chronic inflammation.

Complete resolution of an acute inflammatory response is the ideal outcome following an insult. For resolution to ensue, further leukocyte recruitment must be halted and accompanied by removal of leukocytes from inflammatory sites. These key events governing resolution of inflammation are the focus of our work and collaborators. In particular, we sought to identify mechanisms that regulate these key histological events in resolution using an unbiased systems approach to profile self-limited inflammation using liquid chromatography/tandem mass spectrometry (LC-MS/MS). These analyses identified novel LMs and also provided information regarding their biosynthetic pathways and further metabolic inactivation products. It is noteworthy that, in addition to novel LMs reviewed here, other important components of resolution are emerging (ie, nuclear factor [NF]-κB, annexin-1, specific prostanoids). They are beyond the scope of this concise update and interested readers are directed to.
Proresolving Lipid Mediators: Autacoid Signals in Exudates

Using experimental models of self-resolving acute inflammation, we uncovered a new genus of autacoids that possess potent antiinflammatory and proresolving actions. These include lipoxins, which are generated from arachidonic acid (AA), resolvins, protectins, and newly identified maresins, which are generated from omega-3 fatty acids. The enzymatic generation of these families occurs primarily via transcellular biosynthesis, and in some cases within a single cell type, via lipoxygenase (LOX) enzymes. They are potent, stereoselective agonists controlling both the magnitude and duration of an acute inflammatory response. The biosynthesis and actions of each of these families of endogenous counter-regulatory autacoids are reviewed herein.

**Lipoxins**

Lipoxins are generated in humans from AA via LOX enzymes and comprise 2 distinct regioisomers, lipoxin (LX)A4 and LXB4. Lipoxins were the first mediators recognized to have both antiinflammatory and proresolving actions. They are generated in humans from AA via LOX enzymes and comprise 2 distinct regioisomers, lipoxin (LX)A4 and LXB4. Lipoxins were the first mediators recognized to have both antiinflammatory and proresolving actions.28 Their biosynthesis proceeds via 15-LOX–mediated conversion of AA to 15-hydroxyeicosatetraenoic acid (HETE), which is further transformed via 5-LOX to LXA4 and LXB4 during cell:cell interactions (eg, epithelial:leukocyte, leukocyte:leukocyte). Lipoxins are also generated in the vasculature during platelet:leukocyte interactions, in which the intermediate in leukotriene biosynthesis, leukotriene (LT)A4, is produced within leukocytes and converted to lipoxins by platelet 12-LOX.29 In addition to LOX-initiated lipoxin biosynthesis, an intriguing novel route involving COX-2 was uncovered. In the presence of aspirin, acetylated COX-2 loses activity required to form prostaglandin (PG)H2 but retains oxygenase activity to produce 15R-HETE from arachidonate. This intermediate, like 15S-HETE, is transformed via 5-LOX to generate epimeric lipoxins, termed aspirin-triggered (AT) or 15-epi-lipoxins.30 The 15-epi-lipoxins share the potent bioactions of lipoxins, suggesting that their formation could underlie the antiinflammatory actions of aspirin that cannot be attributed only to the inhibition of prostanoid formation (vide infra). Of note, 15-epi-lipoxin biosynthesis can also be initiated by cytochrome P450 enzymes and this important pathway may underlie the generation of 15-epi-lipoxins in the absence of aspirin.31 In humans, low dose aspirin limits PMN infiltration via local 15-epi-lipoxin formation that in turn stimulates nitric oxide (NO) production (vide infra).32,33

**Table 1. Cellular Actions of Lipoxins, Resolvins, and Maresins**

<table>
<thead>
<tr>
<th>Lipid Mediator and Target Cell</th>
<th>Action(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoxin A4 (or aspirin-triggered lipoxin A4)</td>
<td><strong>Endothelial cells</strong>: Blocks ROS generation; inhibits VEGF-induced migration/proliferation; decreases ICAM-1 expression; stimulates PGi2 and NO formation; stimulates HO-1 expression</td>
<td>33, 93–95, 113</td>
</tr>
<tr>
<td><strong>Vascular smooth muscle cells</strong></td>
<td>Decreases PDGF-stimulated migration</td>
<td>96</td>
</tr>
<tr>
<td><strong>Macrophages</strong></td>
<td>Stimulates nonphlogistic phagocytosis</td>
<td>25, 35</td>
</tr>
<tr>
<td><strong>T cells</strong></td>
<td>Upregulates CCR5 expression; inhibits TNF secretion</td>
<td>69, 123</td>
</tr>
<tr>
<td><strong>Fibroblasts</strong></td>
<td>Blocks MMP-3 production</td>
<td>124</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>Blocks superoxide generation; reduces CD11b/CD18 expression; blocks neutrophil:endothelial interactions; inhibits peroxynitrite formation</td>
<td>125–127</td>
</tr>
<tr>
<td><strong>Resolvin E1</strong></td>
<td><strong>Macrophages</strong>: Stimulates nonphlogistic phagocytosis; binds to ChemR23 in a stereospecific manner</td>
<td>25, 47</td>
</tr>
<tr>
<td><strong>Vascular smooth muscle cells</strong></td>
<td>Decreases PDGF-stimulated migration</td>
<td>96</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>Reduces aggregation/activation</td>
<td>54</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>Blocks transmigration; acts as partial agonist/antagonist of BLT-1 and blocks LTb4-stimulated Ca2+/H1001 mobilization</td>
<td>22</td>
</tr>
<tr>
<td><strong>Dendritic cells</strong></td>
<td>Reduces IL-12 production</td>
<td>47</td>
</tr>
<tr>
<td><strong>Resolvin D1</strong></td>
<td><strong>Neutrophils</strong>: Binds ALX and GPR32 &amp; decreases LTb4-stimulated actin polymerization; Blocks transmigration</td>
<td>61, 63</td>
</tr>
<tr>
<td><strong>Macrophages</strong></td>
<td>Enhances phagocytosis in a receptor-dependent manner</td>
<td>63</td>
</tr>
<tr>
<td><strong>Resolvin D2</strong></td>
<td><strong>Endothelial cells</strong>: Stimulates NO and PGi2 production; blocks leukocyte:endothelial interactions</td>
<td>62</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>Enhances microbial phagocytosis; decreases extracellular ROS generation; reduces CD18 expression and L-selectin shedding</td>
<td>62</td>
</tr>
<tr>
<td><strong>Macrophages</strong></td>
<td>Enhances phagocytosis</td>
<td>62</td>
</tr>
<tr>
<td><strong>Maresin 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Macrophages</strong></td>
<td>Enhances phagocytosis</td>
<td>76</td>
</tr>
</tbody>
</table>

The temporal generation and biological role of endogenous lipoxins was elucidated using animal models of sterile inflammation. The initial formation of leukotrienes corresponds with an increase in PMN infiltration and is followed by a
Table 2. Lipoxins and Aspirin-Triggered Lipoxins in Animal Models of Disease

<table>
<thead>
<tr>
<th>Disease Model</th>
<th>Species</th>
<th>Action(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>Mouse</td>
<td>Attenuates pulmonary inflammation and airway hyperresponsiveness</td>
<td>128</td>
</tr>
<tr>
<td>Ischemia/reperfusion injury</td>
<td>Mouse</td>
<td>Decreases hindlimb ischemia/reperfusion injury in the lung; attenuates renal ischemia/reperfusion injury</td>
<td>34, 129</td>
</tr>
<tr>
<td>Dermal Inflammation</td>
<td>Mouse</td>
<td>Decreases vascular permeability</td>
<td>130</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Rabbit</td>
<td>Decreases bone loss; reduces PMN accumulation</td>
<td>131</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>Mouse</td>
<td>Decreases PMN infiltration; enhances phagocytosis and clearance of apoptotic cells</td>
<td>24, 25</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>Mouse</td>
<td>Decreases disease severity, inflammation, and bacterial burden</td>
<td>81</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>Mouse</td>
<td>Counterregulates VEGF-induced pathological neovascularization</td>
<td>113</td>
</tr>
</tbody>
</table>

progressive increase in prostaglandin formation, namely PGE$_2$ and PGD$_2$. Next, the profile of lipid mediators switches from proinflammatory eicosanoids to lipoxins, whereby PGE$_2$ and PGD$_2$ stimulate upregulation of 15-LOX, a process termed “lipid mediator class switching”. Thus, although COX-2-derived proinflammatory eicosanoids are commonly viewed as harmful, they are critical for positive feed-forward regulation of antiinflammatory LM circuits. Importantly, recent studies have shown that inhibition of COX-2 delays resolution of inflammation. Thus, in addition to inhibiting the formation of protective prostaglandins (eg, prostacyclin), selective COX-2 inhibitors also interfere with endogenous resolution programs. The role of lipoxins in counter-regulating leukocyte trafficking was demonstrated in both animal and human systems, where administration of LXA$_4$ reduces PMN transmigration, adhesion receptor expression, proinflammatory cytokine generation and excessive PMN infiltration into inflamed tissues (Tables 1 and 2).

Proresolving Signals

Along with the antiinflammatorily role of lipoxins in negatively regulating leukocyte infiltration into tissues, lipoxins also stimulate resolution. Within inflammatory exudates, PMNs undergo apoptosis and must be cleared to prevent unwarranted tissue damage. In this regard, lipoxins and 15-epi-lipoxins stimulate phagocytosis of apoptotic PMNs by macrophages in a nonphlogistic (non-fever-causing) manner. Lipoxins also stimulate the production of antiinflammatory cytokines, such as interleukin (IL)-10, in macrophages and promote macrophage efflux to peripheral lymph nodes. Thus, lipoxins are dual acting mediators that not only reduce further leukocyte infiltration, but also promote their removal from inflamed sites.

LXA$_4$ elicits its actions in nanomolar concentrations via agonist signaling by a specific G protein–coupled receptor (GPCR) termed ALX (also denoted formyl peptide receptor 2; FPR2). Specific binding of LXA$_4$ to ALX is stereoselective and ALX signaling mediates the protective actions of LXA$_4$ in myeloid cells, which include inhibition of NF-$\kappa$B activation, blockade of leukotriene biosynthesis, attenuation of superoxide production and regulation of leukocyte chemotaxis (Figure 1 and Table 1). Regulation of leukocyte trafficking by LXA$_4$ is partly dependent on direct stimulation of the suppressor of cytokine synthesis (SOCS-2) pathway. Of note, LXA$_4$ counter-regulates vascular smooth muscle cell migration induced by cysLTs and serves as a cysLT1 receptor antagonist. The role of specific GPCRs in mediating diverse protective actions of lipoxins is evidenced by targeted overexpression and genetic deletion of both human and murine homologs of ALX/FPR2 in murine systems.

The Omega-3 Proresolution Mediators: Resolvins, Protectins, and Maresins

The importance of essential omega-3 polyunsaturated fatty acids in humans is evidenced by multiple studies demonstrating that dietary omega-3 fatty acids have beneficial cardiovascular effects. Of note, a diet rich in omega-3 fatty acids is recommended by the American Heart Association (www.americanheart.org). It was first observed that Greenland Eskimos, who have a diet high in cold water fish, have a low rate of ischemic heart disease. These findings were validated in numerous human and animal studies using purified fish oil extracts rich in omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Most notably, the Grupo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico (GISSI) Prevenzione trial demonstrated that omega-3 dietary supplementation (1 g/d) reduced the risk of cardiovascular death in a patient population of >2,000 that had experienced a previous myocardial infarction. More recently, GISSI Prevenzione determined that omega-3 supplementation also reduces the risk of death from congestive heart failure in a placebo-controlled, double blind, randomized study of more than 6,000 heart failure patients. Although multiple beneficial actions of omega-3 supplementation are widely appreciated, the mechanisms underlying their protection in complex disease remained to be identified. At higher concentrations, omega-3 fatty acids act on ion channels, are metabolized to inactive eicosanoids of the 3-series prostaglandins and 5-series leukotrienes, and change cell membrane physical properties. However, the role of omega-3 fatty acids in resolution of inflammation was unknown.

To address the molecular basis for antiinflammatory properties of omega-3 fatty acids, an unbiased LC-MS/MS-based informatics approach was devised to identify novel mediators generated from omega-3 precursors during acute inflammation in vivo. Using this approach, EPA and DHA were found to be enzymatically converted into novel potent LMs coined resolvins for resolution phase interaction products. Resolvins represent a new distinct family of mediators generated from omega-3 fatty acids during resolution. Importantly, biosynthesis of resolvins gives rise to stereospecific local mediators that have potent actions and activate specific receptors. Therefore, resolvins are distinct from autooxidation products that can arise from EPA and DHA on food spoiling or in vivo during oxidative stress.
E-Series Resolvins
The first evidence that EPA serves as a precursor for bioactive mediators during resolution of inflammation was obtained in sterile, self-limited inflammation in mice. As >90% of patients enrolled in the GISSI Prevenzione trial were taking aspirin in addition to omega-3 fatty acids,44 and our earlier finding that aspirin-acetylated COX-2 gives rise to 15-epi-lipoxins, it was of interest to determine whether this combination would promote the formation of unique chemical mediators generated from EPA during resolution. In mice administered EPA and aspirin, PMN infiltration into inflamed tissues decreased and correlated with conversion of EPA to 18\text{R}-hydroxyeicosapentaenoic acid (18\text{R}-HEPE), as well as other related bioactive compounds. LM-lipidomics revealed that EPA is converted in vivo to a novel bioactive trihydroxyconjugated triene and diene-containing mediator.22 This bio-synthetic pathway was recapitulated with hypoxic human endothelial cells exposed to EPA and aspirin, in which 18\text{R}-HEPE was generated and converted to the mediator by activated human PMNs via 5-LOX.22 The complete structure of this mediator, coined resolin E1 (RvE1), was elucidated via total organic synthesis based on the proposed biosynthesis and basic structure (see abbreviations).22,47 Add back of RvE1 during acute inflammation markedly reduced PMN infiltration and decreased proinflammatory cytokines, results that provided the first evidence for the in vivo molecular basis of antiinflammatory, anti-PMN actions of EPA.

Given these potent actions, we reasoned that RvE1 might activate specific receptors to promote resolution. Screening of candidate GPCRs related in sequence to ALX revealed that RvE1 stereoselectively binds ChemR23, a previous orphan receptor.47 In isolated cells transfected with ChemR23, RvE1 inhibits TNF-\alpha stimulated NF-\kappaB activation, consistent with in vivo actions of RvE1 in blocking TNF-\alpha stimulated leukocyte trafficking. Of note, the endogenous role of ChemR23 in counter-regulating inflammation was recently demonstrated in mice with a genetic deletion of ChemR23.48 Acting via ChemR23, RvE1 stimulates downstream signaling through the phosphatidylinositol-3 kinase (PI3K)/Akt pathway, leading to activation of the translational regulator, ribosomal protein S6.49 This pathway is involved in RvE1 stimulation of macrophage phagocytosis. ChemR23 is highly expressed on dendritic cells and monocytes, although its expression is low on PMNs.47,50 As RvE1 blocks PMN migration in vitro and displays specific binding on human PMNs, this suggested that RvE1 might bind additional...
Dihydroxy-eicosapentaenoic acid, denoted resolvin E2 (RvE2).55 Formed before epoxidation can undergo reduction to 5
involves the formation of an epoxide. The 5
within the vasculature, the importance of this pathway and that E-series resolvins are biosynthesized and have direct actions RvE1 and RvE2 and their respective sites of action. Thus, given studies are warranted to appreciate the specific biosynthesis of and receptors for RvE1 and RvE2 may be distinct. Further
reducing PMN infiltration in peritonitis, and acts in an additive
RvE2 shares some of the potent actions of RvE1, namely
reducing PMN infiltration in peritonitis, and acts in an additive fashion with RvE1. Interestingly, differences in biological activity were observed that depend on the route of administration (ie, intravenous versus intraperitoneal), suggesting that the targets and receptors for RvE1 and RvE2 may be distinct. Further studies are warranted to appreciate the specific biosynthesis of RvE1 and RvE2 and their respective sites of action. Thus, given that E-series resolvins are biosynthesized and have direct actions within the vasculature, the importance of this pathway and related products in cardiovascular disease is an area of ongoing investigation.

**D-Series Resolvins**

DHA also has numerous beneficial actions in the cardiovascular system.42,56–59 Exogenous DHA reduces expression of vascular endothelial adhesion molecules, such as VCAM-1 and ICAM-1, induced by proinflammatory stimuli and thus regulates leukocyte/endothelial interactions.57 However, the amounts of DHA required to elicit these effects are generally high, ie, micromolar range in vitro or gram dose ranges in vivo. Thus, it was important to determine whether DHA might also serve as a precursor to endogenous autacoids. In mice given DHA plus aspirin, a monohydroxy product, namely 17R-hydroxydocosahexaenoic acid, was generated during resolution. Both dihydroxy and trihydroxy structures biosynthesized from DHA were also identified within resolving exudates. These bioactive molecules were coined aspirin-triggered (AT) D-series resolvins because they enhance resolution.56 To identify potential cellular sources of these, the proposed biosynthetic pathway was recapitulated in human cells. Hypoxic endothelial cells treated with aspirin convert DHA to 17R-hydroxydocosahexaenoic acid, which is transformed by leukocytes into D-series resolvins. Importantly, DHA is converted into resolvins in human whole blood in the absence of aspirin.60 Notably, D-series resolvins generated in the absence of aspirin carry the alcohol at the 17 position in predominantly the S configuration, rather than R configuration.60 Addition of this precursor to activated human PMNs also generated D-series resolvins, again highlighting

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**Table 3. Antiinflammatory and Proresolving Actions of Resolvins and Maresins in Animal Models of Disease**

<table>
<thead>
<tr>
<th>Disease Model</th>
<th>Species</th>
<th>Action(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritonitis</td>
<td>Mouse</td>
<td>Reduces neutrophil recruitment; blocks oxidative-stress induced peritonitis</td>
<td>60, 61, 133</td>
</tr>
<tr>
<td>Kidney ischaemia/reperfusion</td>
<td>Mouse</td>
<td>Decreases fibrosis and protects from ischaemia/reperfusion-induced kidney damage and loss of function</td>
<td>72</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Mouse</td>
<td>Reduces pathological neovascularization</td>
<td>52</td>
</tr>
<tr>
<td>Inflammatory Pain</td>
<td>Mouse</td>
<td>Reduces inflammatory pain induced by formalin, carrageenan, or Freund’s complete adjuvant</td>
<td>132</td>
</tr>
<tr>
<td>Sepsis (CLP)</td>
<td>Mouse</td>
<td>Reduces systemic cytokine storm; enhances bacterial clearance; improves survival; regulates leukocyte trafficking; protects from hypothermia</td>
<td>62</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>Mouse</td>
<td>Decreases neutrophil recruitment</td>
<td>62</td>
</tr>
<tr>
<td>Maresin 1</td>
<td>Mouse</td>
<td>Reduces neutrophil recruitment</td>
<td>76</td>
</tr>
</tbody>
</table>
the importance of cell-cell interactions (as occurs during resolution of inflammation). The enzymatic pathway leading to the formation of D-series resolvins is shown in Figure 2. Additional bioactive members of this family were identified and characterized (RvD3-RvD6). Each of these arises by similar biosynthetic routes, but have distinct structures and potentially additional bioactions.13

Following complete structural elucidation, stereochemochemical assignment and total organic synthesis, the bioactions of both native and AT D-series resolvins were elucidated.13,26,61 We recently established the complete structure and stereochemochemical assignment of RvD2.62 D-series resolvins have multiple beneficial actions both in vivo, and in isolated human cells (Figure 1 and Tables 1 and 3). In particular, they reduce excessive PMN infiltration into inflamed tissues, decrease PMN activation and promote phagocytosis and clearance of apoptotic cells and microbes. Protective actions of D-series resolvins have been observed in both acute and chronic inflammatory diseases, such as peritonitis, ischemia/reperfusion injury, and sepsis (Table 3). The unique mechanism of action for resolvins involves both limiting PMN infiltration and enhanced macrophage phagocytosis that uses specific receptors recently identified on human PMNs, monocytes, and macrophages.63 RvD1 signals via a GPCR denoted GPR32 that was an orphan human receptor. Interestingly, RvD1 was also found to activate ALX, in addition to serving as an agonist for GPR32. Signaling through these receptors, RvD1 counter-regulates LTB4-stimulated surface expression of β2 integrins, reduces actin polymerization, and enhances macrophage phagocytosis. Of note, classic GPCR second messengers, cAMP and Ca2+, are not activated by RvD1 signaling in PMNs.

**Protectins: Docosatrienes and Other Novel Products**

In addition to DHA-derived resolvins, resolving exudates also contained novel 10,17S-diHDHA. These dihydroxy-containing products and their regulatory actions in dermal inflammation and peritonitis were first reported in.26 Further experiments revealed that DHA was transformed to 10,17S-docosatriene, 16,17S-docosatriene and D-series resolvins in whole blood, leukocytes, brain and glial cells.60 In human glial cells, 10,17S-docosatriene potently regulated IL-1β and extracellular acidification, providing evidence that this compound is a ligand, evoking rapid cellular responses. Also, an omega-22 hydroxylation product of 10,17S-docosatriene was identified, suggesting that once 10,17S-docosatriene evokes its action, it is inactivated.60 Studies with Bazan and colleagues demonstrated that 10,17S-docosatriene reduces stroke damage in part by limiting neutrophilic infiltration.64 Based on its potent actions in human retinal pigment epithelial cells and neutrophils, this 10,17S-docosatriene was coined NPD1 (neuroprotectin D1) when produced in the vicinity of neural tissues, and PD1 (protectin D1) in the immune system.65,66 The identification of these biologically active endogenous products, isomers and related compounds biosynthesized from DHA provided evidence that a larger family with this basic structure from a 22-carbon backbone was warranted, and the family was coined protectins.67

PD1 is also biosynthesized by T cells and regulates apoptosis.68 During peritonitis, PD1 is formed from endogenous DHA and accumulates during resolution.24 PD1 has a number of potent bioactions evident in the picogram to nanogram range, including the ability to limit PMN infiltration and reduce cytokine/chemokine levels during acute inflammation. Importantly, PD1 shortens the resolution interval.24,25 Hence, it was important to establish the complete stereochemistry of PD1, which was assigned by matching with compounds prepared via total organic synthesis. PD1 proved additive with RvE1, and halted leukocytic infiltration following the initiation of an inflammatory response when administered ≈2 hours following exposure to challenge. LXAn, RvE1, and PD1 stimulate resolution in part by stimulating the sequestration of chemokines on T cells and apoptotic PMNs by their ability to regulate CCR5 expression.69 This event results in macrophage engulfment and clearing of chemokines from inflammatory sites. Transgenic mice overexpressing the fat-1 gene, which encodes a desaturase enzyme that enables the endogenous conversion of omega-6 to omega-3 fatty acids, are protected from a number of inflammatory insults and have higher levels of resolvins and protectins.70 Levels of NPD1/PD1 are also increased in the bone marrow of mice consuming a diet rich in omega-3 fatty acids,71 which is renal-protective via the ability of NPD1/PD1 to regulate leukocyte trafficking.72 Along these lines, PD1 was identified in human breath condensates obtained from healthy subjects and is diminished in exhaled breath condensates obtained from asthmatics. In murine airways, PD1 markedly accelerates resolution of airway inflammation and regulates eosinophil, T lymphocyte, mucus and proinflammatory mediator levels, including IL-13, leukotrienes, and prostaglandins.73 The important role of NPD1/ PD1 in regulating retinal and neural pathophysiology was recently reviewed.74 Trapping products indicate the involvement of an epoxide intermediate in the biosynthesis of NPD1 in retinal pigment epithelial cells, and evidence for stereoselective specific binding sites with human PMNs and retinal pigmented epithelial cells was obtained.75

**Maresins**

Macrophages are key players in resolution and their presence in inflamed tissues is vital for tissue repair, wound healing and the restoration of homeostasis.11 Recently, a new lipid mediator biosynthetic pathway was identified that involves enzymatic conversion of DHA by macrophages during resolution. Late-stage resolving exudates accumulated 14S-hydroxydocosahexaenoic acid, in addition to 17-hydroxydocosahexaenoic acid, a marker of resolving biosynthesis. Given that monohydroxy fatty acids are markers of biosynthetic pathways leading to potent downstream mediators, as is the case for leukotrienes, lipoxins and resolvins, it was reasoned that 14-hydroxydocosahexaenoic acid might be a marker of previously unrecognized mediator pathway operative during resolution and homeostasis. Using isolated human and murine macrophages, we found that these cells convert DHA into 14-hydroxydocosahexaenoic acid and
a novel 7,14-dihydroxy-containing product that showed potent antiinflammatory and proresolving actions. Addition of either DHA or 14\textsuperscript{S}-hydroperoxydocosahexaenoic acid to macrophages transformed these substrates into the dihydroxy mediator, the complete structure of which was established. Given its potent stereoselective actions and in vivo production, we coined this new family the maresins (macrophage mediators in resolving inflammation); specifically the lead mediator as maresin 1 (MaR1). These findings lend further support to the concept that local enzymatic conversion of DHA to bioactive and stereoselective mediators could indeed underlie the essential role of DHA.

**Aspirin, COX-2, and Proresolving Pathway of Local Mediators**

Aspirin is one of the most widely used antiinflammatory drugs, and low-dose aspirin (81 mg) is currently recommended by the American Heart Association (www.americanheart.org) for both primary and secondary prevention of myocardial infarction, stroke, and unstable angina. The beneficial actions of aspirin in the cardiovascular system have been widely attributed to the well-documented ability of aspirin to block prostaglandin and prothrombotic thromboxane (TXA\textsubscript{2}) generation via acetylation of COX-1. Notably, aspirin has additional antiinflammatory actions, such as blocking leukocyte trafficking to inflamed tissues, which cannot be attributed to aspirin’s ability to inhibit prostanoid biosynthesis. As noted above, aspirin acetylation of COX-2 not only inhibits prostanooid formation but alters the active site of COX-2 and thereby permits conversion of AA to 15R-HETE in vascular endothelial cells. This intermediate can be further transformed to epimeric lipoxins by leukocytes (Figure 3). The formation of 15-epi-lipoxins is documented in healthy individuals taking low-dose aspirin and was shown to be both age and gender dependent. Recently, the antiinflammatory actions of aspirin were documented during acute inflammation in humans. Oral administration of low-dose aspirin reduced leukocyte accumulation in cantharidin-induced skin blisters and was associated with both 15-epi-lipoxin biosynthesis and an increase in ALX expression.

As noted, both EPA and DHA are substrates for acetylated COX-2, which generates biosynthetic precursors to AT-resolvins. The AT-resolvins share potent antiinflammatory actions of native resolvins. Thus, it can be considered that aspirin, in addition to blocking proinflammatory lipid mediator production, stimulates resolution via the generation of bioactive epimers of lipoxins and resolvins. These actions could underlie the multiple beneficial effects of aspirin in complex cardiovascular diseases and suggest that formation of proresolving lipid mediators could, in part, explain the distinct antiinflammatory and proresolution actions of aspirin.

**LXA\textsubscript{4} and Aspirin-Triggered 15-Epi-LXA\textsubscript{4} in Animal Models and Human Diseases**

The potent antiinflammatory and proresolving actions of lipoxins and epi-lipoxins have been demonstrated in multiple animal models of human diseases (Table 2). Both native LXA\textsubscript{4} and 15-epi-LXA\textsubscript{4} bind and activate ALX and decrease PMN infiltration in murine and rat models of acute peritonitis. The endogenous protective role of ALX mediating the biological actions of lipoxins has been demonstrated in mice overexpressing the human lipoxin receptor in myeloid cells.
and more recently in mice lacking the murine homolog of ALX, FPR2.40,41 Numerous studies have further demonstrated that the progression of inflammation in chronic diseases, such as asthma, scleroderma lung disease and cystic fibrosis, is associated with deficiencies in lipoxin production and/or an imbalance between proinflammatory eicosanoids and lipoxins.81–83 Accordingly, restoration of these deficiencies resolves inflammation associated with these diseases, decreasing leukocyte accumulation and proinflammatory cytokine generation. In addition to regulating excessive leukocyte responses, lipoxins also decrease fibrosis in lung injury and in renal mesangial cells.84,85 It is noteworthy that several generations of stable analogs of lipoxins and 15-epi-lipoxins were prepared that are longer lived, and share the potent biological actions of endogenous lipoxins in vitro and in vivo.86 Lipoxins are currently in clinical development, and it is clear that their potent actions in regulating leukocyte responses, fibrosis and tissue injury can have far-reaching clinical implications.

Statins and Proresolving Lipid Mediators
Statins (3-hydroxy-3-methylglutaryl [HMG]-CoA reductase inhibitors) represent a widely used class of therapeutics that have well-documented actions in reducing low-density lipoprotein (LDL) cholesterol levels in humans. Although reducing LDL levels provides a prominent mechanism whereby statins reduce the risk of cardiovascular events (eg, myocardial infarction, sudden cardiac death), accumulating evidence suggests that statins have additional antiinflammatory properties that may underlie their diverse protective actions in the cardiovascular system.5 Indeed, numerous studies have demonstrated that statins reduce acute inflammation in vivo, in part via direct regulation of leukocyte-endothelial interactions.5,87 Recently, results of the JUPITER trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) demonstrated that rosuvastatin (20 mg/d) reduced systemic markers of inflammation (eg, C-reactive protein) and provided an additional clinical benefit beyond lowering cholesterol levels in patients, namely reducing major cardiovascular events.88 Recent results demonstrate that, as with aspirin, formation of 15-epi-lipoxins may underlie some of the beneficial actions of statins. Studies from Birnbaum et al demonstrate that atorvastatin promotes the myocardial generation of 15-epi-LXA4 via S-nitrosylation of COX-2.89 Similar to aspirin acetylation of COX-2, S-nitrosylated COX-2 produces 15R-HETE, which is converted by leukocyte 5-LOX to generate 15-epi-LXA4 (Figure 3). It was further elucidated that the antiatherogenic thiadizolidinedione pioglitazone also promotes the generation of 15-epi-lipoxins in the myocardium and is additive when given together with statins.90 COX-2 and 5-LOX coprecipitate in adult rat hearts after treatment with these commonly-used therapeutics.90 In isolated adult rat cardiac myocytes, 5-LOX cellular distribution was regulated by statin and thiazolidinedione treatment, and it was proposed that protein kinase A-dependent phosphorylation of 5-LOX induced by these therapeutics promotes its association with COX-2 to generate 15-epi-lipoxins, whereas in absence of phosphorylation, 5-LOX associates with membranous cytosolic phospholipase A2 to promote generation of leukotrienes.90 Thus, these findings illustrate the delicate balance between proinflammatory and antiinflammatory-proresolving LM pathways and suggest that commonly used therapeutics may exert antiinflammatory effects and potentially proresolving actions via the regulation of LM biosynthesis.

The enhanced formation of 15-epi-LXA4 by statins was recently confirmed and extended in a report showing that lovastatin promotes 15-epi-LXA4 formation and coincides with protection from murine lung inflammation.91 It is noteworthy that potential adverse interactions can occur when COX-2 is both acetylated and S-nitrosylated, in that the activity of COX-2 in promoting 15-epi-LXA4 formation is inhibited.92 Given that many cardiovascular disease patients are on both antiplatelet therapy and cholesterol-lowering therapy, further studies on the impact of this combination therapy on inflammation-resolution pathways will be important. It remains of interest whether these results, from both in vitro and in vivo studies, translate to humans. It is likely that this mechanism will also impact the biosynthesis of epimeric forms of resolvins.

Vascular Actions of Proresolving Lipid Mediators
Lipoxins and resolvins each exhibit direct actions on endothelial cells and regulate leukocyte:endothelial interactions in isolated cells and in vivo (Figure 4). Recent evidence demonstrates that receptors for these mediators, namely ALX and GPR32, are expressed on human endothelial cells.79,80 Lipoxins directly stimulate the endothelial production of vaso-protective and antiinflammatory mediators, NO and prostacyclin (PGI2).78,93 Of interest, aspirin-stimulated NO production was found to be dependent on the formation of 15-epi-LXA4 in vivo. Notably, the antiinflammatory actions of aspirin are dependent on both constitutive and inducible nitric oxide synthase (eNOS and iNOS)-derived NO, and both aspirin and 15-epi-LXA4 have reduced effects on leukocyte:endothelial interactions in eNOS and iNOS knockout mice.78 These results provide further support that 15-epi-lipoxin production underlies some of the beneficial cardiovascular effects of aspirin treatment. Lastly, 15-epi-lipoxin potently reduces the formation of reactive oxygen species (ROS) in endothelial cells by preventing NADPH-oxidase activation.94 Recently, we determined that resolvin D2 (RvD2) directly stimulates the endothelial production of NO and that RvD2 potently reduces leukocyte infiltration in a murine model of peritonitis in an eNOS-dependent manner.62 Leukocyte adhesion to postcapillary venules was largely abolished by RvD2, as assessed by intravital microscopy, and this is also partially dependent on endogenous NO production, as a nonselective NOS inhibitor reversed this effect.59 In addition to regulating the vascular production of NO and prostacyclin, 15-epi-lipoxins have been shown to underlie aspirin-induced heme oxygenase (HO)-1 expression in endothelial cells. A stable analog of 15-epi-LXA4, denoted ATL-1, directly stimulates HO-1 expression in isolated human endothelial cells to a similar extent as aspirin alone.95 These effects of ATL-1 were shown to be receptor-dependent and translated to reduced surface expression of VCAM-1 in a HO-1–dependent manner.95 Lastly, recent evidence indicates
that both lipoxins and resolvins have direct actions on vascular smooth muscle cells (VSMCs). Receptors for both LXA₄ and RvE1, ALX, and ChemR23, respectively, were identified on human saphenous vein SMC. Both RvE1 and 15-epi-LXA₄ counter-regulate platelet-derived growth factor (PDGF)-stimulated VSMC migration in a dose-dependent manner and decrease PDGF receptor phosphorylation. ⁹⁶ Thus, these and related results suggest that proresolution LMs may have multiple diverse and protective actions beyond myeloid cells. It will be important to determine whether these novel actions of proresolution lipid mediators translate into protective actions in complex cardiovascular pathologies.

**Atherosclerosis**

Atherosclerosis is widely viewed as a chronic inflammatory disease characterized by the excessive recruitment and activation of peripheral blood mononuclear cells, such as monocytes and T-cells. ¹⁸ Monocytes differentiate into macrophages within the plaque milieu and attempt to clear excess oxidized lipoproteins and cholesterol from the tissue. This precipitates the generation of lipid-laden foam cells that fail to clear from the plaque, continue to secrete proinflammatory cytokines/chemokines and adhesion receptors (VCAM-1 and P-selectin). ⁹⁷ Notably, LXA₄ and RvD1 display defects in their ability to phagocytose apoptotic cells. ¹⁶ Moreover, defective phagocytosis and clearance of apoptotic cells was observed in advanced atherosclerotic lesions from these mice. Interestingly, saturated fatty acids (palmitic and stearic) were increased in obese mice relative to endogenous omega-3 fatty acids and were implicated in defective macrophage efferocytosis. Accordingly, supplementation of omega-3 fatty acids, EPA and DHA, reversed deficits in macrophage efferocytosis in obese/LDLR⁻/⁻ mice. ¹⁶ These studies highlight that progression of chronic inflammatory diseases could result in part because of altered resolution and implicates a role for endogenous lipid mediator pathways.

In accordance with the view that altered resolution contributes to atherogenesis, mice lacking both 12/15-LOX and apoE display exacerbated atherosclerotic lesion formation compared to apoE-null mice. ⁹⁷ Similarly, targeted macrophage-specific overexpression of 12/15-LOX protected from lesion development. Importantly, 12/15-LOX gene dosage correlated with LXA₄ formation in isolated macrophages, as well as the production of 17-hydroxydocosahexaenoic acid, a marker of the D-series resolvin biosynthetic pathway. Both lipoxins and resolvins display potent actions on isolated macrophages and endothelial cells, regulating production of proinflammatory cytokines/chemokines and adhesion receptors (VCAM-1 and P-selectin). ⁹⁷ Notably, LXA₄ and RvD1 each enhance macrophage phagocytosis of apoptotic cells. These results corroborate earlier findings in rabbits demonstrating the atheroprotective effect of macrophage-specific transgenic overexpression of 15-LOX. ⁹⁶ The antiinflammatory proresolving role of this pathway was recently independently confirmed. ⁹⁹–¹⁰¹

**Containing and Clearing Microbes**

Prevalence of polymicrobial sepsis is increasing and mortality rates associated with septic shock remain as high as 20%
Angiogenesis

Vessel sprouting induced by mitogenic stimuli, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), plays an important role in wound healing, recovery from myocardial ischemia/reperfusion injury, and organ regeneration. In contrast, pathological angiogenesis, as occurs during retinopathy and tumor growth, can be detrimental if not properly regulated. Lipid mediators are key players in angiogenesis, with proinflammatory eicosanoids such as 12-HETE and prostaglandins (PGE2) promoting VEGF-stimulated angiogenesis. Like most biological processes, angiogenesis is tightly controlled by both positive and negative inputs and, although multiple chemical mediators have been shown to operate in this regard, we have recently learned that lipoxins and resolvins also regulate pathological angiogenesis. In isolated human endothelial cells, lipoxins and their AT-epimers regulate VEGF, as well as cysteinyl leukotriene (LTD4)-stimulated migration and proliferation. Godson et al elucidated the mechanistic basis for this regulation and found that LXA4 regulates VEGF receptor-2 phosphorylation and downstream signaling. This concept of growth factor receptor transinhbition was extended to other growth factor receptors, including PDGF receptor β in mesangial cells. These potent actions of lipoxins were validated in vivo, where 15-epi-lipoxin stable analog (ATL-1) blocked angiogenesis in a granuloma model of inflammatory angiogenesis. Groenert and colleagues demonstrated the role of endogenous lipoxin circuits in protecting from pathological neovascularization induced by corneal injury. Both the murine homolog of the lipoxin receptor and 15-LOX were upregulated during corneal injury, and genetic ablation of either 15-LOX or 5-LOX (both enzymes involved in lipoxin biosynthesis) exacerbated pathological neovascularization and correlated with increased VEGF-A and VEGF-3 receptor expression. Along these lines, topical administration of synthetic lipoxins decreases VEGF-A expression and protects from pathological angiogenesis.

In addition to the role of AA-derived eicosanoids in regulating angiogenesis, recent evidence also indicates a protective role for omega-3-derived LMs in pathological angiogenesis. Transgenic mice overexpressing the fat-1 gene (as noted above) are protected from hypoxia-induced neovascularization. Interestingly, resolvins are biosynthesized in fat-1 transgenic mice and administration of synthetic resolvins protect from pathological neovascularization. The protective actions of resolvins in this context were mediated in part through the direct regulation of retinal TNF-α production. These results were extended in further studies which showed that both lipoxins and resolvins modulate leukocyte infiltration into injured corneas, regulate proinflammatory cytokine generation, VEGF-A production, and decrease angiogenesis. Importantly, receptors for both LXA4 and RvE1, ALX and ChemR23, are expressed in epithelial cells, stromal keratinocytes and infiltrated CD11b+ cells. Overall, these results suggest that lipoxins and resolvins have diverse protective actions on multiple cell types and endogenous pro-resolution circuits protect against pathological angiogenesis.

Ischemia/Reperfusion Injury

Several recent reports demonstrate that proresolution LMs elicit organ-protective actions during ischemia/reperfusion. In the kidney, reperfusion following an ischemic insult results in increased circulating DHA and correlates with local production of D-series resolvins, including RvD2. This is consistent with recent results demonstrating that free plasma DHA is rapidly delivered to inflammatory sites for local generation of resolvins. Synthetic resolvins largely abolish leukocyte infiltration into reperfused kidneys and protect from second-organ reperfusion injury in a murine model of hind-limb ischemia. Importantly, the protective actions of synthetic resolvins and their stable analogs are retained after therapeutic administration (ie, after the initiation of reperfusion). The endogenous protective role of proresolution mediators was demonstrated in mice lacking the murine homolog of the LXA4 receptor (ALX/FPR2), in which ischemia/reperfusion...
resulted in excessive leukocyte adhesion and emigration in the mesenteric microcirculation. Of note, in addition to regulating excessive leukocyte-mediated tissue injury in response to reperfusion, proresolution LMs also reduce organ fibrosis. Recently, it was shown that RvE1 stimulates phosphorylation of eNOS and Akt, and prevents apoptosis in cardiac myocytes exposed to hypoxia/reoxygenation by attenuating the level of activated caspase-3. These direct actions were demonstrated in vivo where RvE1 decreased infarct size in a rat model of myocardial ischemia/reperfusion injury. Of note, these protective actions of RvE1 are dependent on activation of the epidermal growth factor receptor (EGFR) and are consistent with a recent report documenting transactivation of EGFR by RvE1 in corneal epithelial cells.

**Obesity and Diabetes: Lipid Mediator Interplay**

Obesity is one of the most robust risk factors for the development of type 2 diabetes, and chronic low-grade inflammation is currently held to be a prominent link between the two syndromes. Given the indispensable role of LMs in orchestrating macrophage-dependent inflammatory responses, it is not surprising that operational LM pathways were recently found to play both positive and negative regulatory roles within the context of obesity-induced diabetes. Claria et al recently found that the leukotriene pathway plays a role in the development of adipose tissue inflammation in experimental obesity. Enzymes involved in LTB4 biosynthesis, including 5-LOX and 5-LOX activating protein (FLAP), were expressed in adipose tissue, and the level of FLAP increases with high fat feeding. Importantly, receptors for LTB4 (BLT-1 and 2), as well as the cysteinyl leukotrienes (cysLT1 and -2) are expressed in adipocytes and stromal vascular cells. Modulation of the leukotriene biosynthetic pathway with a FLAP inhibitor decreased systemic proinflammatory cytokines, adipose tissue macrophage content and systemic insulin resistance. In a separate study, omega-3 feeding was protective in obesity-induced inflammation and associated with resolvin biosynthesis in adipose tissue. Synthetic RvE1 increased mRNA expression of genes known to be protective against systemic insulin resistance, such as adiponectin, PPARγ, and insulin-receptor substrate-1.

**Summary and Future Directions**

Proresolving LMs, including the lipoxins, resolvins, protectins, and recently identified maresins, represent a new genus of endogenous LMs that carry out multilevel antiinflammatory and proresolution actions to hasten the return to homeostasis. These novel mediators possess unique and specific protective functions demonstrated in animal models of acute and chronic diseases, and also have potent actions on, and are biosynthesized by, human cells. The discovery of these opens up entirely new terrain for therapeutics based on endogenous protective LMs and thereby exert antiinflammatory and proresolving actions, other commonly used therapeutics can delay resolution. In ongoing studies, it will be important to elucidate how proresolution pathways are modulated with the progression of chronic diseases and the impact of blocking these pathways in humans. Although both aspirin and statins can promote the formation of endogenous protective LMs and thereby exert antiinflammatory and proresolving actions, other commonly used therapeutics can delay resolution. In summation, this new area of “resolution pharmacology” is likely to lead to better targeted approaches to treat inflammatory diseases without precipitating sustained immunosuppression that may be relevant in vascular medicine.

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