Inflammation and Cardiovascular Disease

Persistent unresolved inflammation has long been recognized to play a fundamental role in the development of chronic diseases including arthritis, autoimmune diseases, and asthma. In recent decades, it has emerged that maladaptive inflammation is causally involved in the development of cardiovascular disease (CVD) and other metabolic disorders including diabetes mellitus and obesity. Indeed, inflammation is a central feature in the development of cardiovascular diseases, an understanding of the endogenous processes that govern normal resolution of acute inflammation is critical for determining why sterile maladaptive cardiovascular inflammation perpetuates. Here, we provide an overview of the process of resolution with a focus on the enzymatic biosynthesis and receptor-dependent actions of resolvins and related proresolving mediators in immunity, thrombosis, and vascular biology. We discuss how nutritional and current therapeutic approaches modulate resolution and propose that harnessing resolution concepts could potentially lead to the development of new approaches for treating chronic cardiovascular inflammation in a manner that is not host disruptive. (Circ Res. 2016;119:113-130.

**Key Words:** cardiovascular diseases ■ fatty acids, omega-3 ■ homeostasis ■ inflammation ■ lipids

Resolution of Acute Inflammation and the Role of Resolvins in Immunity, Thrombosis, and Vascular Biology

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**Abstract:** Acute inflammation is a host-protective response that is mounted in response to tissue injury and infection. Initiated and perpetuated by exogenous and endogenous mediators, acute inflammation must be resolved for tissue repair to proceed and for homeostasis to be restored. Resolution of inflammation is an actively regulated process governed by an array of mediators as diverse as those that initiate inflammation. Among these, resolvins have emerged as a genus of evolutionarily conserved proresolving mediators that act on specific cellular receptors to regulate leukocyte trafficking and blunt production of inflammatory mediators, while also promoting clearance of dead cells and tissue repair. Given that chronic unresolved inflammation is emerging as a central causative factor in the development of cardiovascular diseases, an understanding of the endogenous processes that govern normal resolution of acute inflammation is critical for determining why sterile maladaptive cardiovascular inflammation perpetuates. Here, we provide an overview of the process of resolution with a focus on the enzymatic biosynthesis and receptor-dependent actions of resolvins and related proresolving mediators in immunity, thrombosis, and vascular biology. We discuss how nutritional and current therapeutic approaches modulate resolution and propose that harnessing resolution concepts could potentially lead to the development of new approaches for treating chronic cardiovascular inflammation in a manner that is not host disruptive. (Circ Res. 2016;119:113-130.

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Mounting evidence indicates that the presence of other chronic inflammatory diseases (eg, periodontitis and rheumatoid arthritis) can exacerbate CVD. Atherosclerosis is perhaps the CVD that has been most closely linked with chronic unresolved inflammation. Although the development of statins revolutionized the treatment of atherosclerosis by lowering low-density lipoprotein cholesterol, the burden of ischemic cardiovascular conditions has continued to rise globally and CVD remains the leading cause of death among Americans. Furthermore, intolerance and adverse effects of statins in a growing number of patients have emphasized the need for novel therapies.
Acute Inflammation and Its Resolution

In response to injury or infection, the protective program of acute inflammation and its complete and timely resolution are critical for the restoration of tissue homeostasis. This highly coordinated and synergistic program combines the distinct actions of multiple cell types to achieve pathogen eradication and subsequent tissue repair. The acute inflammatory response can be divided into 2 general phases: initiation and resolution (Figure 1). Initiation is marked by tissue edema resulting from increased blood flow and permeability of the microvasculature; processes that are mediated, in part, by lipid mediators (eg, cysteinyl leukotrienes and prostaglandins) and other vasoactive products (eg, histamine and bradykinin). Subsequently, polymorphonuclear neutrophils (PMN) migrate to the area to defend against microbial invasion. Drawn to the site of injury by exuded chemical signals including proinflammatory lipid mediators (eg, leukotriene B4 [LTB4]) and chemokines, PMN traverse the vasculature through precise interactions with endothelial adhesion receptors and subsequently engulf and degrade pathogens within phagolysosomes. The resolution phase is already being enacted at this early point as the influx of PMN is halted at a level appropriate for the insult and is accompanied by their timely apoptosis. Monocytes subsequently infiltrate the tissue where they differentiate into macrophages that avidly respond to pathogen-associated molecular patterns and damage-associated molecular patterns present in the injured tissue. Importantly, macrophages are highly responsive to the so-called find-me and eat-me signals (eg, nucleotides and externalized phosphatidylserine) released or presented by apoptotic cells such as PMN. Uptake of apoptotic cells by macrophages (ie, efferocytosis) is an anti-inflammatory process associated with decreased production of inflammatory mediators, thus coupling the initiation of inflammation with its ultimate resolution (Figure 1). The timely clearance of microbes and apoptotic cells is required to prevent bystander tissue damage and to set the stage for tissue repair and regeneration, allowing for the return to homeostasis. Indeed, active clearance of apoptotic cells is a key defining feature of resolution, as failed clearance can lead to cellular necrosis and exacerbated inflammation beyond the initial insult, impeding tissue repair. Macrophages persist in injured tissues longer than short-lived PMN, during which time they are continuously reprogrammed in response to local cues to facilitate tissue repair and orchestrate the delicate balance of fibrosis. Like innate immune cells, adaptive immune cells also play critical roles in the host response to infection, resolution of inflammation, and in tissue repair. Their accumulation defines the postresolution phase of the inflammatory response and assures a more rapid response to subsequent exposure to the same antigens. Interruption of this process at any point (eg, prolonged leukocyte recruitment and survival, impairments in apoptotic cell removal, and alterations in macrophage phenotype switching) could potentially lead to chronic inflammation with resultant tissue damage, excessive fibrosis, and loss of function, as is seen in many CVDs such as atherosclerosis and heart failure.

By its nature, the acute inflammatory response is self-limiting in part because of inherent negative feedback regulation of inflammatory signaling pathways (eg, transcriptional repressors and endogenous receptor antagonists) when the trigger has been eliminated. However, it has recently become evident that active resolution of inflammation involves the biosynthesis of proresolving mediators that, as a genus, are just as diverse as the initiators of inflammation. Thus, critical to determining the fate of an inflammatory response is the balance of...
proinflammatory and proresolving mediators that are produced in the exudate in a temporal manner. Traditionally, it has been held that an excess production of proinflammatory mediators underlies chronic inflammation; however, mounting evidence supports the view that disruptions in endogenous proresolving circuits may be an equally important mechanism. These proresolving mediators not only actively terminate the production of proinflammatory mediators but also directly stimulate macrophage phagocytosis of both apoptotic cells and bacteria, promote egress of phagocytes from sites of inflammation, regulate PMN apoptosis, promote chemokine scavenging, and stimulate tissue repair and regeneration.

Proresolving Mediators

The discovery of bioactive mediators with potent inflammation-resolving actions in experimental models of acute inflammation was a seminal development that provided compelling evidence that resolution is an active process rather than a passive one as traditionally thought. Self-resolving inflammatory exudates were shown to contain structurally unique families of signaling molecules that are temporally produced and when added back in experimental models of acute inflammation, potently enhance the resolution phase. Like the initial phases of inflammation, enzymatically produced lipid mediators derived from polyunsaturated fatty acids (PUFAs) play a central role in resolution, in part because of their rapid production by distinct immune cell subsets. During the transition from inflammation to resolution, lipid mediator class switching takes place in which initial formation of proinflammatory mediators induce the production of enzymes that enable the reprogramming of leukocytes to generate specialized proresolving lipid mediators (SPMs) from the same PUFAs.

Figure 1. The coordinated temporal events of self-limited acute inflammation. The ideal outcome of an acute inflammatory response is complete resolution. The inflammatory response can be divided into 2 general phases: initiation and resolution. Critical to progressing from initiation to resolution is the temporal switch in lipid mediators that are biosynthesized by leukocytes in the tissue, a process known as lipid mediator class switching. The earliest stage of the inflammatory response is marked by tissue edema caused by increased blood flow and microvascular permeability and is mediated by the release of proinflammatory lipid mediators including the cysteinyl leukotrienes and prostaglandins. Polymorphonuclear neutrophils (PMN) infiltrate in response to lipid mediators including leukotriene $\text{B}_4$, and engulf and degrade pathogens. Subsequently, PMN undergo apoptosis and also switch from releasing proinflammatory mediators to proresolving mediators (eg, resolvins) that signal the clearance of apoptotic cells by macrophages (MΦ) in an anti-inflammatory process termed efferocytosis. In addition to promoting efferocytosis, proresolving lipids halt further PMN recruitment and stimulate a proresolving macrophage phenotype that is important for tissue repair.
conserved from flatworms (eg, *Planaria*) to humans, and several studies have documented endogenous formation of these mediators in healthy individuals, with lower levels observed in patients with Alzheimer disease, peripheral artery disease, and asthma. In addition to lipid mediators of resolution, other important mediators of the resolution program have emerged and include proteins and bioactive peptides (eg, annexin A1, galectin 1, and melanocortins) and gases (nitric oxide, hydrogen sulfide, and carbon monoxide). Readers are referred to recent reviews covering each of these mediators in detail. Here, we focus primarily on the biosynthesis and biological actions of resolvins in the inflammation-resolution program.

### Biosynthesis of Resolvins

The use of liquid chromatography–tandem mass spectrometry–based approaches were critical in the identification and structural elucidation of resolvins and related SPMs. Applying these methods to generate a lipid mediator profile of self-resolving inflammatory exudates in mice yielded the first evidence of a novel bioactive mediator generated from EPA during resolution. Later named resolin E1 (RvE1), its biosynthetic pathway proceeds via conversion of EPA to 18R-hydroperoxyeicosapentaenoic acid by acetylated cyclooxygenase-2 (COX-2) or cytochrome P450 enzymes, which is subsequently converted to RvE1 by 5-LOX (Figure 2). The epoxide intermediate formed during RvE1 biosynthesis is hydrolyzed by leukotriene A4 hydrolase, the same enzyme that produces proinflammatory LTB4. This mechanism provides an additional point of control in the balance between proinflammatory mediators and SPMs. The formation of RvE1 precursor, 18R-hydroperoxyeicosapentaenoic acid, was identified in hypoxic endothelial cells (ECs) and can be transformed to RvE1 through leukocyte–endothelial interactions. The complete structural elucidation of RvE1 was performed via total organic synthesis and matching with endogenous material, which enabled the assessment of the individual bioactions of RvE1. These actions include the reduction in PMN infiltration to sites of acute inflammation, blunting of inflammatory cytokine production, and the enhancement of macrophage effecrocytosis. Additional members of the EPA-derived (E-series) resolvins have since been characterized. Similar to RvE1, RvE2 is generated from EPA via the intermediate 18R-hydroxyeicosapentaenoic acid (HEPE) and its resultant 5-LOX–dependent conversion to a 5S-hydroperoxide. However, instead of undergoing an epoxidation step, the 5S-hydroperoxide is directly converted to RvE2 (Figure 2). More recently, a third member, RvE3, was also identified and its biosynthetic pathway proceeds via 5-LOX. Specifically, the hydroperoxide formed via 5-LOX can undergo epoxidation to generate a 7S,8S epoxide that is then enzymatically hydrolyzed to produce RvD1 or RvD2 (Figure 2). As with the E-series resolvins, these steps can take place within a single cell (eg, PMN or macrophages) or can occur during cell–cell interactions (eg, leukocyte–endothelial, PMN–macrophage). Additional structurally unique D-series resolvins, including RvD3–D6, have also been identified, and the complete structures and stereochemical assignments of RvD3 and RvD4 have recently been reported. In murine models of acute inflammation, distinct temporal patterns of resolin biosynthesis have been observed, highlighting additional control points in their biosynthesis (eg, potentially novel regulation by epoxide hydro-lases). Interestingly, within a single cell type, lipid mediator class switching can occur and proresolving lipid mediators can regulate each other. For instance, RvD1 reduces nuclear localization of 5-LOX in macrophages and diverts arachidonic acid metabolism from proinflammatory lipid mediator, LTB4, to SPM, LXA4. Mechanistically, RvD1 inhibits the activation of calcium-calmodulin–dependent kinase II and downstream activation of p38 and a mitogen-activated kinase, which results in reduced phosphorylation of 5-LOX. Thus, SPM biosynthesis can be amplified by positive feed-forward mechanisms, helping to tip the balance from inflammation to resolution. Indeed, in several distinct models of acute inflammation and infection, administration of resolvins blunts production of proinflammatory eicosanoids and increases the formation of other proresolving mediators. It is important to note that, like most autacoids, resolvins are rapidly inactivated after they have elicited their biological actions in resolution. For instance, RvE1 is a substrate for 15-hydroxyprostaglandin dehydrogenase, generating the inactive product, 18oxo-RvE1. This suggests that accelerated inactivation could potentially underlie defective resolution in some cases and as a way to circumvent metabolic inactivation from a therapeutic standpoint, a variety of stable resolin analogs have been formulated and shown to have enhanced biological activity in vivo. Recently, a new family of mediators was identified where-in the epoxide intermediate in RvD1 and RvD2 biosynthesis can be enzymatically conjugated to glutathione to generate sulfido-conjugated resolvins. They contain glutathione at the C8 position and can be subsequently converted to cysteinylglycinyl conjugates by γ-glutamyl transferase. These novel mediators were identified in infectious inflammatory exudates, human spleens, and in human leukocytes (eg, PMN and macrophages), as well as apoptotic PMN. Like other SPMs, these resolin conjugates enhance bacterial clearance in vivo and macrophage phagocytosis. Interestingly, they have distinct roles in promoting tissue regeneration in Planaria. Given these new roles, they were named resolin conjugates in tissue regeneration. It is noteworthy that similar biosynthetic routes were also identified for maresin and protectin families of SPMs.

### Proresolving Receptors

Like other classic lipid mediators and chemokines, SPMs elicit their biological actions through the activation of specific G-protein–coupled receptors in a stereoselective manner (Figure 2). The proresolving and anti-inflammatory actions of LXA4 and RvD1 are mediated via specific signaling through ALX (Alox receptor; also termed formyl peptide receptor 2 [FPR2]) and previous orphan, GPR32
(G-protein–coupled receptor 32; also termed D-resolvin receptor 1). Given that LXA₄ and RvD1 are structurally similar but generated from different PUFA precursors and that they activate the same receptors and promote reciprocal biosynthesis of each other provides additional evidence that proresolving pathways are intimately linked. Specific
binding to these receptors was unequivocally demonstrated using radiolabeled material and both homo- and heteroligand competition. Moreover, their proresolving actions were validated in mouse models of acute inflammation with targeted overexpression or genetic deletion of ALX/FPR2. We note that there is no murine homolog of human GPR32 identified to date. The actions of RvD1 in counter-regulating PMN recruitment during acute peritonitis are ablated in ALX/FPR2-deficient mice. In human macrophages, RvD1 enhances phagocytosis of both apoptotic PMN and opsonized zymosan, actions that are potentiated by overexpression of ALX/FPR2 and GPR32 and attenuated with their knockdown.

Recently, it has been shown that RvD3 and RvD5 also activate human GPR32. Given that these mediators are generated at distinct times during resolution of inflammation, this mechanism may serve to preserve GPR32 ligands during different stages of resolution. Interestingly, ALX/FPR2 is also activated by other proresolving mediators, such as annexin A1, along with proinflammatory mediators (e.g., serum amyloid A). Depending on the stimulus, signaling through this receptor can elicit either proinflammatory or proresolving effects. Emerging evidence indicates that dimerization underlies, in part, these distinct and unconventional roles.

Specifically, annexin A1 and LXA4 induce ALX homodimer formation, and this leads to increased production of IL-10 in monocytes. In contrast, proinflammatory agonists, such as serum amyloid A, can block this homodimerization. In PMN, annexin A1, an annexin A1 peptide mimetic (Ac2-26), and LXA4 can induce formation of ALX heterodimers with FPR1. These heterodimers elicit distinct signaling pathways (JNK/caspase-3) that override proinflammatory signals in PMN, such as serum amyloid A, through the same receptor. As noted, this is important because timely apoptosis and clearance of PMN is required for resolution of inflammation, and prolonged PMN survival can cause excessive tissue damage.

A distinct G-protein-coupled receptor denoted chemotactrant receptor 23 (ChemR23; also termed E-resolvin receptor), which shares 36% sequence identity with ALX/FPR2, is activated by RvE1 in a stereoselective manner (Figure 2). The anti-inflammatory signaling properties of ChemR23 were also demonstrated in studies interrogating a different agonist, chemerin peptide, and in mice with genetic deletion of the receptor. Subsequent investigations further substantiated ChemR23-mediated signaling by RvE1, including stimulation of macrophage phagocytosis via the phosphatidylinositol 3-kinase/Akt pathway and activation of the translational regulator, ribosomal protein S6. Interestingly, not all of RvE1’s actions can be ascribed to ChemR23. Indeed, RvE1 potently blocks PMN migration in vitro, yet these cells do not express ChemR23. Additional studies determined that RvE1 binding to human PMN is displaced by LTβ2 and an antagonist to the LTB4 receptor (BLT1), but not by chemerin peptide, indicating that BLT1 is a target for RvE1 on PMN. This study also demonstrated that the actions of RvE1 on PMN may be, in part, because of an attenuation of proinflammatory LTB4 signaling, which was subsequently confirmed in Blt1-deficient mice. Like RvE1, RvE2 also binds BLT1 and blocks LTβ4-stimulated actin polymerization in PMN. Importantly, although many of the downstream actions of RvD1 are similar to those of RvE1, it does not bind ChemR23 or BLT1.

Using an unbiased β-arrestin-based screening approach, a recent study identified that RvD2 activates a distinct G-protein–coupled receptor denoted GPR18 (also termed D-resolvin receptor 2), which among innate immune cells, is expressed on human PMN, monocytes, and macrophages. Specific binding to GPR18 was confirmed using radiolabeled RvD2 and binding competition studies. Similar to the actions of RvD1, RvD2 increases phagocytosis of apoptotic PMN by macrophages, which is enhanced with GPR18 overexpression and decreased with GPR18 knockdown. Similarly, the potent actions of RvD2 in limiting PMN infiltration and enhancing clearance of Escherichia coli and Staphylococcus aureus in vivo are abolished in Gpr18-deficient mice. Taken together, it is clear that the actions of resolvins have significant overlap yet remain specific and are mediated by distinct receptors. The multiplicity of cellular sites of action of resolvins reinforces the concept that these mediators are vital in orchestrating the complex, coordinated response that governs the active process of inflammation-resolution. The identification of these proresolving receptors has, and will continue to, illuminate the mechanisms governing resolution, including the specific signaling pathways and transcriptional programs engaged by proresolving mediators.

**Increasing Proresolving Lipid Mediators With Nutritional and Pharmacological Approaches**

Given the potent bioactions of resolvins and related SPMs during acute inflammation, there has been significant attention and effort dedicated to developing strategies for increasing their production endogenously. Perhaps the most obvious approach would be to increase the substrate. As discussed, the omega-3 PUFA EPA and DHA serve as the precursors for resolvins and other SPMs. In rodent models of inflammation, administration of EPA or DHA increases resolvin production. Mice with transgenic expression of a gene from Caenorhabditis elegans that enables endogenous generation of omega-3 PUFA from omega-6 PUFA (denoted Fat-1) are protected from inflammatory conditions such as colitis and pathological retinal angiogenesis and have elevated endogenous levels of resolvins. These protective actions were recapitulated with exogenous delivery of resolvins, establishing causality in this relationship. There have been numerous studies investigating the protective effects of increased dietary intake of omega-3s on a diverse range of inflammatory diseases in humans, including asthma and arthritis, as well as CVD, based in large part on the fact that the typical Western-type diet is deficient in omega-3 PUFA. One landmark clinical trial, the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico (GISSI) Prevenzione study, enrolled >11,000 recent MI patients and reported that 1 g of daily omega-3 supplementation for three and half years significantly reduced the risk of cardiovascular death. More recently, GISSI also found greater survival in patients with heart failure treated with omega-3s. Although these trials were able to demonstrate the beneficial effects of omega-3
supplementation, the mechanism of this protection remains unclear. In humans, several studies have shown that intake of omega-3 PUFAs increases production of resolvins and their biosynthetic pathway markers (e.g., 18-HEPE and 17-hydroxy-docosahexaenoic acid [DHA]) in plasma or serum. In a recent study, treatment with Lovaza (omega-3 ethyl esters) in patients with stable coronary artery disease increased levels of RvD6, 17R-PD1, and RvE2. Whether production of SPMs is causally related to the protective actions of omega-3s in humans requires further clinical studies, and it is also important to note that some chronic inflammatory diseases are associated with defects in SPMs production. Given that, unlike hormones, SPMs are locally produced and have actions locally in inflammatory exudates (i.e., autacoids); the relevance of their measurement in plasma is still unclear. Given that some clinical trials have failed to show improvements in cardiovascular end points with omega-3 PUFAs supplementation, it is intriguing to speculate that altered downstream conversion to bioactive SPMs could be related to variable results in such trials. This variation could also arise from the diversity in dosing and preparations (e.g., ethyl esters versus triglycerides). This is particularly relevant when one considers that the appearance of SPMs is dependent on multiple biosynthetic checkpoints and enzymatic degradation, rather than a passive linear increase with omega-3 intake. Moreover, high intake of omega-3s could also bias the utilization of EPA and DHA away from SPMs and toward the formation of free radical-initiated auto-oxidation products. Although the evidence to date indicates that SPMs and their intermediate precursors are indeed produced in humans in vivo and are demonstrable by liquid chromatography–tandem mass spectrometry, it is noteworthy that with inadequate approaches and methodology, some studies were unable to identify endogenous SPMs in plasma, which have specific stereochemistry and require the use of commercially available deuterium-labeled standards for their definitive identification in human tissues. These are important considerations for the development and implementation of new therapeutic approaches and also suggest that targeted delivery of specific SPMs may be a better therapeutic approach.

One important design aspect of the GISSI trials was the instruction of enrolled patients to adhere to their recommended preventive medications in addition to the omega-3 PUFAs supplement. Included in the preventive medications for many of these patients was aspirin. Aspirin is one of the most widely used anti-inflammatory drugs, and low-dose aspirin is currently recommended by the American Heart Association (www.americanheart.org) for people at high risk of heart attack and heart attack survivors. It was traditionally held that the salubrious effects of an aspirin regimen stemmed solely from halting prostaglandin and thromboxane production. More recently, however, additional anti-inflammatory actions of aspirin have been described. Aspirin-acetylation of COX-2 not only blocks the production of prostaglandin precursors but also switches the activity of COX-2 such that it generates 15R-hydroxyeicosatetraenoic acid (HETE) with arachidonic acid as a substrate or 18-HEPE and 17R-HDHA with EPA and DHA as substrates, respectively. These intermediates are subsequently converted into epimeric (i.e., aspirin-triggered) lipoxins and resolvins that share the potent biological actions of the native mediators in which the chirality of the C15 or C17 hydroxyl groups in 15-HETE and 17-HDHA are predominantly in the S configuration. Thus, aspirin could be considered to be resolution friendly because in addition to blocking inflammation, it jump starts resolution by shifting the lipid mediator profile from proinflammatory eicosanoids to SPMs. Interestingly, some of these mediators (i.e., RvE1) regulate platelet activation as well (vide infra). Of interest, a recent study demonstrated that aspirin treatment enhances reverse cholesterol transport in hypercholesterolemic mice, which reduces atherosclerosis. This was associated with increased production of lipoxins in the liver and exogenous delivery of a stable analog of LXB4 recapitulated these protective effects. These results indicate that in addition to the inflammation-resolving roles of SPMs, they may have additional atheroprotective actions.

In addition to aspirin, statins are a widely prescribed class of lipid-lowering therapeutics with anti-inflammatory effects in the cardiovascular system, as discussed above. Results of the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial demonstrated that the administration of rosuvastatin decreases systemic markers of inflammation and that the observed reduction in major cardiovascular events is independent of the reduction in low-density lipoprotein cholesterol. Although it is clear that statins have anti-inflammatory effects, their mechanism of action is not entirely clear. Similar to aspirin, statins have been shown to target COX-2, however, rather than acetyling the enzyme; statins increase S-nitrosylation and bias the enzyme toward the formation of 15-epimeric lipoxins as well. More recently, it was demonstrated that S-nitrosylation of COX-2 via administration of atorvastatin initiates the biosynthesis of a novel group of 13-series resolvins from omega-3 docosapentaenoic acid (DPA). Human and mouse leukocytes were shown to convert DPA to these novel bioactive products analogous to other SPMs including the D-series resolvins. These bioactive compounds are produced in PMN-endothelial cocultures and are present in human and mouse tissues during sterile inflammation or infection. The structures of 4 members of this family have recently been elucidated and contain conjugated triene and diene double bonds, as well as an alcohol at C13 for which they have been termed 13-series resolvins. Although their biosynthetic pathway remains to be fully detailed, it has been shown that endothelial COX-2 initiates their production during PMN–EC interactions and S-nitrosylation of the enzyme by atorvastatin further increases their production. Conversely, selective COX-2 inhibitors diminish their formation. Along these lines, with the widespread use of these and other therapeutics that target the inflammatory response, it is critical to underscore the importance of avoiding drugs that are resolution toxic. Broad anti-inflammatory treatments are insufficient, in that many of the signals produced during the inflammatory phase actually play key roles in initiating resolution (e.g., prostanoids-vide supra). Indeed, selective COX-2 inhibitors that are detrimental for patients with CVD because they perturb production of
protective prostanoids (ie, prostacyclin) also impair resolution and resolvin production during acute inflammation, as do LOX inhibitors.39 Ideal therapies to treat inflammatory diseases would be dual in nature and blunt the inflammatory response, while promoting the resolution program.10

Cellular Actions of Resolvins in the Context of CVD

Neutrophils
As the first responders to sites of microbial invasion and tissue injury, the fundamental role of PMN as drivers of inflammation has been thoroughly described.9 The capacity of the initiators of resolution, however, is just beginning to be appreciated.29,96 In response to prostaglandin E₂, PMN transition from releasing proinflammatory LTB₄ to proresolving LXA₄ in a process known as lipid mediator class switching (vide supra).42 PMN are also a prominent source of resolvins, and thus, similar to macrophage subsets, PMN can be reprogrammed to promote inflammation or enact its resolution. LXA₄ is critical in halting further PMN recruitment and was shown to inhibit chemotaxis, as well as transendothelial and transepithelial migration, in isolated PMN (Figure 3).97 As noted above, SPMs (eg, LXA₄, RvE1) can also override survival signals in PMN, ensuring their timely apoptosis.41,63 In addition, both lipoxins and resolvins act on macrophages to promote the effecrotic process of apoptotic PMN,49 a process that reciprocally stimulates the production of both E- and D-series resolvins, maresins, and protectins, thereby promoting the propagation of resolution, rather than proinflammatory signaling (Figure 3).96,99 Resolvins also act directly on PMN to decrease chemotaxis toward chemokine gradients (eg, IL-8) and to reduce the surface expression of adhesion receptors (eg, CD11b/CD18) while preventing L-selectin (CD62L) shedding.40,58 These selective actions of SPMs on PMN activation, rolling, and adhesion to the activated endothelium have been demonstrated both in vitro and in vivo using human ECs under flow or intravital microscopy–based imaging of the inflamed microvasculature.40,59,100 Accordingly, all SPMs have been shown to blunt PMN infiltration into sites of acute inflammation, including bacterial peritonitis, dermal inflammation, and lung inflammation.25 In addition to blunting PMN activation and regulating apoptosis, SPMs have also been shown to increase the expression of chemokine receptors (ie, CCR5) on PMN.

Figure 3 continued. In addition, they stimulate production of nitric oxide (NO) and prostacyclin (PGI₂) from EC. In the tissue, SPMs promote the protective actions of leukocytes by enhancing the phagocytosis of bacteria and cellular debris and macrophage effecrotic process of apoptotic PMN. In the context of atherosclerosis, SPMs decrease EC production of leukocyte chemoattractants and adhesion molecules, potentially halting further recruitment of leukocytes to the vessel wall. They also potently prevent platelet aggregation and thrombosis. In the subendothelium, enhanced macrophage effecrotic process and promotion of a resolving macrophage phenotype contributes to a more stable plaque with decreased necrosis. During pathological inward remodeling (ie, restenosis), SPMs combat neointimal hyperplasia by inhibiting vascular smooth muscle cell (VSMC) proliferation and migration while simultaneously preventing monocyte adhesion and inflammatory signaling. IL indicates interleukin; and MCP, monocyte chemoattractant protein.
apoptotic PMN, thereby facilitating chemokine scavenging during resolution as these apoptotic cell/chemokine clusters are subsequently cleared by macrophages. Interestingly, in concert with blunting PMN recruitment, SPMs enhance PMN-mediated bacterial containment (Figure 3), which translates into increased survival and reduced systemic inflammation in microbial sepsis, a condition when uncontrolled, has detrimental cardiovascular implications. In response to a sterile ischemic injury in tissues such as the heart, PMN rapidly infiltrate as they would during an infection. During this process, however, excessive generation of proinflammatory mediators, as well as reactive oxygen species, causes further injury to the surviving myocytes. Therefore, promoting a timely removal of PMN is paramount in protecting the healthy myocardium during ischemia. Recently, it was demonstrated that treatment with RvD1 after MI in mice reduced accumulation of PMN in the ischemic myocardium, reduced fibrosis, and improved fractional shortening when compared with untreated mice. These actions were associated with increased SPM biosynthesis in the spleen and reduced splenic PMN numbers at day 5 post MI. Similarly, it was previously shown that RvE1 decreases infarct size in a rat model of MI/reperfusion injury. Proresolving mediators have also been described to be protective in ischemia/reperfusion injury outside the heart. In the kidney, in response to reperfusion after an ischemic insult, there is increased production of D-series resolvins and protectins. Treatment with resolvins before ischemia decreases PMN infiltration, tissue levels of myeloperoxidase, and tissue fibrosis, while administration shortly after reperfusion was also organ protective. In a model of hindlimb ischemia/reperfusion, resolvins protect against second-organ reperfusion injury by blocking PMN recruitment in the lung. The endogenous protective role of SPMs was demonstrated in mice lacking the ALX/Fpr2 receptor, in which ischemia/reperfusion resulted in excessive leukocyte adhesion and emigration in the mesenteric microcirculation. As noted above, there is also emerging evidence that PMN participate in distinct phases of tissue repair as well. For instance, a recent study demonstrated that PMN depletion impairs the development of a tissue reparative macrophage phenotype, resulting in increased apoptotic cell accumulation in the heart after MI. Along these lines, recent studies by Gao et al identified a distinct tissue-resident and lymphoid homing PMN subset that produces SPMs to blunt activation of Th1 and Th17 T cells in a murine model of immune-driven dry eye disease. Clearly, the multifaceted roles of PMN in inflammation, resolution, and tissue repair deserve further study.

Although mature human atherosclerotic plaques largely contain macrophages and T cells, recent studies have begun to elucidate the roles of PMN in the initiation and progression of atherosclerotic plaques, as well as in plaque rupture. In humans, peripheral blood PMN counts correlate with coronary syndromes. Initiation of atherosclerosis is marked by subendothelial retention of low-density lipoprotein and endothelial activation at sites of disturbed flow. This dysfunction is accompanied by the upregulation of adhesion molecules and production of chemokines by ECs and leukocytes (eg, interleukin 8, C-C chemokine ligand 3), which subsequently recruit PMN (in addition to other leukocytes; vide infra) to the vessel wall. In mice, atherosclerotic lesion size is positively correlated with circulating PMN levels and conversely smaller lesions with PMN depletion. Importantly, recruitment of monocytes is diminished in the absence of PMN, which is, in part, because of PMN-derived granule protein, cathelicidin. Diabetes mellitus exacerbates atherosclerosis, in part, by increasing PMN production of S100A8/S100A9, which leads to enhanced myelopoiesis and monocyte accumulation in lesions. Moreover, a recent study found that cholesterol crystals active PMN to release neutrophil extracellular traps, which amplify immune cell activation in atherosclerosis. As noted above, PMN are a rich source 5-LOX–derived LTβ which promotes further leukocyte recruitment. Mice lacking the LTβ receptor, BLT1, have a significant reduction in plaque formation and macrophage recruitment as did mice that received bone marrow transplants with genetically reduced levels of 5-LOX. Importantly, SPMs including RvD1 block LTβ-stimulated expression of adhesion molecules and actin polymerization in PMN, and the E-series resolvins block the activation of BLT1 by LTB. PMN have also been linked with later stages of atherosclerosis, including plaque destabilization. Plaques that are highly inflamed will continue to attract PMN to infiltrate, and the proinflammatory mediators and oxidants they produce may contribute to endothelial erosion and rupture of the fibrous cap. Although most studies have primarily focused on other cell types (ie, macrophages, vascular smooth muscle cells [VSMCs]) to combat atherosclerosis, targeting PMN may offer valuable insights into halting the initiation or the eventual rupture of a plaque. All of these aforementioned features are indicative of defective resolution. In this context, it has previously been shown that RvE1 reduces PMN activation and tissue destruction in periodontitis, which is a risk factor for atherosclerosis. In rabbits, periodontitis increases atherosclerosis, and administration of RvE1 simultaneously decreases both periodontitis and atherosclerosis. Additional studies revealed that RvE1 reduces atherosclerosis even in the absence of periodontitis, which was associated with a reduction in systemic levels of C-reactive protein. Interestingly, platelet–PMN aggregates formed during inflammation lead to SPM biosynthesis, which have been shown to reduce human platelet–PMN aggregates. Given that platelet–PMN aggregates contribute to plaque inflammation, these results highlight that SPMs may have additional protective roles in regulating PMN activation during inflammation associated with atherosclerosis.

Macrophages

Both tissue macrophages and monocytes that differentiate into macrophages after being recruited to the site of injury biosynthesize and respond to SPMs during resolution. Several SPMs, including E-series and D-series resolvins, potently enhance macrophage phagocytosis of bacteria and effectorcytosis, essential functions fundamental to host-defense and resolution (Figure 3). Stimulation of phagocytosis by SPMs is at least partially dependent on downstream activation of Akt, phosphatidylinositol 3-kinase, and ERK (extracellular signal-regulated kinase) and involves cytoskeletal remodeling associated
with the activation of Rho-GTPases (ie, Cdc42, RhoA, Rac) in a receptor-dependent manner.65,122–125 Interestingly, during efferocytosis, SPM (eg, RvD1, RvD2, RvE2) production increases, which potentiates further clearance of debris and apoptotic cells in an autocrine and paracrine manner.66,99 However, in several chronic inflammatory diseases (eg, asthma, obesity/diabetes mellitus), SPM production is disrupted, and these conditions are also associated with defective resolution and efferocytosis.81 Indeed, in diabetic wounds, apoptotic and these conditions are also associated with defective resolution and efferocytosis.81 Indeed, in diabetic wounds, apoptotic and inflammatory mediators, and eventually undergo postapoptotic secondary necrosis.22 Atherosclerosis could, therefore, be viewed as a state of failed resolution of inflammation, and defective clearance of plaque macrophages may underlie the progression of advanced atherosclerotic lesions, characterized by macrophage necrosis (Figure 3).22 In a mouse model of diabetic atherosclerosis, isolated peritoneal macrophages were shown to have impaired phagocytosis and in atherosclerotic lesions there was defective macrophage efferocytosis that was reversed by a fish oil diet rich in SPM precursors, EPA and DHA.21 Interestingly, it was also recently shown that RvD1, acting through ALX/FPR2, protects macrophages from oxidative stress–induced apoptosis during efferocytosis, in part, by regulating nicotinamide adenine dinucleotide phosphate oxidase activation and expression of apoptotic proteins, Bel-XL and Bcl-2.131 In the context of atherosclerotic lesions, the ability of macrophages to survive and continue to phagocytose debris despite the highly oxidative environment is critically important. Furthermore, atherosclerotic apoE-null mice lacking SPM biosynthetic enzyme, 12/15-LOX, displays exacerbated lesion formation when compared with apoE-null mice, and macrophage-specific overexpression of 12/15-LOX protects against lesion development.132 Macrophages isolated from these mice produce higher amounts of SPMs.132 Similarly, in rabbit models of atherosclerosis, it was shown that macrophage-specific overexpression of 15-LOX is atheroprotective, resulting in decreased lesion area.133 It should be noted that these enzymes produce both proinflammatory and resolutor mediators in a temporal manner, and both atheroprotective and atherogenic roles of this enzyme have been reported.134 However, as noted above, RvE1 used as an oral/topical agent decreases aortic atherogenesis, the intima/media ratio, and inflammatory cell infiltration into plaques.118 In addition, recent studies documented that the ALX/FPR2 receptor has an endogenous antiatherosclerotic role, and that selective targeting of this receptor improves lesion stability and reduces lesion necrosis in mice with pre-existing atherosclerosis.135,136 These results collectively demonstrate that chronic macrophage-mediated inflammation in atherosclerosis may be, in part, because of a failure of endogenous resolution programs.

In addition to macrophage efferocytosis, the phenotype of both monocytes and macrophages is an important determinant of resolution (Figure 3). Recruited by specific chemokines (eg, C-C chemokine ligand 2) in the injured myocardium or atherosclerotic lesions, inflammatory Ly6Cint monocytes infiltrate the tissue shortly after the initial PMN wave, as occurs during any normal acute inflammatory response.101 After this first phase of recruitment, monocytes can differentiate into Ly6Clow monocytes/macrophages to facilitate tissue repair in a process dependent, in part, on the transcription factor, Nr4a1.135 These monocytes/macrophages facilitate repair because they are a rich source of growth factors and cytokines that promote tissue vascularization and matrix remodeling, whereas inflammatory monocytes/macrophages secrete inflammatory cytokines (eg, IL-1β, IL-6, tumor necrosis

This precipitates the generation of lipid-laden foam cells that fail to clear from the plaque, continue to secrete proinflammatory mediators, and eventually undergo postapoptotic secondary necrosis.22 Defective efferocytosis has also been implicated as a crucial underlying contributor to the pathophysiology of atherosclerosis.22 Macrophages play essential roles in all aspects of the life of an atherosclerotic plaque (Figure 3). They are important both in the pathology of the lesion from initiation of formation to expansion, necrosis, and eventual rupture and in the resolution and regression of lesions.3,130 Monocytes infiltrate the subendothelium of the arterial wall in response to inflammatory cytokines. Once in the plaque environment, monocytes differentiate into macrophages and attempt to clear excess oxidized lipoproteins and cholesterol from the tissue.23 At this early stage of lesion development, efferocytosis seems to function normally, and the majority of plaques do not progress to a vulnerable state. In the plaques that do progress, however, both key events of resolution (cessation of additional immune cell entry and efferocytosis) are impaired.22
factor-α) and are highly microbicidal. Too many Ly6C<sup>high</sup> cells or a delayed switch into reparative macrophages can lead to excessive proteolysis of the healthy myocardium surrounding the infarct, whereas increased production of IL-1β enhances proliferation of hematopoietic stem and progenitor cells to promote leukocytosis. In addition, a population of embryonic-derived resident cardiac macrophages may contribute to homeostasis, as well as tissue repair in the heart during injury, and can be replaced by circulating monocytes and through local proliferation. Similar to the heart, the phenotype of lesional macrophages in atherosclerosis has become an important indicator of the overall plaque environment. Macrophages have traditionally been lumped into general classically activated (M1) or alternatively activated (M2) designations based largely on in vitro model systems in which macrophages are polarized with lipopolysaccharide/interferon-γ or IL-4, respectively, although this nomenclature has been revised because macrophages largely exist in a phenotype spectrum dependent on the local milieu. This is true for disease states like atherosclerosis, where distinct M1 versus M2 populations are difficult to discern. With this inherent plasticity, agents that induce proresolving characteristics in macrophages may be beneficial in breaking the feed-forward proinflammatory cycle occurring in the lesion. Notably, SPM biosynthetic enzyme, ALOX15, is highly induced in macrophages stimulated with IL-4, and these IL-4–stimulated macrophages make more SPMs overall than lipopolysaccharide/interferon-γ–polarized macrophages. SPMs have also been implicated in promoting a switch from an inflammatory macrophage phenotype to a tissue reparative phenotype. For instance, it was recently shown that RvD1 blunts macrophage chemotaxis toward C-C chemokine ligand 2, while enhancing phagocytic function. In human macrophages, RvD1 potently blocks production of proinflammatory cytokines (eg, IL-1β, IL-8, and C-C chemokine ligand 2) in a GPR32-dependent manner, which could help to prevent further recruitment of inflammatory monocytes. In inflamed obese adipose tissue, RvD1 decreases the proportion of inflammatory macrophages and enhances macrophage phagocytosis and expression of arginase 1. In isolated macrophages, RvD1 increases expression of Arg I, Ym1, IL-10, and CD206, typical of an anti-inflammatory macrophage phenotype. RvD2 similarly increases surface expression of CD206, as well as CD163, in human macrophages in a GPR18-dependent manner. In murine peritonitis, both RvE1 and RvD1 have been shown to decrease CD11b expression on macrophages. CD11b<sup>hi</sup> macrophages have been reported to express lower levels of inducible nitric oxide synthase (iNOS), COX-2, and MMP-9 (matrix metalloproteinase-9) and engulf more apoptotic cells than CD11b<sup>lo</sup> macrophages. There have also been reports of a previously undescribed resolution-phase macrophage subset with similar features of the CD11b<sup>lo</sup> population and possessing a hybrid of the M1/M2 phenotypes (M2: mannose receptor expression, IL-10, TGF-β, and arginase 1; M1: high iNOS and COX-2 expression). These resolution-phase macrophages also express Alox15, indicating that they may contribute to SPM production during resolution. As critical regulators of the resolution program, targeting the actions of macrophages may be an effective strategy to address chronic maladaptive inflammation in CVD.

**Endothelial Cells**

As the interface between the blood and surrounding tissues, the vascular endothelium plays an important early role in the inflammatory response by regulating the passage of macromolecules and immune cells across the vessel wall. As such, the temporal regulation of endothelial permeability and activation is a critical determinant of resolution of the inflammatory response. Indeed, CVDs such as atherosclerosis are associated with EC dysfunction and chronic vascular inflammation. Analogous to the dynamic regulation of leukocytes during resolution, EC activation is a self-limited process that is uniquely positioned to control the magnitude and duration of the acute inflammatory response. Indeed, continued EC activation, characterized by the expression of specific leukocyte adhesion receptors such as vascular cell adhesion molecule 1, is associated with chronic inflammatory diseases such as atherosclerosis and diabetes. Accordingly, ECs participate in the local biosynthesis of SPMs during acute inflammation and are also direct cellular targets of SPMs (Figure 3). As noted above, the biosynthesis of most SPMs occurs during cell–cell interactions in which the biosynthetic intermediates, such as 18-HEPE, can be generated in EC and transformed by adhering leukocytes expressing 5-LOX to generate SPMs such as RvE1. Furthermore, it was in the coculture of human PMN and EC that the novel 13-series resolvins were recently identified. A recent study documented that RvD1 and RvE1 are also biosynthesized during coculture of choroid-retinal EC (CREC) with leukocytes (both PMN and monocytes), but not by CREC alone. This mechanism likely serves as a local circuit to ensure timely termination of leukocyte adhesion to EC. Indeed, ECs express the known receptors for SPMs, including ALX/FPR2, GPR32, and GPR18. Acting through these receptors, SPMs regulate both recruitment and adhesion of leukocytes. Arachidonic acid–derived LXA<sub>4</sub> not only downregulates vascular cell adhesion molecule 1 and additional adhesion receptors, E-selectin, and intercellular adhesion molecule-1, but also increases nitric oxide and prostacyclin that potent counter-regulate leukocyte–EC interactions and platelet activation. Lipoxins have further EC-specific effects including the downregulation of nicotinamide adenine dinucleotide phosphate oxidase, which results in the decreased generation of reactive oxygen species. Similarly, resolvins have also been shown to regulate leukocyte–EC interactions. For instance, RvD2 limits leukocyte–EC adhesion in a nitric oxide–dependent manner, and the regulation of PMN recruitment during microbial peritonitis by RvD2 is attenuated in mice deficient in endothelial nitric oxide synthase. Both RvD1 and RvD2 limit PMN interaction with human EC prestimulated with inflammatory cytokines (eg, TNFα, IL-1β) under shear conditions at a physiological flow rate in a receptor-specific manner. In this context, resolvins block PMN capture, rolling, and firm adhesion to EC, and they attenuate EC production of chemokines (eg, IL-8 and C-C chemokine ligand 2) that recruit additional leukocytes. A recent study of the mechanisms whereby resolvins regulate inflammatory signaling in human EC uncovered that D-series
resolvins stimulate the activation of phosphatidylinositol 3-kinase and Akt in a receptor-dependent manner and thereby regulate the production of developmental endothelial locus 1, an anti-inflammatory protein secreted by EC that prevents PMN transmigration.166

In addition to regulating EC-dependent adhesion of leukocytes during inflammation, resolvins also regulate permeability of the microvasculature. In vitro, RvD1 protects against endotoxin-induced impairment of barrier function in human EC,157 and aspirin-triggered-RvD1 enhances the restitution of endothelial barrier function after acute lung injury in vivo.158 These results build on previous studies showing that lipoxins counter-regulate leukocyte-mediated microvascular permeability in vivo during acute inflammation stimulated by proinflammatory lipid mediators.159 Permeability of the microvasculature is increased by several inflammatory mediators that also regulate angiogenesis. Along these lines, LXA₄ decreases vascular endothelial growth factor receptor 2 phosphorylation and its downstream signaling in human EC, thereby blocking VEGF-induced angiogenesis.152 Similarly, in a model of murine retinal neovascularization, administration of exogenous SPM (LXA₄, RvD1, RvE1, and PD1) protects against pathological angiogenesis,89,160,161 This is in contrast to some proinflammatory lipid mediators (ie, 12-HETE and prostaglandin E₃) that promote angiogenesis.162,163 Collectively, ECs have emerged as important cellular targets of SPMs during resolution, suggesting that engagement of SPM receptors might offer a new approach to reversing EC dysfunction that is associated with CVD. Additional studies will be required to understand fully the role of SPMs in EC biology, and whether dysfunctional EC in chronic diseases is associated with deficits in resolution circuits at the level of EC-leukocyte interactions.

Vascular Smooth Muscle Cells
In addition to their physiological role in regulating blood vessel tone, VSMCs also contribute to the pathophysiology of atherosclerosis and restenosis. In response to vascular injury, VSMCs undergo a phenotype switch from a contractile to a synthetic phenotype; synthetic VSMCs are more proliferative and chemotactic and are characterized by a decreased expression of contractile proteins and increased production of proinflammatory mediators.164 During chronic vascular inflammation (eg, atherosclerosis), this proinflammatory environment can lead to successive contractile to synthetic phenotype switching thereby propagating the proinflammatory environment. Moreover, VSMC can assume a phenotype similar to macrophages and express macrophage markers, such as CD68 and F4/80 in lesions.164 Interruption of this proinflammatory signaling cycle may, therefore, be a useful strategy to combat inflammatory diseases of the vessel wall. Receptors of SPMs, including ALX/Fpr2 and ChemR23, are expressed in human saphenous vein SMC, and administration of RvE1 and 15-epi-LXA₄ counter-regulate platelet-derived growth factor–stimulated VSMC migration in a dose-dependent manner (Figure 3).165 These actions are, in part, because of direct regulation of platelet-derived growth factor receptor phosphorylation. Building on these in vitro studies, it was recently found that 15-epi-LXA₄ inhibits VSMC migration and intimal hyperplasia after murine carotid artery ligation, and this protection afforded by 15-epi-LXA₄ is abolished in mice lacking the ALX/Fpr2 receptor.166 Interestingly, genetic deficiency of ALX/Fpr2 decreases the stability of atherosclerotic lesions; effects that were attributed to decreased collagen production by VSMC.165 Given that VSMC can also contribute to stability of atherosclerotic lesions164 and that it is their phenotype that dictates their contribution to atherosclerosis, these results suggest that SPMs may regulate the balance of VSMC phenotypes important for regulating lesion stability. More recently, it was demonstrated that RvD1 and RvD2 inhibit proliferation, migration, monocyte adhesion, superoxide production, and proinflammatory gene expression in human,168 rat,169 and mouse170 VSMC. Similar results were observed with another SPM, maresin 1 (Mar1).171 In a rabbit model of balloon angioplasty, local delivery of RvD2 reduces cell proliferation, leukocyte recruitment, and neointimal hyperplasia.169 Interestingly, RvD2 and Mar1 increase M2 macrophage polarization during vascular injury, which may help to promote vessel remodeling and repair.172 In addition, RvD1, delivered perivascularly via a novel biodegradable wrap reduces carotid artery neointimal formation without promoting thrombosis after carotid angioplasty in rats.169 In a model of human pulmonary artery hypertension, RvD1 and RvE1 are able to normalize arterial hyper-reactivity induced by proinflammatory mediators.172,173 These results add further support to the concept that most CVDs are driven by abhorrent inflammation and that stimulating resolution of inflammation could potentially restore physiological function of the vasculature.

Platelets
In response to blood vessel damage, platelets are rapidly activated and are a critical component of the coagulation process that serves to re-establish a physical barrier and prevent blood loss. This process is tightly coupled with the inflammatory program and like the regulation of leukocyte trafficking, platelet activation must be tightly controlled to prevent further damage to the host. Platelet aggregation and ensuing thrombosis are significant vascular events that are linked to inflammation and atherogenesis and causative to the clinical manifestations of atherosclerosis, namely stroke and MI.174 In a randomized clinical trial involving healthy subjects receiving aspirin, prothrombotic thromboxane was reduced, and there was a concomitant increase in 15-epi-LXA₄.175,176 Plasma levels of 15-epi-LXA₄ were increased most significantly with low-dose aspirin (ie, 81 mg), the current recommended dose for patients with CVD. Interestingly, human platelets express ChemR23, and RvE1 blocks ADP and thromboxane-stimulated platelet aggregation (Figure 3).176,177 Further mechanistic studies revealed that RvE1 blocks ADP-mediated signals downstream of its receptor, P2Y₁₂, and that the effects of RvE1 are ChemR23-dependent.177 In a mouse burn wound model, exogenous systemic administration of RvD2 prevents thrombosis of the deep dermal vascular network and subsequent dermal necrosis. Treatment with RvD2 allowed enhanced neutrophil access to the dermis, while preventing neutrophil-mediated damage caused by an abrogation of proinflammatory mediator secretion.178 Finally, it was recently shown that
SPMs increase macrophage uptake of blood clots in vitro, indicating that SPMs may play a role in clot remodeling during resolution.\(^6\) As noted above, SPMs are biosynthesized during interactions of platelets and leukocytes during inflammation. Thus, these results collectively demonstrate that SPMs may play key roles in regulating the resolution of thrombosis during inflammation, which could have important therapeutic implications for CVD.

**Summary and Future Directions**

A significant effort has been dedicated to the structural elucidation of proresolving mediators and the identification of their receptors.\(^8\) It is becoming apparent that alterations in specific resolution pathways could be beneficial for clinical management of CVD beyond the current standard of care (eg, aspirin, statins, antiplatelet therapies).\(^4\) In contrast to traditional anti-inflammatory strategies that blunt the production of inflammation initiators and could potentially lead to immunosuppression, proresolving mediators described in this review activate distinct cellular programs to resolve inflammation without compromising host-defense.\(^9\) In fact, proresolving mediators enhance host defense to both viral and bacterial infections and lower the threshold for antibiotic therapy.\(^17\,19\)

Thus, new strategies to promote resolution of inflammation may offer unique opportunities to combat chronic inflammation associated with CVD. Proresolving mediators blunt excessive inflammation that can be detrimental to healthy tissues, particularly in sterile inflammation, and they also stimulate distinct processes necessary for tissue repair and regeneration. They have multiple cellular targets in the inflammatory response, including immune cells, platelets, and vascular cells. Collectively, the structural elucidation of these mediators and identification of the signaling pathways that they engage could inform the development of therapeutic strategies encompassing a novel resolution pharmacology approach. Indeed, clinical trials with proresolving mediators in humans with other inflammatory pathologies are currently underway.\(^18\)

In regards to therapeutically stimulating resolution of inflammation in CVD, there are several things to consider. First, can SPMs be effectively targeted to sites of local inflammation? In a rabbit model of atherosclerosis, delivery of RvE1 to the oral cavity decreased lesion formation in the aorta, suggesting that resolvins can get absorbed and have systemic effects.\(^18\) Our group has also shown that microparticles released from activated immune cells carry SPMs, and as a form of biomimicry, we formulated humanized nanoparticles that showed enhanced bioactivity; these could potentially be vehicles for targeted delivery.\(^18\) Moreover, recent studies have begun to highlight other methodologies for local sustained SPM delivery (eg, vascular wraps) that could be used in vascular surgery, for example.\(^19\) Second, what are the mechanisms underlying failed resolution of inflammation? As noted in this review, several chronic diseases are associated with lower levels of SPMs. Does this occur because of altered biosynthesis or enhanced metabolic inactivation? How are SPM receptors modulated in chronic diseases like CVD? Finally, are nutritional-based approaches (eg, omega-3 PUFA) sufficient to promote resolution, or are there contexts in which resolution is perturbed regardless of substrate availability? These are all important questions for future work in the area and given that the field of resolution physiology is relatively new, an understanding of the mechanisms underlying failed resolution of inflammation in CVD is likely to be a fruitful area of future investigation.

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**Disclosures**

None.

**References**


Resolution of Acute Inflammation and the Role of Resolvins in Immunity, Thrombosis, and Vascular Biology

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