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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 pro-resolving mediators that act on specific cellular receptors to regulate leukocyte trafficking and blunt production of inflammatory mediators, while 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 pro-resolving 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.

Keywords

Resolution of inflammation; Lipid mediators; Omega-3 fatty acids

Subject terms

Inflammation; Vascular Biology; Physiology

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Disclosures

None

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 and obesity. Indeed, inflammation is a central feature of virtually all pathologies afflicting both the heart and the vasculature, including chronic heart failure (HF), atherosclerosis, myocardial infarction (MI), and stroke[1], as discussed in greater depth in the accompanying reviews of this series. Moreover, mounting evidence indicates that the presence of other chronic inflammatory diseases (e.g., periodontitis, rheumatoid arthritis) can exacerbate CVD[1]. Atherosclerosis is perhaps the CVD that has been most closely linked with chronic unresolved inflammation[2, 3]. While the development of statins revolutionized the treatment of atherosclerosis by lowering low-density lipoprotein (LDL) cholesterol, the burden of ischemic cardiovascular conditions has continued to rise globally and CVD remains the leading cause of death among Americans[4]. Furthermore, intolerance and adverse effects of statins in a growing number of patients has emphasized the need for novel therapies[2, 4]. Many new strategies, therefore, have looked beyond lipid lowering alone and have focused instead on halting aberrant inflammatory signaling[5]. Several clinical trials are currently underway testing the efficacy of anti-inflammatory drugs in the context of CVD. One large trial, the Cardiovascular Inflammation Reduction Trial (CIRT), using low dose methotrexate, is currently recruiting patients and is specifically designed to assess whether inhibition of inflammation will lower vascular events rates[6, 7]. Another trial, the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS), has enrolled over 10,000 patients with stable coronary artery disease (CAD) and a history of MI. Preliminary results show treatment with the IL-1β receptor antagonist decreased measures of inflammation without affecting lipids[4, 8]. These clinical trials will provide valuable insights into the role of inflammation in CVD and may herald a new direction in treatment strategies. While invasive procedures will remain indispensable for the most severe CVD cases, innovative therapies targeting the body's innate immune system may indeed be a promising alternative for many patients. Inherent in the discovery and development of such new, effective and less invasive therapies is a more thorough delineation of the endogenous mechanisms that govern the natural resolution of acute inflammation. A novel genus of "pro-resolving mediators" potently resolve inflammation, but also enhance certain aspects of host defense, potentially overcoming limitations to traditional therapeutic anti-inflammatory agents that can be immunosuppressive[9]. This review focuses on the role of these endogenous mediators in promoting the resolution of acute inflammation, highlighting their biosynthesis and cellular targets, and discusses how defective resolution may contribute to CVD.

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[10]. 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 two general phases: initiation and resolution (Fig 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 (e.g., cysteinyl leukotrienes and prostaglandins) and other vasoactive products (e.g., histamine, 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 pro-inflammatory lipid mediators (e.g., leukotriene B_4; LTB_4) and chemokines, PMN traverse the vasculature through precise interactions with endothelial adhesion receptors and subsequently engulf and degrade pathogens within phagolysosomes[11–13]. 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[14]. Monocytes subsequently infiltrate the tissue where they differentiate into macrophages that avidly respond to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) present in the injured tissue. Importantly, macrophages are highly responsive to so-called find-me and eat-me signals (e.g., nucleotides, externalized phosphatidylserine) released or presented by apoptotic cells such as PMN[10, 15]. Uptake of apoptotic cells by macrophages (i.e., efferocytosis) is an anti-inflammatory process associated with decreased production of inflammatory mediators, thus coupling the initiation of inflammation to its ultimate resolution (Fig. 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[9, 15]. 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[16–18]. Like innate immune cells, adaptive immune cells also play critical roles in the host response to infection, resolution of inflammation and in tissue repair[19, 20]. Their accumulation defines the “post-resolution” phase of the inflammatory response and assures a more rapid response to subsequent exposure to the same antigens[19]. Interruption of this process at any point (e.g., prolonged leukocyte recruitment and survival, impairments in apoptotic cell removal, 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 like atherosclerosis and HF[10, 17, 21–23].

By its nature, the acute inflammatory response is self-limiting in part because of inherent negative feedback regulation of inflammatory signaling pathways (e.g., transcriptional repressors, endogenous receptor antagonists) when the trigger has been eliminated. However, it has recently become evident that active resolution of inflammation involves the biosynthesis of pro-resolving mediators that, as a genus, are just as diverse as the initiators of inflammation[24–33]. Thus, critical to determining the fate of an inflammatory response is the balance of pro-inflammatory and pro-resolving mediators that are produced in the exudate in a temporal manner. Traditionally, it has been held that an excess production of pro-inflammatory mediators underlies chronic inflammation[34], however, mounting evidence supports the view that disruptions in endogenous pro-resolving circuits may be an equally important mechanism[10, 34, 35]. These pro-resolving mediators actively terminate the

production of pro-inflammatory 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[9, 36–41]. These agonist-based actions distinguish pro-resolving mediators from intrinsic negative feedback pathways and other antagonists that terminate inflammatory signaling pathways. Systems-based approaches have played a crucial role in the identification of the principal mediators of resolution[9]. As a result, a complex and ever-expanding network of interrelated mediators and the cellular targets and pathways that they engage has been assembled. The discovery of these novel bioactive pro-resolving mediators represents a paradigm shift in our understanding of the dynamic regulation of acute inflammation and has led to a new era of "resolution physiology"[9].

Pro-resolving 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 (PUFA) play a central role in resolution, due in part to 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 pro-inflammatory mediators induce the production of enzymes that enable the reprogramming of leukocytes to generate specialized pro-resolving lipid mediators (SPM) from the same PUFA precursors (Fig. 1)[42]. For instance, 5-lipoxygenase (5-LOX) is required for the generation of potent leukocyte chemoattractant, LTB_4 from omega-6 PUFA arachidonic acid (AA). When 12-LOX is also expressed (e.g., during platelet:leukocyte interactions), the epoxide intermediate in LTB_4 biosynthesis (i.e., LTA_4) can be converted into the family of lipoxins. Lipoxins can also be produced with cooperation between 5-LOX and 15-LOX, the latter of which can be induced in PMN by prostaglandin E_2 (PGE_2), or in macrophages by Th2 cytokines (e.g., IL-4). Lipoxin A_4 (LXA_4), was the first lipid mediator shown to possess both anti-inflammatory and pro-resolving actions[43] and, along with subsequently discovered members of the lipoxin family (i.e., LXB_4), has been the subject of extensive and rigorous study. In addition to omega-6 PUFA serving as precursors to lipoxins, omega-3 PUFAs emerged as an additional source of novel SPM. Indeed, after the initial discovery of LXA_4, the enzymatic conversion of the omega-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to novel families of SPM – resolvins, protectins and maresins – was identified[44–47]. This mechanism could have evolved to ensure adequate production of SPM in organisms with different PUFA in their diets. Importantly, SPM are evolutionarily conserved from flatworms (e.g., *Planaria*) to humans and several studies have documented endogenous formation of these mediators in healthy individuals, with lower levels observed in patients with Alzheimer's disease, peripheral artery disease and asthma (for review, see [25]). In addition to lipid mediators of resolution, other important mediators of

the resolution program have emerged and include proteins and bioactive peptides (e.g., annexin A1, galectin 1, melanocortins) and gases (nitric oxide, hydrogen sulfide, carbon monoxide). Readers are referred to recent reviews covering each of these mediators in detail[24–33]. 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 (LC-MS/MS)-based approaches were critical in the identification and structural elucidation of resolvins and related SPM[9]. 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[44]. Later named resolvin E1 (RvE1), its biosynthetic pathway proceeds via conversion of EPA to 18*R*-hydroperoxyeicosapentaenoic acid (18*R*-H(p)EPE) by acetylated cyclooxygenase-2 (COX-2) or cytochrome P_{450} enzymes, which is subsequently converted to RvE1 by 5-LOX (Fig. 2)[44, 48]. The epoxide intermediate formed during RvE1 biosynthesis is hydrolyzed by leukotriene A_4 hydrolase (LTA_4H), the same enzyme that produces pro-inflammatory LTB_4[49]. This mechanism provides an additional point of control in the balance between pro-inflammatory mediators and SPM. The formation of RvE1 precursor, 18-H(p)EPE, was identified in hypoxic endothelial cells and can be transformed to RvE1 through leukocyte:endothelial interactions. The complete structural elucidation of RvE1 was carried out via total organic synthesis and matching with endogenous material, which enabled 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 efferocytosis[44, 48]. Additional members of the EPA-derived (E-series) resolvins have since been characterized. Similar to RvE1, RvE2 is generated from EPA via the intermediate 18*R*-HEPE and its subsequent 5-LOX-dependent conversion to a 5*S*-hydroperoxide[50, 51]. However, instead of undergoing an epoxidation step, the 5*S*-hydroperoxide is directly converted to RvE2 (Fig. 2). More recently, a third member, RvE3, was also identified and its structure determined[52]. Unlike RvE1 and RvE2, the formation of this product does not require 5-LOX.

In addition to EPA, the other prominent omega-3 PUFA in humans and marine oils is DHA. This fatty acid serves as the precursor to the D-series resolvins, as well as the protectins and maresins[44–48]. In the presence of 15-LOX, DHA is converted to a 17-hydroperoxide intermediate (17-H(p)DHA) which can subsequently be converted to D-series resolvins via 5-LOX. Specifically, the hydroperoxide formed via 5-LOX can undergo epoxidation to generate a 7*S*,8*S* epoxide that is then enzymatically hydrolyzed to produce resolvin D1 (RvD1) or resolvin D2 (RvD2; see Fig. 2)[40, 53]. As with the E-series resolvins, these steps can take place within a single cell (e.g., PMN or macrophages), or can occur during cell:cell interactions (e.g., leukocyte:endothelial, PMN:macrophage). Additional structurally unique D-series resolvins, including RvD3-6, have also been identified and the complete structures and stereochemical assignments of RvD3 and RvD4 have recently been reported[54, 55]. In murine models of acute inflammation, distinct temporal patterns of resolvin biosynthesis have been observed, highlighting additional control points in their biosynthesis (e.g., potentially novel regulation by epoxide hydrolases)[54]. Interestingly, within a single cell type,

lipid mediator class switching can occur and pro-resolving lipid mediators can regulate each other. For instance, RvD1 reduces nuclear localization of 5-LOX in macrophages and diverts AA metabolism from pro-inflammatory lipid mediator, LTB_4, to SPM, LXA_4. Mechanistically, RvD1 inhibits the activation of calcium-calmodulin-dependent kinase II (CaMKII) and downstream activation of p38 and a mitogen-activated kinase (MK2), which results in reduced phosphorylation of 5-LOX[56]. 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 pro-inflammatory eicosanoids and increases formation of other pro-resolving mediators[9]. 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 (PGDH), 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 resolvin analogs have been formulated and shown to have enhanced biological activity *in vivo*[25].

Recently, a new family of mediators was identified wherein the epoxide intermediate in RvD1 and RvD2 biosynthesis can be enzymatically conjugated to glutathione to generate sulfido-conjugated resolvins[38]. 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 (e.g., PMN and macrophages), as well as apoptotic PMN. Like other SPM, these resolvin-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 “resolvin-conjugates in tissue regeneration; RCTRs”. It is noteworthy that similar biosynthetic routes were also identified for maresin and protectin families of SPM[38, 57].

Pro-resolving receptors

Like other classic lipid mediators and chemokines, SPM elicit their biological actions through activation of specific G-protein coupled receptors (GPCRs) in a stereoselective manner (Fig. 2). The pro-resolving and anti-inflammatory actions of LXA_4 and RvD1 are mediated via specific signaling through ALX (also termed formyl peptide receptor 2; FPR2) and previous orphan, GPR32 (also termed D-resolvin receptor 1; DRV1)[25, 58]. Given that LXA_4 and RvD1 are structurally similar but generated from different PUFA precursors, that they activate the same receptors and promote reciprocal biosynthesis of each other provides additional evidence that pro-resolving pathways are intimately linked. Specific binding to these receptors was unequivocally demonstrated using radiolabeled material and both homo- and heteroligand competition. Moreover, their pro-resolving actions were validated in mouse models of acute inflammation with targeted overexpression or genetic deletion of the *ALX/Fpr2*[59–61]. 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[59]. 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[58]. Recently, it has been shown that RvD3 and RvD5 also activate human GPR32[37, 54]. Given that these mediators are generated at distinct times during resolution of inflammation[54], this mechanism may serve to preserve GPR32 ligands during different stages of resolution. Interestingly, ALX/FPR2 is also activated by other pro-resolving mediators, such as annexin A1, as well as pro-inflammatory mediators (e.g., serum amyloid A; SAA). Depending on the stimulus, signaling through this receptor can elicit either pro-inflammatory or pro-resolving effects. Emerging evidence indicates that dimerization underlies in part these distinct and unconventional roles[62]. Specifically, annexin A1 and LXA_4 induce ALX homodimer formation and this leads to increased production of IL-10 in monocytes. In contrast, pro-inflammatory agonists, such as SAA, can block this homodimerization. In PMN, annexin A1, an annexin A1 peptide mimetic (Ac_{2-26}), and LXA_4 can induce formation of ALX heterodimers with FPR1. These heterodimers elicit distinct signaling pathways (JNK/caspase-3) that override pro-survival signals in PMN, such as SAA, through the same receptor[62, 63]. 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 GPCR denoted chemoattractant receptor 23 (ChemR23; also termed E-resolvin receptor, ERV), which shares 36% sequence identity with ALX/FPR2, is activated by RvE1 in a stereoselective manner[48]. 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[64]. Subsequent investigations further substantiated ChemR23-mediated signaling by RvE1, including stimulation of macrophage phagocytosis via the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and activation of the translational regulator, ribosomal protein S6[65]. 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[48]. Additional studies determined that RvE1 binding to human PMN is displaced by LTB_4 and an antagonist to the LTB_4 receptor (BLT1), but not by chemerin peptide, indicating that BLT1 is a target for RvE1 on PMN[66]. This study also demonstrated that the actions of RvE1 on PMN may be due, in part, to an attenuation of pro-inflammatory LTB_4 signaling, which was subsequently confirmed in *Blt1*-deficient mice[66]. Like RvE1, RvE2 also binds BLT1 and blocks LTB_4-stimulated actin polymerization in PMN[50, 51]. Importantly, although many of the downstream actions of RvD1 are similar to those of RvE1, it does not bind ChemR23 or BLT1[58].

Using an unbiased β-arrestin based screening approach, a recent study identified that RvD2 activates a distinct GPCR denoted GPR18 (also termed D-resolvin receptor 2; DRV2), which among innate immune cells, is expressed on human PMN, monocytes and macrophages[67]. 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 *E. coli* and *S. aureus in vivo* are abolished in *Gpr18*-deficient mice[67]. 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 pro-resolving receptors has, and will continue to, illuminate the mechanisms governing resolution, including the specific signaling pathways and transcriptional programs engaged by pro-resolving mediators.

Increasing pro-resolving lipid mediators with nutritional and pharmacologic approaches

Given the potent bioactions of resolvins and related SPM 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 SPM. In rodent models of inflammation, administration of EPA or DHA increases resolvin production[25]. 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[68, 69]. These protective actions were recapitulated with exogenous delivery of resolvins, establishing causality in this relationship[68, 69]. 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[70, 71]. One landmark clinical trial, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) Prevenzione study, enrolled more than 11,000 recent MI patients and reported that 1g of daily omega-3 supplementation for three and half years significantly reduced the risk of cardiovascular death[72]. More recently, GISSI also found greater survival in HF patients treated with omega-3s[73]. Though 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 PUFA increases production of resolvins and/or their biosynthetic pathway markers (e.g., 18-HEPE and 17-HDHA) in plasma and/or serum[74–79]. In a very recent study, treatment with Lovaza (omega-3 ethyl esters) in patients with stable CAD increased levels of RvD6, 17*R*-PD1 and RvE2[80]. Whether production of SPM 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 SPM production[25, 81]. Given that, unlike hormones, SPM 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 endpoints with omega-3 PUFA supplementation[71], it is intriguing to speculate that altered downstream conversion to bioactive SPM could be related to variable results in such trials. Such variation could also arise from the diversity in dosing and preparations (e.g., ethyl esters vs. triglycerides). This is particularly relevant when one considers that the appearance of SPM is dependent upon 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 SPM and toward the formation of free radical-initiated auto-oxidation products[82]. While the evidence to date indicates that SPM and their intermediate precursors are indeed produced in humans *in vivo* and are demonstrable by LC-MS/MS[74–76, 79, 83, 84], it is noteworthy that with inadequate approaches and methodology, some studies were unable to identify endogenous SPM in plasma[85], 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 SPM 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 PUFA supplement[86]. 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[87]. More recently, however, additional anti-inflammatory actions of aspirin have been described. Aspirin-acetylation of COX-2 blocks the production of prostaglandin precursors, but also switches the activity of COX-2 such that it generates 15*R*-HETE with AA as a substrate, or 18-HEPE and 17*R*-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[44, 45, 49, 53, 88]. 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 pro-inflammatory eicosanoids to SPM. 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[89]. This was associated with increased production of lipoxins in the liver and exogenous delivery of a stable analog of LXB_4 recapitulated these protective effects. These results indicate that in addition to the inflammation-resolving roles of SPM, 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[4]. Results of the JUPITER trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) demonstrated that administration of Rosuvastatin decreases systemic markers of inflammation and that the observed reduction in major cardiovascular events is independent of the reduction in LDL cholesterol[90]. Though 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 acetylating the enzyme, statins increase *S*-nitrosylation and bias the enzyme toward the formation of 15-epimeric lipoxins as well[91, 92]. 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)[93]. Human and mouse leukocytes were shown to convert DPA to these novel bioactive products analogous to other SPM including the D-series resolvins[93]. These bioactive compounds are produced in PMN-endothelial co-cultures and are present in human and mouse tissues during sterile inflammation or infection[93]. The structures of four 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 (RvTs)[93]. Though their biosynthetic pathway remains to be fully detailed, it has been shown that endothelial COX-2 initiates their production during PMN-endothelial cell interactions and *S*-nitrosylation of the enzyme by Atorvastatin further increases their production. Conversely, selective COX-2 inhibitors diminish their formation[93]. 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”[10]. 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*)[94]. Indeed, selective COX-2 inhibitors that are detrimental for CVD patients because they perturb production of protective prostanoids (i.e., 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[95]. Their capacity as the initiators of resolution, however, is just beginning to be appreciated[29, 96]. In response to PGE_2, PMN transition from releasing pro-inflammatory LTB_4 to pro-resolving LXA_4 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. LXA_4 is critical in halting further PMN recruitment and was shown in isolated PMN to inhibit chemotaxis, as well as transendothelial and transepithelial migration (Fig. 3)[97]. As noted above, SPM (e.g., LXA_4, RvE1) can also override survival signals in PMN, ensuring their timely apoptosis[41, 63]. Additionally, both lipoxins and resolvins act on macrophages to promote the efferocytosis of apoptotic PMN[98], 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 pro-inflammatory signaling (Fig. 3)[96, 99]. Resolvins also act directly on PMN to decrease chemotaxis toward chemokine gradients (e.g., IL-8) and to reduce the surface expression of adhesion receptors (e.g., CD11b/CD18) while preventing L-selectin (CD62L) shedding[40, 58]. These selective actions of SPM on PMN activation, rolling, and adhesion to the activated endothelium have been demonstrated both *in vitro* and *in vivo* using human endothelial cells under flow or intravital microscopy-based imaging of the inflamed microvasculature[40, 59, 100]. Accordingly, all SPM 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, SPM have also been shown to increase the expression of chemokine receptors (i.e., CCR5) on apoptotic PMN, thereby facilitating chemokine scavenging during resolution as these apoptotic cell/chemokine clusters are subsequently cleared by macrophages[36]. Interestingly, in concert with blunting PMN recruitment, SPM enhance PMN-mediated bacterial containment (Fig. 3), which translates into increased survival and reduced systemic inflammation in microbial sepsis, a condition that when uncontrolled, has detrimental cardiovascular implications[37, 40].

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 pro-inflammatory mediators, as well as reactive oxygen species (ROS), are released and cause further injury to the surviving myocytes[101]. 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, compared with untreated mice[102]. 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[103]. Pro-resolving 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 prior to ischemia decreases PMN infiltration, tissue levels of myeloperoxidase (MPO), and tissue fibrosis, while administration shortly after reperfusion was also organ protective[104]. In a model of hind limb ischemia/reperfusion, resolvins protect against second-organ reperfusion injury by blocking PMN recruitment in the lung[67, 105]. The endogenous protective role of SPM was demonstrated in mice lacking the *ALX/Fpr2* receptor, in which ischemia/reperfusion resulted in excessive leukocyte adhesion and emigration in the mesenteric microcirculation[106]. As noted above, there is also emerging evidence that PMN participate in distinct phases of tissue repair as well[29]. For instance, a very 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[107]. Along these lines, recent studies by Gronert et al. identified a distinct tissue resident and lymphoid homing PMN subset that produces SPM to blunt activation of Th1 and Th17 T cells in a murine model of immune-driven dry eye disease[108]. Clearly, the multifaceted roles of PMN in inflammation, resolution and tissue repair deserve further study.

While 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[109]. In humans, peripheral blood PMN counts correlate with coronary syndromes[110]. Initiation of atherosclerosis is marked by sub-endothelial retention of LDL and endothelial activation at sites of disturbed flow[11]. This dysfunction is accompanied by the upregulation of adhesion molecules and production of chemokines by endothelial cells (EC) and leukocytes (e.g., 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[111]. Importantly, recruitment of monocytes is diminished in the absence of PMN, which is due in part to PMN-derived granule protein, cathelicidin[112]. Diabetes exacerbates atherosclerosis in part by increasing PMN production of S100A8/S100A9, which leads to enhanced myelopoiesis and monocyte accumulation in lesions[113]. Moreover, a recent study found that cholesterol crystals active PMN to release neutrophil extracellular traps (NETs), which amplify immune cell activation in atherosclerosis[109, 114]. As noted above, PMN are a rich source 5-LOX-derived LTB_4, which promotes further leukocyte recruitment. Mice lacking the LTB_4 receptor, BLT1 have a significant reduction in plaque formation and macrophage recruitment[115] as did mice that received bone marrow transplants with genetically reduced levels of 5-LOX[116]. Importantly, SPM including RvD1 block LTB_4-stimulated expression of adhesion molecules and actin polymerization in PMN, and the E-series resolvins block activation of BLT1 by LTB_4[58, 66]. PMN have also been linked with latter stages of atherosclerosis, including plaque destabilization. Plaques that are highly inflamed will continue to attract PMN to infiltrate, and the pro-inflammatory mediators and oxidants they produce may contribute to endothelial erosion and rupture of the fibrous cap. While most studies have primarily focused on other cell types (i.e., macrophages, vascular smooth muscle cells) 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[117]. In rabbits, periodontitis increases atherosclerosis and administration of RvE1 simultaneously decreases both periodonitits and atherosclerosis[118]. Further studies revealed that RvE1 reduces atherosclerosis even in absence of periodontitis, which was associated with a reduction in systemic levels of C-reactive protein (CRP). Interestingly, platelet:PMN aggregates formed during inflammation lead to SPM biosynthesis, which have been shown to reduce human platelet:PMN aggregates[119, 120]. Given that platelet:PMN aggregates contribute to plaque inflammation[121], these results highlight that SPM 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 SPM during resolution. Several SPM, including E-series and D-series resolvins potently enhance macrophage phagocytosis of bacteria and efferocytosis, essential functions fundamental to host-defense and resolution (Fig. 3)[25]. Stimulation of phagocytosis by SPM is at least partially dependent upon downstream activation of Akt, PI3K and ERK, and involves cytoskeletal remodeling associated with activation of Rho-GTPases (i.e., Cdc42, RhoA, Rac) in a receptor-dependent manner [65, 122–125]. Interestingly, during efferocytosis, SPM (e.g., RvD1, RvD2, RvE2) production increases, which potentiates further clearance of debris and apoptotic cells in an autocrine and paracrine manner[39, 99]. However, in several chronic inflammatory diseases (e.g., asthma, obesity/diabetes), SPM production is disrupted and these conditions are also associated with defective resolution and efferocytosis[81]. Indeed, in diabetic wounds,

apoptotic cells accumulate and are associated with defective wound healing[126]. RvD1 enhances wound healing in diabetic mice in part by enhancing efferocytosis[125]. The delayed clearance of apoptotic cells in the thymus was also rescued by RvD1 and in isolated diabetic macrophages, RvD1 rescues defective phagocytosis in a receptor (ALX/Fpr2) and PI3K-dependent manner[125]. The specific mechanisms underlying impaired SPM production and efferocytosis in these diseases are incompletely understood. It is known however that the cellular location of the key 5-LOX enzyme plays an important role in dictating the predominant lipid mediators that macrophages produce. As noted above, RvD1 promotes cytoplasmic localization of 5-LOX (rather than nuclear) and shifts the production in favor of pro-resolving LXA_4 rather than the pro-inflammatory LTB_4[56]. More recently, the mechanism of this nuclear localization was found to be governed by signaling through the myeloid-epithelial-reproductive tyrosine kinase (MerTK) receptor, mechanistically coupling efferocytosis to SPM biosynthesis[127]. In mice genetically engineered to resist cleavage (and therefore inactivation) of this receptor, SPM biosynthesis is increased and resolution of acute inflammation and efferocytosis are improved[127]. In acute MI, mice deficient in efferocytosis receptors, *Mertk* and/or *Mfge8*, have decreased cardiac function and remodeling that was specifically due to abrogation of efferocytosis and not to an alteration in the circulating or tissue resident macrophages, nor the ability of monocytes to infiltrate the tissue[128, 129]. It is, therefore, reasonable to consider that defective efferocytosis in CVD could be related to defective production of SPM, but that these alterations could be potentially rescued by exogenous administration of SPM. Whether there is a causal relationship between SPM production and efferocytosis in the heart during MI requires further study.

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 (Fig. 3). They are important both in the pathology of the lesion from initiation of formation to expansion, necrosis and eventual rupture, as well as 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[22]. 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]. This precipitates the generation of lipid-laden foam cells that fail to clear from the plaque, continue to secrete pro-inflammatory mediators and eventually undergo post-apoptotic 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 (Fig. 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 NADPH oxidase activation and expression of apoptotic proteins, Bcl-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 display exacerbated lesion formation 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 SPM[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 pro-inflammatory and pro-resolving 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]. Additionally, recent studies documented that the ALX/FPR2 receptor has an endogenous anti-atherosclerotic 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 due in part to a failure of endogenous resolution programs.

In addition to macrophage efferocytosis, the phenotype of both monocytes and macrophages is an important determinant of resolution (Fig. 3). Recruited by specific chemokines (e.g., C-C chemokine ligand 2; CCL2) in the injured myocardium or atherosclerotic lesions, inflammatory Ly6C^{high} 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 Ly6C^{low} monocytes/macrophages to facilitate tissue repair in a process dependent in part upon the transcription factor, Nr4a1[137]. These monocytes/macrophages facilitate repair because they are a rich source of growth factors and cytokines that promote tissue vascularization and matrix remodeling, while inflammatory monocytes/macrophages secrete inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α) and are highly microbicidal[138]. Too many Ly6C^{high} cells or a delayed switch into reparative macrophages can lead to excessive proteolysis of the healthy myocardium surrounding the infarct, while increased production of IL-1β enhances proliferation of hematopoietic stem and progenitor cells to promote leukocytosis[139, 140]. 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[17, 141]. 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 LPS/IFNγ or IL-4, respectively, although this nomenclature has been revised because macrophages largely exist in a phenotype spectrum dependent upon the local milieu[16]. This is true for disease states like atherosclerosis, where distinct M1 vs. M2 populations are difficult to discern[130]. With this inherent plasticity, agents that induce pro-resolving characteristics in macrophages may be beneficial in breaking the feed forward pro-inflammatory 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 SPM overall than LPS/IFNγ-polarized macrophages[99]. 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 CCL2, while enhancing phagocytic function. In human macrophages, RvD1 potently blocks production of pro-inflammatory cytokines (e.g., IL-1β, IL-8 and CCL2) in a GPR32-dependent manner, which could help to prevent further recruitment of inflammatory monocytes[142]. In inflamed obese adipose tissue, RvD1 decreases the proportion of inflammatory macrophages and enhances macrophage phagocytosis and expression of arginase 1[143, 144]. In isolated macrophages, RvD1 increases expression of *Arg1, Ym1*, IL-10 and CD206, typical of an anti-inflammatory macrophage phenotype[143]. RvD2 similarly increases surface expression of CD206, as well as CD163, in human macrophages in a GPR18-dependent manner[67]. In murine peritonitis, both RvE1 and RvD1 have been shown to decrease CD11b expression on macrophages. $CD11b^{low}$ macrophages have been reported to express lower levels of iNOS, COX-2 and MMP-9 and engulf more apoptotic cells compared with $CD11b^{high}$ macrophages[145]. There have also been reports of a previously undescribed resolution-phase macrophage (rM) subset with similar features of the $CD11b^{low}$ 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)[146]. These rM 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[11]. 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 expression of specific leukocyte adhesion receptors such as vascular cell adhesion molecule 1 (VCAM-1), is associated with chronic inflammatory diseases such as atherosclerosis and diabetes[11]. Accordingly, EC participate in the local biosynthesis of SPM during acute inflammation and are also direct cellular targets of SPM (Fig. 3). As noted above, the biosynthesis of most SPM 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 SPM such as RvE1[44, 45]. Furthermore, it was in the co-culture of human PMN and EC that the novel 13-series resolvins (RvTs) were recently identified[93]. A recent study documented that RvD1 and RvE1 are also biosynthesized during co-culture of choroid-retinal (CR) EC with leukocytes (both PMN and monocytes), but not by CREC alone[147]. This mechanism likely serves as a local circuit to ensure timely termination of leukocyte adhesion to EC. Indeed, EC express the known receptors for SPM, including ALX/FPR2, GPR32 and

GPR18[58, 148, 149]. Acting through these receptors, SPM regulate both recruitment and adhesion of leukocytes. AA-derived LXA_4 downregulates VCAM-1 and additional adhesion receptors, E-selectin[150] and ICAM-1[151, 152], but also increases nitric oxide (NO) and prostacyclin (PGI_2) that potently counter-regulate leukocyte:EC interactions and platelet activation[153, 154]. Lipoxins have further EC-specific effects including the downregulation of NADPH oxidase, which results in the decreased generation of ROS[155]. Similarly, resolvins have also been shown to regulate leukocyte:EC interactions. For instance, RvD2 limits leukocyte:EC adhesion in a NO-dependent manner and the regulation of PMN recruitment during microbial peritonitis by RvD2 is attenuated in mice deficient in endothelial nitric oxide synthase (eNOS)[40]. Both RvD1 and RvD2 limit PMN interaction with human EC pre-stimulated with inflammatory cytokines (e.g., TNFα, IL-1β) under shear conditions at a physiological flow rate in a receptor-specific manner[40, 59]. In this context, resolvins block PMN capture, rolling and firm adhesion to EC and they attenuate EC production of chemokines (e.g., IL-8 and CCL2) that recruit additional leukocytes[132, 147]. A recent study of the mechanisms whereby resolvins regulate inflammatory signaling in human EC uncovered that D-series resolvins stimulate the activation of PI3K and Akt in a receptor-dependent manner and thereby regulate the production of developmental endothelial locus 1 (Del-1), an anti-inflammatory protein secreted by EC that prevents PMN transmigration[156].

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 following acute lung injury i*n vivo*[158]. These results build on prior studies showing that lipoxins counter-regulate leukocyte-mediated microvascular permeability *in vivo* during acute inflammation stimulated by pro-inflammatory lipid mediators[159]. Permeability of the microvasculature is increased by several inflammatory mediators that also regulate angiogenesis. Along these lines, LXA_4 decreases vascular endothelial growth factor receptor 2 (VEGFR-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 (LXA_4, RvD1, RvE1 and PD1) protects against pathologic angiogenesis[69, 160, 161]. This is in contrast to some pro-inflammatory lipid mediators (i.e., 12-HETE and PGE_2) that promote angiogenesis[162, 163]. Collectively, EC have emerged as important cellular targets of SPM during resolution, suggesting that engagement of SPM receptors might offer a new approach to reversing EC dysfunction that is associated with CVD. Future studies will be required to understand fully the role of SPM 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, vascular smooth muscle cells (VSMC) also contribute to the pathophysiology of atherosclerosis and restenosis. In response to vascular injury, VSMC undergo a phenotype switch from a contractile to a synthetic phenotype; synthetic VSMC are more proliferative and chemotactic and are characterized by a decreased expression of contractile proteins and increased

production of pro-inflammatory mediators[164]. During chronic vascular inflammation (e.g., atherosclerosis) this pro-inflammatory environment can lead to successive contractile to synthetic phenotype switching thereby propagating the pro-inflammatory 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 pro-inflammatory signaling cycle may, therefore, be a useful strategy to combat inflammatory diseases of the vessel wall. Receptors for SPM, including ALX/FPR2 and ChemR23, are expressed in human saphenous vein SMC and administration of RvE1 and 15-epi-LXA_4 counter-regulate platelet-derived growth factor (PDGF)-stimulated VSMC migration in a dose-dependent manner (Fig. 3)[165]. These actions are due in part to direct regulation of PDGF receptor phosphorylation. Building on these *in vitro* studies, it was recently found that 15-epi-LXA_4 inhibits VSMC migration and intimal hyperplasia following murine carotid artery ligation and this protection afforded by 15-epi-LXA_4 is abolished in mice lacking the *ALX/Fpr2* receptor[166]. Interestingly, genetic deficiency of *ALX/Fpr2* decreases stability of atherosclerotic lesions, effects that were attributed to decreased collagen production by VSMC[167]. Given that VSMC can also contribute to stability of atherosclerotic lesions[164] and that it is their phenotype that dictates their contribution to atherosclerosis, these results suggest that SPM 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 pro-inflammatory gene expression in human[168], rat[169] and mouse[170] 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[168]. Interestingly, RvD2 and Mar1 increase M2 macrophage polarization during vascular injury, which may help to promote vessel remodeling and repair[170]. Additionally, 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 pro-inflammatory 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, pro-thrombotic thromboxane was reduced and there was a concomitant increase in 15-epi LXA_4[175, 176]. Plasma levels of 15-epi-LXA_4 were increased most significantly with low dose aspirin (i.e., 81mg), the currently recommended dose for patients with CVD. Interestingly, human platelets express ChemR23

and RvE1 blocks ADP and thromboxane-stimulated platelet aggregation (Fig. 3)[100, 177]. Further mechanistic studies revealed that RvE1 blocks ADP-mediated signals downstream of its receptor, $P2Y_{12}$, 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 due to an abrogation of pro-inflammatory mediator secretion[178]. Lastly, it was very recently shown that SPM increase macrophage uptake of blood clots *in vitro*, indicating that SPM may play a role in clot remodeling during resolution[80]. As noted above, SPM are biosynthesized during interactions of platelets and leukocytes during inflammation. Thus, these results collectively demonstrate that SPM 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 pro-resolving mediators and the identification of their receptors[9]. It is becoming apparent that alterations in specific resolution pathways are associated with a wide range of chronic diseases. Chronic unresolved inflammation is now widely documented to play a causal role in the development of CVD. As such, a large effort is underway to determine whether targeting specific pro-inflammatory pathways could be beneficial for clinical management of CVD beyond the current standard of care (e.g., aspirin, statins, anti-platelet therapies)[4]. In contrast to traditional anti-inflammatory strategies that blunt the production of inflammation “initiators” and could potentially lead to immunosuppression, pro-resolving mediators described in this review activate distinct cellular programs to resolve inflammation without compromising host-defense[9]. In fact, pro-resolving mediators enhance host defense to both viral and bacterial infections and lower the threshold for antibiotic therapy[37, 179]. Thus, new strategies to promote resolution of inflammation may offer unique opportunities to combat chronic inflammation associated with CVD. Pro-resolving 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 pro-resolving mediators in humans with other inflammatory pathologies are currently underway[180].

In regards to therapeutically stimulating resolution of inflammation in CVD, there are several things to consider. First, can SPM 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[118]. Our group has also shown that microparticles released from activated immune cells carry SPM and as a form of biomimicry, we formulated humanized nanoparticles that showed enhanced bioactivity; these could potentially be vehicles for

targeted delivery[181]. Moreover, recent studies have begun to highlight other methodologies for local sustained SPM delivery (e.g., vascular wraps) that could be used in vascular surgery for example[169]. Second, what are the mechanisms underlying failed resolution of inflammation? As noted in this review, several chronic diseases are associated with lower levels of SPM. Does this occur because of altered biosynthesis or enhanced metabolic inactivation? How are SPM receptors modulated in chronic diseases like CVD? Lastly, are nutritional based approaches (e.g., 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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Non-standard Abbreviations and Acronyms

LXA_4	lipoxin A_4, 5*S*,6*R*,15*S*-trihydroxy-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid
LXB_4	lipoxin B_4, 5*S*,14*R*,15*S*-trihydroxy-6,10,12-*trans*-8-*cis*-eicosatetraenoic acid
RvD1	resolvin D1, 7*S*,8*R*,17*S*-trihydroxy-docosa-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-hexaenoic acid
RvD2	resolvin D2, 7*S*,16*R*,17*S*-trihydroxy-docosa-4*Z*,8*E*,10*Z*,12*E*,14*E*,19*Z*-hexaenoic acid
RvE1	resolvin E1, 5*S*,12*R*,18*R*-trihydroxy-eicosa-6*Z*,8*E*,10*E*,14*Z*,16*E*-pentaenoic acid
RvE2	resolvin E2, 5*S*,18*R*-dihydroxy-eicosa-6*E*,8*Z*,11*Z*,14*Z*,16*E*-pentaenoic acid
AA	arachidonic acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
SPM	specialized pro-resolving lipid mediators
LOX	lipoxygenase
COX	cyclooxygenase
PMN	polymorphonuclear neutrophils
PUFA	polyunsaturated fatty acids
CVD	cardiovascular disease

EC	endothelial cells
VSMC	vascular smooth muscle cells

References

1. Libby P, Nahrendorf M, Swirski FK. Leukocytes Link Local and Systemic Inflammation in Ischemic Cardiovascular Disease: An Expanded “Cardiovascular Continuum”. J Am Coll Cardiol. 2016; 67:1091–103. [PubMed: 26940931]
2. Libby P, Bornfeldt KE, Tall AR. Atherosclerosis: Successes, Surprises, and Future Challenges. Circ Res. 2016; 118:531–4. [PubMed: 26892955]
3. Libby P, Tabas I, Fredman G, Fisher EA. Inflammation and its resolution as determinants of acute coronary syndromes. Circ Res. 2014; 114:1867–79. [PubMed: 24902971]
4. Shapiro MD, Fazio S. From Lipids to Inflammation: New Approaches to Reducing Atherosclerotic Risk. Circ Res. 2016; 118:732–49. [PubMed: 26892970]
5. Ridker PM. From C-Reactive Protein to Interleukin-6 to Interleukin-1: Moving Upstream To Identify Novel Targets for Atheroprotection. Circ Res. 2016; 118:145–56. [PubMed: 26837745]
6. Ridker PM. Testing the inflammatory hypothesis of atherothrombosis: scientific rationale for the cardiovascular inflammation reduction trial (CIRT). J Thromb Haemost. 2009; 7(Suppl 1):332–9. [PubMed: 19630828]
7. Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, Gupta M, Clearfield M, Libby P, Hasan AA, Glynn RJ, Ridker PM. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. Am Heart J. 2013; 166:199–207. e15. [PubMed: 23895801]
8. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, Thuren T, Group CPI. Effects of interleukin-1beta inhibition with canakinumab on hemoglobin A1c, lipids, C-reactive protein, interleukin-6, and fibrinogen: a phase IIb randomized, placebo-controlled trial. Circulation. 2012; 126:2739–48. [PubMed: 23129601]
9. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014; 510:92–101. [PubMed: 24899309]
10. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O’Neill LA, Perretti M, Rossi AG, Wallace JL. Resolution of inflammation: state of the art, definitions and terms. FASEB J. 2007; 21:325–32. [PubMed: 17267386]
11. Gimbrone MA Jr, Garcia-Cardena G. Endothelial Cell Dysfunction and the Pathobiology of Atherosclerosis. Circ Res. 2016; 118:620–36. [PubMed: 26892962]
12. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013; 13:159–75. [PubMed: 23435331]
13. Granger DN, Rodrigues SF, Yildirim A, Senchenkova EY. Microvascular responses to cardiovascular risk factors. Microcirculation. 2010; 17:192–205. [PubMed: 20374483]
14. Buckley CD, Gilroy DW, Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. Immunity. 2014; 40:315–27. [PubMed: 24656045]
15. Poon IK, Lucas CD, Rossi AG, Ravichandran KS. Apoptotic cell clearance: basic biology and therapeutic potential. Nat Rev Immunol. 2014; 14:166–80. [PubMed: 24481336]
16. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014; 41:14–20. [PubMed: 25035950]
17. Frangogiannis NG. Inflammation in cardiac injury, repair and regeneration. Curr Opin Cardiol. 2015; 30:240–5. [PubMed: 25807226]
18. Cohen HB, Mosser DM. Extrinsic and intrinsic control of macrophage inflammatory responses. J Leukoc Biol. 2013; 94:913–9. [PubMed: 23964115]

19. Fullerton JN, Gilroy DW. Resolution of inflammation: a new therapeutic frontier. Nat Rev Drug Discov. 2016
20. Kupper TS, Fuhlbrigge RC. Immune surveillance in the skin: mechanisms and clinical consequences. Nat Rev Immunol. 2004; 4:211–22. [PubMed: 15039758]
21. Li S, Sun Y, Liang CP, Thorp EB, Han S, Jehle AW, Saraswathi V, Pridgen B, Kanter JE, Li R, Welch CL, Hasty AH, Bornfeldt KE, Breslow JL, Tabas I, Tall AR. Defective phagocytosis of apoptotic cells by macrophages in atherosclerotic lesions of ob/ob mice and reversal by a fish oil diet. Circ Res. 2009; 105:1072–82. [PubMed: 19834009]
22. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol. 2010; 10:36–46. [PubMed: 19960040]
23. Hansson GK, Libby P. The immune response in atherosclerosis: a double-edged sword. Nat Rev Immunol. 2006; 6:508–19. [PubMed: 16778830]
24. Perretti M. The resolution of inflammation: New mechanisms in patho-physiology open opportunities for pharmacology. Semin Immunol. 2015; 27:145–8. [PubMed: 26209504]
25. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: Novel pro-resolving lipid mediators in resolution. Semin Immunol. 2015; 27:200–15. [PubMed: 25857211]
26. Headland SE, Norling LV. The resolution of inflammation: Principles and challenges. Semin Immunol. 2015; 27:149–60. [PubMed: 25911383]
27. Gilroy D, De Maeyer R. New insights into the resolution of inflammation. Semin Immunol. 2015; 27:161–8. [PubMed: 26037968]
28. Crean D, Godson C. Specialised lipid mediators and their targets. Semin Immunol. 2015; 27:169–76. [PubMed: 26048571]
29. Colgan SP. Neutrophils and inflammatory resolution in the mucosa. Semin Immunol. 2015; 27:177–83. [PubMed: 25818531]
30. Viola J, Soehnlein O. Atherosclerosis - A matter of unresolved inflammation. Semin Immunol. 2015; 27:184–93. [PubMed: 25865626]
31. Haworth O, Buckley CD. Pathways involved in the resolution of inflammatory joint disease. Semin Immunol. 2015; 27:194–9. [PubMed: 25944272]
32. Montero-Melendez T. ACTH: The forgotten therapy. Semin Immunol. 2015; 27:216–26. [PubMed: 25726511]
33. Wallace JL, Ianaro A, Flannigan KL, Cirino G. Gaseous mediators in resolution of inflammation. Semin Immunol. 2015; 27:227–33. [PubMed: 26095908]
34. Nathan C, Ding A. Nonresolving inflammation. Cell. 2010; 140:871–82. [PubMed: 20303877]
35. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005; 6:1191–7. [PubMed: 16369558]
36. Ariel A, Fredman G, Sun YP, Kantarci A, Van Dyke TE, Luster AD, Serhan CN. Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. Nat Immunol. 2006; 7:1209–16. [PubMed: 17013391]
37. Chiang N, Fredman G, Backhed F, Oh SF, Vickery T, Schmidt BA, Serhan CN. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature. 2012; 484:524–8. [PubMed: 22538616]
38. Dalli J, Ramon S, Norris PC, Colas RA, Serhan CN. Novel proresolving and tissue-regenerative resolvin and protectin sulfido-conjugated pathways. FASEB J. 2015; 29:2120–36. [PubMed: 25713027]
39. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature. 2007; 447:869–74. [PubMed: 17568749]
40. Spite M, Norling LV, Summers L, Yang R, Cooper D, Petasis NA, Flower RJ, Perretti M, Serhan CN. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. Nature. 2009; 461:1287–91. [PubMed: 19865173]
41. El Kebir D, Gjorstrup P, Filep JG. Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation. Proc Natl Acad Sci U S A. 2012; 109:14983–8. [PubMed: 22927428]

42. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2001; 2:612–9. [PubMed: 11429545]
43. Serhan CN, Hamberg M, Samuelsson B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proc Natl Acad Sci U S A. 1984; 81:5335–9. [PubMed: 6089195]
44. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med. 2000; 192:1197–204. [PubMed: 11034610]
45. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med. 2002; 196:1025–37. [PubMed: 12391014]
46. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. J Exp Med. 2009; 206:15–23. [PubMed: 19103881]
47. Hong S, Gronert K, Devchand PR, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. J Biol Chem. 2003; 278:14677–87. [PubMed: 12590139]
48. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med. 2005; 201:713–22. [PubMed: 15753205]
49. Oh SF, Pillai PS, Recchiuti A, Yang R, Serhan CN. Pro-resolving actions and stereoselective biosynthesis of 18S E-series resolvins in human leukocytes and murine inflammation. J Clin Invest. 2011; 121:569–81. [PubMed: 21206090]
50. Tjonahen E, Oh SF, Siegelman J, Elangovan S, Percarpio KB, Hong S, Arita M, Serhan CN. Resolvin E2: identification and anti-inflammatory actions: pivotal role of human 5-lipoxygenase in resolvin E series biosynthesis. Chem Biol. 2006; 13:1193–202. [PubMed: 17114001]
51. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. Resolvin E2 formation and impact in inflammation resolution. J Immunol. 2012; 188:4527–34. [PubMed: 22450811]
52. Isobe Y, Arita M, Matsueda S, Iwamoto R, Fujihara T, Nakanishi H, Taguchi R, Masuda K, Sasaki K, Urabe D, Inoue M, Arai H. Identification and structure determination of novel anti-inflammatory mediator resolvin E3, 17,18-dihydroxyeicosapentaenoic acid. J Biol Chem. 2012; 287:10525–34. [PubMed: 22275352]
53. Sun YP, Oh SF, Uddin J, Yang R, Gotlinger K, Campbell E, Colgan SP, Petasis NA, Serhan CN. Resolvin D1 and its aspirin-triggered 17R epimer. Stereochemical assignments, anti-inflammatory properties, and enzymatic inactivation. J Biol Chem. 2007; 282:9323–34. [PubMed: 17244615]
54. Dalli J, Winkler JW, Colas RA, Arnardottir H, Cheng CY, Chiang N, Petasis NA, Serhan CN. Resolvin D3 and aspirin-triggered resolvin D3 are potent immunoresolvents. Chem Biol. 2013; 20:188–201. [PubMed: 23438748]
55. Winkler JW, Orr SK, Dalli J, Cheng CY, Sanger JM, Chiang N, Petasis NA, Serhan CN. Resolvin D4 stereoassignment and its novel actions in host protection and bacterial clearance. Sci Rep. 2016; 6:18972. [PubMed: 26743932]
56. Fredman G, Ozcan L, Spolitu S, Hellmann J, Spite M, Backs J, Tabas I. Resolvin D1 limits 5-lipoxygenase nuclear localization and leukotriene B4 synthesis by inhibiting a calcium-activated kinase pathway. Proc Natl Acad Sci U S A. 2014; 111:14530–5. [PubMed: 25246560]
57. Dalli J, Chiang N, Serhan CN. Identification of 14-series sulfido-conjugated mediators that promote resolution of infection and organ protection. Proc Natl Acad Sci U S A. 2014; 111:E4753–61. [PubMed: 25324525]
58. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, Petasis NA, Serhan CN. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. Proc Natl Acad Sci U S A. 2010; 107:1660–5. [PubMed: 20080636]

59. Norling LV, Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 limits polymorphonuclear leukocyte recruitment to inflammatory loci: receptor-dependent actions. Arterioscler Thromb Vasc Biol. 2012; 32:1970–8. [PubMed: 22499990]
60. Devchand PR, Arita M, Hong S, Bannenberg G, Moussignac RL, Gronert K, Serhan CN. Human ALX receptor regulates neutrophil recruitment in transgenic mice: roles in inflammation and host defense. FASEB J. 2003; 17:652–9. [PubMed: 12665478]
61. Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, Serhan CN, Murphy PM. International Union of Basic and Clinical Pharmacology. LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. Pharmacol Rev. 2009; 61:119–61. [PubMed: 19498085]
62. Cooray SN, Gobbetti T, Montero-Melendez T, McArthur S, Thompson D, Clark AJ, Flower RJ, Perretti M. Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses. Proc Natl Acad Sci U S A. 2013; 110:18232–7. [PubMed: 24108355]
63. El Kebir D, Jozsef L, Khreiss T, Pan W, Petasis NA, Serhan CN, Filep JG. Aspirin-triggered lipoxins override the apoptosis-delaying action of serum amyloid A in human neutrophils: a novel mechanism for resolution of inflammation. J Immunol. 2007; 179:616–22. [PubMed: 17579083]
64. Cash JL, Hart R, Russ A, Dixon JP, Colledge WH, Doran J, Hendrick AG, Carlton MB, Greaves DR. Synthetic chemerin-derived peptides suppress inflammation through ChemR23. J Exp Med. 2008; 205:767–75. [PubMed: 18391062]
65. Ohira T, Arita M, Omori K, Recchiuti A, Van Dyke TE, Serhan CN. Resolvin E1 receptor activation signals phosphorylation and phagocytosis. J Biol Chem. 2010; 285:3451–61. [PubMed: 19906641]
66. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. J Immunol. 2007; 178:3912–7. [PubMed: 17339491]
67. Chiang N, Dalli J, Colas RA, Serhan CN. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. J Exp Med. 2015; 212:1203–17. [PubMed: 26195725]
68. Hudert CA, Weylandt KH, Lu Y, Wang J, Hong S, Dignass A, Serhan CN, Kang JX. Transgenic mice rich in endogenous omega-3 fatty acids are protected from colitis. Proc Natl Acad Sci U S A. 2006; 103:11276–81. [PubMed: 16847262]
69. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A, Hong S, Pravda EA, Majchrzak S, Carper D, Hellstrom A, Kang JX, Chew EY, Salem N Jr, Serhan CN, Smith LE. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med. 2007; 13:868–73. [PubMed: 17589522]
70. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015; 1851:469–84. [PubMed: 25149823]
71. De Caterina R. n-3 fatty acids in cardiovascular disease. N Engl J Med. 2011; 364:2439–50. [PubMed: 21696310]
72. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet. 1999; 354:447–55. [PubMed: 10465168]
73. Tavazzi L, Maggioni AP, Marchioli R, Barlera S, Franzosi MG, Latini R, Lucci D, Nicolosi GL, Porcu M, Tognoni G, Gissi HFI. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. Lancet. 2008; 372:1223–30. [PubMed: 18757090]
74. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. Am J Physiol Cell Physiol. 2014; 307:C39–54. [PubMed: 24696140]
75. Barden A, Mas E, Croft KD, Phillips M, Mori TA. Short-term n-3 fatty acid supplementation but not aspirin increases plasma proresolving mediators of inflammation. J Lipid Res. 2014; 55:2401–7. [PubMed: 25187667]

76. Mas E, Croft KD, Zahra P, Barden A, Mori TA. Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation. Clin Chem. 2012; 58:1476–84. [PubMed: 22912397]
77. Barden AE, Mas E, Mori TA. n-3 Fatty acid supplementation and proresolving mediators of inflammation. Curr Opin Lipidol. 2016; 27:26–32. [PubMed: 26655290]
78. Grenon SM, Owens CD, Nosova EV, Hughes-Fulford M, Alley HF, Chong K, Perez S, Yen PK, Boscardin J, Hellmann J, Spite M, Conte MS. Short-Term, High-Dose Fish Oil Supplementation Increases the Production of Omega-3 Fatty Acid-Derived Mediators in Patients With Peripheral Artery Disease (the OMEGA-PAD I Trial). J Am Heart Assoc. 2015; 4:e002034. [PubMed: 26296857]
79. Barden AE, Moghaddami M, Mas E, Phillips M, Cleland LG, Mori TA. Specialised pro-resolving mediators of inflammation in inflammatory arthritis. Prostaglandins Leukot Essent Fatty Acids. 2016; 107:24–9. [PubMed: 27033423]
80. Elajami TK, Colas RA, Dalli J, Chiang N, Serhan CN, Welty FK. Specialized proresolving lipid mediators in patients with coronary artery disease and their potential for clot remodeling. FASEB J. 2016
81. Spite M, Claria J, Serhan CN. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. Cell Metab. 2014; 19:21–36. [PubMed: 24239568]
82. Roberts LJ 2nd, Montine TJ, Markesbery WR, Tapper AR, Hardy P, Chemtob S, Dettbarn WD, Morrow JD. Formation of isoprostane-like compounds (neuroprostanes) in vivo from docosahexaenoic acid. J Biol Chem. 1998; 273:13605–12. [PubMed: 9593698]
83. Arnardottir H, Orr SK, Dalli J, Serhan CN. Human milk proresolving mediators stimulate resolution of acute inflammation. Mucosal Immunol. 2016; 9:757–66. [PubMed: 26462421]
84. Sasaki A, Fukuda H, Shiida N, Tanaka N, Furugen A, Ogura J, Shuto S, Mano N, Yamaguchi H. Determination of omega-6 and omega-3 PUFA metabolites in human urine samples using UPLC/MS/MS. Anal Bioanal Chem. 2015; 407:1625–39. [PubMed: 25577352]
85. Murphy RC. Specialized pro-resolving mediators: do they circulate in plasma? J Lipid Res. 2015; 56:1641–2. [PubMed: 26232500]
86. Marchioli R, Schweiger C, Tavazzi L, Valagussa F. Efficacy of n-3 polyunsaturated fatty acids after myocardial infarction: results of GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. Lipids. 2001; 36(Suppl):S119–26. [PubMed: 11837985]
87. Vane JR, Botting RM. The mechanism of action of aspirin. Thromb Res. 2003; 110:255–8. [PubMed: 14592543]
88. Claria J, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A. 1995; 92:9475–9. [PubMed: 7568157]
89. Demetz E, Schroll A, Auer K, Heim C, Patsch JR, Eller P, Theurl M, Theurl I, Theurl M, Seifert M, Lener D, Stanzl U, Haschka D, Asshoff M, Dichtl S, Nairz M, Huber E, Stadlinger M, Moschen AR, Li X, Pallweber P, Scharnagl H, Stojakovic T, Marz W, Kleber ME, Garlaschelli K, Uboldi P, Catapano AL, Stellaard F, Rudling M, Kuba K, Imai Y, Arita M, Schuetz JD, Pramstaller PP, Tietge UJ, Trauner M, Norata GD, Claudel T, Hicks AA, Weiss G, Tancevski I. The arachidonic acid metabolome serves as a conserved regulator of cholesterol metabolism. Cell Metab. 2014; 20:787–98. [PubMed: 25444678]
90. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM Jr, Kastelein JJ, Koenig W, Libby P, Lorenzatti AJ, MacFadyen JG, Nordestgaard BG, Shepherd J, Willerson JT, Glynn RJ. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med. 2008; 359:2195–207. [PubMed: 18997196]
91. Birnbaum Y, Ye Y, Lin Y, Freeberg SY, Nishi SP, Martinez JD, Huang MH, Uretsky BF, Perez-Polo JR. Augmentation of myocardial production of 15-epi-lipoxin-a4 by pioglitazone and atorvastatin in the rat. Circulation. 2006; 114:929–35. [PubMed: 16908763]
92. Planaguma A, Pfeffer MA, Rubin G, Croze R, Uddin M, Serhan CN, Levy BD. Lovastatin decreases acute mucosal inflammation via 15-epi-lipoxin A4. Mucosal Immunol. 2010; 3:270–9. [PubMed: 20130564]

93. Dalli J, Chiang N, Serhan CN. Elucidation of novel 13-series resolvins that increase with atorvastatin and clear infections. Nat Med. 2015; 21:1071–5. [PubMed: 26236990]

94. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med. 1999; 5:698–701. [PubMed: 10371510]

95. Scapini P, Cassatella MA. Social networking of human neutrophils within the immune system. Blood. 2014; 124:710–9. [PubMed: 24923297]

96. Jones HR, Robb CT, Perretti M, Rossi AG. The role of neutrophils in inflammation resolution. Semin Immunol. 2016

97. Fierro IM, Colgan SP, Bernasconi G, Petasis NA, Clish CB, Arita M, Serhan CN. Lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 inhibit human neutrophil migration: comparisons between synthetic 15 epimers in chemotaxis and transmigration with microvessel endothelial cells and epithelial cells. J Immunol. 2003; 170:2688–94. [PubMed: 12594298]

98. Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. J Immunol. 2000; 164:1663–7. [PubMed: 10657608]

99. Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood. 2012; 120:e60–72. [PubMed: 22904297]

100. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN. Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. Blood. 2008; 112:848–55. [PubMed: 18480426]

101. Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science. 2013; 339:161–6. [PubMed: 23307733]

102. Kain V, Ingle KA, Colas RA, Dalli J, Prabhu SD, Serhan CN, Joshi M, Halade GV. Resolvin D1 activates the inflammation resolving response at splenic and ventricular site following myocardial infarction leading to improved ventricular function. J Mol Cell Cardiol. 2015; 84:24–35. [PubMed: 25870158]

103. Keyes KT, Ye Y, Lin Y, Zhang C, Perez-Polo JR, Gjorstrup P, Birnbaum Y. Resolvin E1 protects the rat heart against reperfusion injury. Am J Physiol Heart Circ Physiol. 2010; 299:H153–64. [PubMed: 20435846]

104. Duffield JS, Hong S, Vaidya VS, Lu Y, Fredman G, Serhan CN, Bonventre JV. Resolvin D series and protectin D1 mitigate acute kidney injury. J Immunol. 2006; 177:5902–11. [PubMed: 17056514]

105. Kasuga K, Yang R, Porter TF, Agrawal N, Petasis NA, Irimia D, Toner M, Serhan CN. Rapid appearance of resolvin precursors in inflammatory exudates: novel mechanisms in resolution. J Immunol. 2008; 181:8677–87. [PubMed: 19050288]

106. Dufton N, Hannon R, Brancaleone V, Dalli J, Patel HB, Gray M, D'Acquisto F, Buckingham JC, Perretti M, Flower RJ. Anti-inflammatory role of the murine formyl-peptide receptor 2: ligand-specific effects on leukocyte responses and experimental inflammation. J Immunol. 2010; 184:2611–9. [PubMed: 20107188]

107. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, Weber C, Soehnlein O, Steffens S. Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. Eur Heart J. 2016

108. Gao Y, Min K, Zhang Y, Su J, Greenwood M, Gronert K. Female-Specific Downregulation of Tissue Polymorphonuclear Neutrophils Drives Impaired Regulatory T Cell and Amplified Effector T Cell Responses in Autoimmune Dry Eye Disease. J Immunol. 2015; 195:3086–99. [PubMed: 26324767]

109. Doring Y, Drechsler M, Soehnlein O, Weber C. Neutrophils in atherosclerosis: from mice to man. Arterioscler Thromb Vasc Biol. 2015; 35:288–95. [PubMed: 25147339]

110. Weber C, Zernecke A, Libby P. The multifaceted contributions of leukocyte subsets to atherosclerosis: lessons from mouse models. Nat Rev Immunol. 2008; 8:802–15. [PubMed: 18825131]

111. Drechsler M, Megens RT, van Zandvoort M, Weber C, Soehnlein O. Hyperlipidemia-triggered neutrophilia promotes early atherosclerosis. Circulation. 2010; 122:1837–45. [PubMed: 20956207]

112. Doring Y, Drechsler M, Wantha S, Kemmerich K, Lievens D, Vijayan S, Gallo RL, Weber C, Soehnlein O. Lack of neutrophil-derived CRAMP reduces atherosclerosis in mice. Circ Res. 2012; 110:1052–6. [PubMed: 22394519]

113. Nagareddy PR, Murphy AJ, Stirzaker RA, Hu Y, Yu S, Miller RG, Ramkhelawon B, Distel E, Westerterp M, Huang LS, Schmidt AM, Orchard TJ, Fisher EA, Tall AR, Goldberg IJ. Hyperglycemia promotes myelopoiesis and impairs the resolution of atherosclerosis. Cell Metab. 2013; 17:695–708. [PubMed: 23663738]

114. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science. 2015; 349:316–20. [PubMed: 26185250]

115. Subbarao K, Jala VR, Mathis S, Suttles J, Zacharias W, Ahamed J, Ali H, Tseng MT, Haribabu B. Role of leukotriene B4 receptors in the development of atherosclerosis: potential mechanisms. Arterioscler Thromb Vasc Biol. 2004; 24:369–75. [PubMed: 14656734]

116. Mehrabian M, Allayee H, Wong J, Shi W, Wang XP, Shaposhnik Z, Funk CD, Lusis AJ. Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. Circ Res. 2002; 91:120–6. [PubMed: 12142344]

117. Dave S, Van Dyke T. The link between periodontal disease and cardiovascular disease is probably inflammation. Oral Dis. 2008; 14:95–101. [PubMed: 18302669]

118. Hasturk H, Abdallah R, Kantarci A, Nguyen D, Giordano N, Hamilton J, Van Dyke TE. Resolvin E1 (RvE1) Attenuates Atherosclerotic Plaque Formation in Diet and Inflammation-Induced Atherogenesis. Arterioscler Thromb Vasc Biol. 2015; 35:1123–33. [PubMed: 25792445]

119. Abdulnour RE, Dalli J, Colby JK, Krishnamoorthy N, Timmons JY, Tan SH, Colas RA, Petasis NA, Serhan CN, Levy BD. Maresin 1 biosynthesis during platelet-neutrophil interactions is organ-protective. Proc Natl Acad Sci U S A. 2014; 111:16526–31. [PubMed: 25369934]

120. Norris PC, Arnardottir H, Sanger JM, Fichtner D, Keyes GS, Serhan CN. Resolvin D3 multi-level proresolving actions are host protective during infection. Prostaglandins Leukot Essent Fatty Acids. 2016

121. Gerdes N, Seijkens T, Lievens D, Kuijpers MJ, Winkels H, Projahn D, Hartwig H, Beckers L, Megens RT, Boon L, Noelle RJ, Soehnlein O, Heemskerk JW, Weber C, Lutgens E. Platelet CD40 Exacerbates Atherosclerosis by Transcellular Activation of Endothelial Cells and Leukocytes. Arterioscler Thromb Vasc Biol. 2016; 36:482–90. [PubMed: 26821950]

122. Maderna P, Cottell DC, Toivonen T, Dufton N, Dalli J, Perretti M, Godson C. FPR2/ALX receptor expression and internalization are critical for lipoxin A4 and annexin-derived peptide-stimulated phagocytosis. FASEB J. 2010; 24:4240–9. [PubMed: 20570963]

123. Maderna P, Cottell DC, Berlasconi G, Petasis NA, Brady HR, Godson C. Lipoxins induce actin reorganization in monocytes and macrophages but not in neutrophils: differential involvement of rho GTPases. Am J Pathol. 2002; 160:2275–83. [PubMed: 12057930]

124. Reville K, Crean JK, Vivers S, Dransfield I, Godson C. Lipoxin A4 redistributes myosin IIA and Cdc42 in macrophages: implications for phagocytosis of apoptotic leukocytes. J Immunol. 2006; 176:1878–88. [PubMed: 16424219]

125. Tang Y, Zhang MJ, Hellmann J, Kosuri M, Bhatnagar A, Spite M. Proresolution therapy for the treatment of delayed healing of diabetic wounds. Diabetes. 2013; 62:618–27. [PubMed: 23043160]

126. Khanna S, Biswas S, Shang Y, Collard E, Azad A, Kauh C, Bhasker V, Gordillo GM, Sen CK, Roy S. Macrophage dysfunction impairs resolution of inflammation in the wounds of diabetic mice. PLoS One. 2010; 5:e9539. [PubMed: 20209061]

127. Cai B, Thorp EB, Doran AC, Subramanian M, Sansbury BE, Lin C, Spite M, Fredman G, Tabas I. MerTK cleavage limits pro-resolving mediator biosynthesis and exacerbates tissue inflammation. Proc Natl Acad Sci U S A. 2016 In Press.

128. Wan E, Yeap XY, Dehn S, Terry R, Novak M, Zhang S, Iwata S, Han X, Homma S, Drosatos K, Lomasney J, Engman DM, Miller SD, Vaughan DE, Morrow JP, Kishore R, Thorp EB. Enhanced

efferocytosis of apoptotic cardiomyocytes through myeloid-epithelial-reproductive tyrosine kinase links acute inflammation resolution to cardiac repair after infarction. Circ Res. 2013; 113:1004–12. [PubMed: 23836795]

129. Howangyin KY, Zlatanova I, Pinto C, Ngkelo A, Cochain C, Rouanet M, Vilar J, Lemitre M, Stockmann C, Fleischmann BK, Mallat Z, Silvestre JS. Myeloid-Epithelial-Reproductive Receptor Tyrosine Kinase and Milk Fat Globule Epidermal Growth Factor 8 Coordinately Improve Remodeling After Myocardial Infarction via Local Delivery of Vascular Endothelial Growth Factor. Circulation. 2016; 133:826–39. [PubMed: 26819373]
130. Tabas I, Bornfeldt KE. Macrophage Phenotype and Function in Different Stages of Atherosclerosis. Circ Res. 2016; 118:653–67. [PubMed: 26892964]
131. Lee HN, Surh YJ. Resolvin D1-mediated NOX2 inactivation rescues macrophages undertaking efferocytosis from oxidative stress-induced apoptosis. Biochem Pharmacol. 2013; 86:759–69. [PubMed: 23856291]
132. Merched AJ, Ko K, Gotlinger KH, Serhan CN, Chan L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. FASEB J. 2008; 22:3595–606. [PubMed: 18559988]
133. Shen J, Herderick E, Cornhill JF, Zsigmond E, Kim HS, Kuhn H, Guevara NV, Chan L. Macrophage-mediated 15-lipoxygenase expression protects against atherosclerosis development. J Clin Invest. 1996; 98:2201–8. [PubMed: 8941635]
134. Wittwer J, Hersberger M. The two faces of the 15-lipoxygenase in atherosclerosis. Prostaglandins Leukot Essent Fatty Acids. 2007; 77:67–77. [PubMed: 17869078]
135. Drechsler M, de Jong R, Rossaint J, Viola JR, Leoni G, Wang JM, Grommes J, Hinkel R, Kupatt C, Weber C, Doring Y, Zarbock A, Soehnlein O. Annexin A1 counteracts chemokine-induced arterial myeloid cell recruitment. Circ Res. 2015; 116:827–35. [PubMed: 25520364]
136. Fredman G, Kamaly N, Spolitu S, Milton J, Ghorpade D, Chiasson R, Kuriakose G, Perretti M, Farokhzad O, Tabas I. Targeted nanoparticles containing the proresolving peptide Ac2-26 protect against advanced atherosclerosis in hypercholesterolemic mice. Sci Transl Med. 2015; 7:275ra20.
137. Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, Iwamoto Y, Liao R, Zirlik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Ly-6Chigh monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. Circ Res. 2014; 114:1611–22. [PubMed: 24625784]
138. Varga T, Mounier R, Horvath A, Cuvellier S, Dumont F, Poliska S, Ardjoune H, Juban G, Nagy L, Chazaud B. Highly Dynamic Transcriptional Signature of Distinct Macrophage Subsets during Sterile Inflammation, Resolution, and Tissue Repair. J Immunol. 2016; 196:4771–82. [PubMed: 27183604]
139. Sager HB, Heidt T, Hulsmans M, Dutta P, Courties G, Sebas M, Wojtkiewicz GR, Tricot B, Iwamoto Y, Sun Y, Weissleder R, Libby P, Swirski FK, Nahrendorf M. Targeting Interleukin-1beta Reduces Leukocyte Production After Acute Myocardial Infarction. Circulation. 2015; 132:1880–90. [PubMed: 26358260]
140. Panizzi P, Swirski FK, Figueiredo JL, Waterman P, Sosnovik DE, Aikawa E, Libby P, Pittet M, Weissleder R, Nahrendorf M. Impaired infarct healing in atherosclerotic mice with Ly-6C(hi) monocytosis. J Am Coll Cardiol. 2010; 55:1629–38. [PubMed: 20378083]
141. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, Brija T, Gautier EL, Ivanov S, Satpathy AT, Schilling JD, Schwendener R, Sergin I, Razani B, Forsberg EC, Yokoyama WM, Unanue ER, Colonna M, Randolph GJ, Mann DL. Embryonic and adult-derived resident cardiac macrophages are maintained through distinct mechanisms at steady state and during inflammation. Immunity. 2014; 40:91–104. [PubMed: 24439267]
142. Schmid M, Gemperle C, Rimann N, Hersberger M. Resolvin D1 Polarizes Primary Human Macrophages toward a Proresolution Phenotype through GPR32. J Immunol. 2016; 196:3429–37. [PubMed: 26969756]
143. Titos E, Rius B, Gonzalez-Periz A, Lopez-Vicario C, Moran-Salvador E, Martinez-Clemente M, Arroyo V, Claria J. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. J Immunol. 2011; 187:5408–18. [PubMed: 22013115]

144. Hellmann J, Tang Y, Kosuri M, Bhatnagar A, Spite M. Resolvin D1 decreases adipose tissue macrophage accumulation and improves insulin sensitivity in obese-diabetic mice. FASEB J. 2011; 25:2399–407. [PubMed: 21478260]

145. Schif-Zuck S, Gross N, Assi S, Rostoker R, Serhan CN, Ariel A. Saturated-efferocytosis generates pro-resolving CD11b low macrophages: modulation by resolvins and glucocorticoids. Eur J Immunol. 2011; 41:366–79. [PubMed: 21268007]

146. Bystrom J, Evans I, Newson J, Stables M, Toor I, van Rooijen N, Crawford M, Colville-Nash P, Farrow S, Gilroy DW. Resolution-phase macrophages possess a unique inflammatory phenotype that is controlled by cAMP. Blood. 2008; 112:4117–27. [PubMed: 18779392]

147. Tian H, Lu Y, Sherwood AM, Hongqian D, Hong S. Resolvins E1 and D1 in choroid-retinal endothelial cells and leukocytes: biosynthesis and mechanisms of anti-inflammatory actions. Invest Ophthalmol Vis Sci. 2009; 50:3613–20. [PubMed: 19443724]

148. Chiang N, Arita M, Serhan CN. Anti-inflammatory circuitry: lipoxin, aspirin-triggered lipoxins and their receptor ALX. Prostaglandins Leukot Essent Fatty Acids. 2005; 73:163–77. [PubMed: 16125378]

149. Offertaler L, Mo FM, Batkai S, Liu J, Begg M, Razdan RK, Martin BR, Bukoski RD, Kunos G. Selective ligands and cellular effectors of a G protein-coupled endothelial cannabinoid receptor. Mol Pharmacol. 2003; 63:699–705. [PubMed: 12606780]

150. Nascimento-Silva V, Arruda MA, Barja-Fidalgo C, Villela CG, Fierro IM. Novel lipid mediator aspirin-triggered lipoxin A4 induces heme oxygenase-1 in endothelial cells. Am J Physiol Cell Physiol. 2005; 289:C557–63. [PubMed: 15901601]

151. Souza MC, Padua TA, Torres ND, Souza Costa MF, Candea AP, Maramaldo T, Seito LN, Penido C, Estato V, Antunes B, Silva L, Pinheiro AA, Caruso-Neves C, Tibirica E, Carvalho L, Henriques MG. Lipoxin A4 attenuates endothelial dysfunction during experimental cerebral malaria. Int Immunopharmacol. 2015; 24:400–7. [PubMed: 25576659]

152. Baker N, O'Meara SJ, Scannell M, Maderna P, Godson C. Lipoxin A4: anti-inflammatory and anti-angiogenic impact on endothelial cells. J Immunol. 2009; 182:3819–26. [PubMed: 19265161]

153. Brezinski ME, Gimbrone MA Jr, Nicolaou KC, Serhan CN. Lipoxins stimulate prostacyclin generation by human endothelial cells. FEBS Lett. 1989; 245:167–72. [PubMed: 2494071]

154. Paul-Clark MJ, Van Cao T, Moradi-Bidhendi N, Cooper D, Gilroy DW. 15-epi-lipoxin A4-mediated induction of nitric oxide explains how aspirin inhibits acute inflammation. J Exp Med. 2004; 200:69–78. [PubMed: 15238606]

155. Nascimento-Silva V, Arruda MA, Barja-Fidalgo C, Fierro IM. Aspirin-triggered lipoxin A4 blocks reactive oxygen species generation in endothelial cells: a novel antioxidative mechanism. Thromb Haemost. 2007; 97:88–98. [PubMed: 17200775]

156. Maekawa T, Hosur K, Abe T, Kantarci A, Ziogas A, Wang B, Van Dyke TE, Chavakis T, Hajishengallis G. Antagonistic effects of IL-17 and D-resolvins on endothelial Del-1 expression through a GSK-3beta-C/EBPbeta pathway. Nat Commun. 2015; 6:8272. [PubMed: 26374165]

157. Zhang X, Wang T, Gui P, Yao C, Sun W, Wang L, Wang H, Xie W, Yao S, Lin Y, Wu Q. Resolvin D1 reverts lipopolysaccharide-induced TJ proteins disruption and the increase of cellular permeability by regulating IkappaBalpha signaling in human vascular endothelial cells. Oxid Med Cell Longev. 2013; 2013:185715. [PubMed: 24381712]

158. Eickmeier O, Seki H, Haworth O, Hilberath JN, Gao F, Uddin M, Croze RH, Carlo T, Pfeffer MA, Levy BD. Aspirin-triggered resolvin D1 reduces mucosal inflammation and promotes resolution in a murine model of acute lung injury. Mucosal Immunol. 2013; 6:256–66. [PubMed: 22785226]

159. Takano T, Clish CB, Gronert K, Petasis N, Serhan CN. Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A4 and novel lipoxin B4 stable analogues. J Clin Invest. 1998; 101:819–26. [PubMed: 9466977]

160. Jin Y, Arita M, Zhang Q, Saban DR, Chauhan SK, Chiang N, Serhan CN, Dana R. Anti-angiogenesis effect of the novel anti-inflammatory and pro-resolving lipid mediators. Invest Ophthalmol Vis Sci. 2009; 50:4743–52. [PubMed: 19407006]

161. Leedom AJ, Sullivan AB, Dong B, Lau D, Gronert K. Endogenous LXA4 circuits are determinants of pathological angiogenesis in response to chronic injury. Am J Pathol. 2010; 176:74–84. [PubMed: 20008149]

162. Nie D, Tang K, Diglio C, Honn KV. Eicosanoid regulation of angiogenesis: role of endothelial arachidonate 12-lipoxygenase. Blood. 2000; 95:2304–11. [PubMed: 10733500]

163. Finetti F, Donnini S, Giachetti A, Morbidelli L, Ziche M. Prostaglandin E(2) primes the angiogenic switch via a synergic interaction with the fibroblast growth factor-2 pathway. Circ Res. 2009; 105:657–66. [PubMed: 19713533]

164. Bennett MR, Sinha S, Owens GK. Vascular Smooth Muscle Cells in Atherosclerosis. Circ Res. 2016; 118:692–702. [PubMed: 26892967]

165. Ho KJ, Spite M, Owens CD, Lancero H, Kroemer AH, Pande R, Creager MA, Serhan CN, Conte MS. Aspirin-triggered lipoxin and resolvin E1 modulate vascular smooth muscle phenotype and correlate with peripheral atherosclerosis. Am J Pathol. 2010; 177:2116–23. [PubMed: 20709806]

166. Petri MH, Laguna-Fernandez A, Tseng CN, Hedin U, Perretti M, Back M. Aspirin-triggered 15-epi-lipoxin A(4) signals through FPR2/ALX in vascular smooth muscle cells and protects against intimal hyperplasia after carotid ligation. Int J Cardiol. 2015; 179:370–2. [PubMed: 25464488]

167. Petri MH, Laguna-Fernandez A, Gonzalez-Diez M, Paulsson-Berne G, Hansson GK, Back M. The role of the FPR2/ALX receptor in atherosclerosis development and plaque stability. Cardiovasc Res. 2015; 105:65–74. [PubMed: 25341894]

168. Miyahara T, Runge S, Chatterjee A, Chen M, Mottola G, Fitzgerald JM, Serhan CN, Conte MS. D-series resolvin attenuates vascular smooth muscle cell activation and neointimal hyperplasia following vascular injury. FASEB J. 2013; 27:2220–32. [PubMed: 23407709]

169. Wu B, Mottola G, Chatterjee A, Lance KD, Chen M, Siguenza IO, Desai TA, Conte MS. Perivascular delivery of resolvin D1 inhibits neointimal hyperplasia in a rat model of arterial injury. J Vasc Surg. 2016

170. Akagi D, Chen M, Toy R, Chatterjee A, Conte MS. Systemic delivery of proresolving lipid mediators resolvin D2 and maresin 1 attenuates intimal hyperplasia in mice. FASEB J. 2015; 29:2504–13. [PubMed: 25777995]

171. Chatterjee A, Sharma A, Chen M, Toy R, Mottola G, Conte MS. The pro-resolving lipid mediator maresin 1 (MaR1) attenuates inflammatory signaling pathways in vascular smooth muscle and endothelial cells. PLoS One. 2014; 9:e113480. [PubMed: 25409514]

172. Hiram R, Rizcallah E, Sirois C, Sirois M, Morin C, Fortin S, Rousseau E. Resolvin D1 reverses reactivity and Ca2+ sensitivity induced by ET-1, TNF-alpha, and IL-6 in the human pulmonary artery. Am J Physiol Heart Circ Physiol. 2014; 307:H1547–58. [PubMed: 25281570]

173. Hiram R, Rizcallah E, Marouan S, Sirois C, Sirois M, Morin C, Fortin S, Rousseau E. Resolvin E1 normalizes contractility, Ca2+ sensitivity and smooth muscle cell migration rate in TNF-alpha- and IL-6-pretreated human pulmonary arteries. Am J Physiol Lung Cell Mol Physiol. 2015; 309:L776–88. [PubMed: 26320154]

174. Foley JH, Conway ME. Cross Talk Pathways Between Coagulation and Inflammation. Circ Res. 2016 In Press.

175. Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci U S A. 2004; 101:15178–83. [PubMed: 15471991]

176. Chiang N, Hurwitz S, Ridker PM, Serhan CN. Aspirin has a gender-dependent impact on antiinflammatory 15-epi-lipoxin A4 formation: a randomized human trial. Arterioscler Thromb Vasc Biol. 2006; 26:e14–7. [PubMed: 16293793]

177. Fredman G, Van Dyke TE, Serhan CN. Resolvin E1 regulates adenosine diphosphate activation of human platelets. Arterioscler Thromb Vasc Biol. 2010; 30:2005–13. [PubMed: 20702811]

178. Bohr S, Patel SJ, Sarin D, Irimia D, Yarmush ML, Berthiaume F. Resolvin D2 prevents secondary thrombosis and necrosis in a mouse burn wound model. Wound Repair Regen. 2013; 21:35–43. [PubMed: 23110665]

179. Morita M, Kuba K, Ichikawa A, Nakayama M, Katahira J, Iwamoto R, Watanebe T, Sakabe S, Daidoji T, Nakamura S, Kadowaki A, Ohto T, Nakanishi H, Taguchi R, Nakaya T, Murakami M, Yoneda Y, Arai H, Kawaoka Y, Penninger JM, Arita M, Imai Y. The lipid mediator protectin D1

inhibits influenza virus replication and improves severe influenza. Cell. 2013; 153:112–25. [PubMed: 23477864]

180. Auven Therapeutics. http://www.auventherapeutics.com/auven/products/RX10045.php

181. Norling LV, Spite M, Yang R, Flower RJ, Perretti M, Serhan CN. Cutting edge: Humanized nano-proresolving medicines mimic inflammation-resolution and enhance wound healing. J Immunol. 2011; 186:5543–7. [PubMed: 21460209]

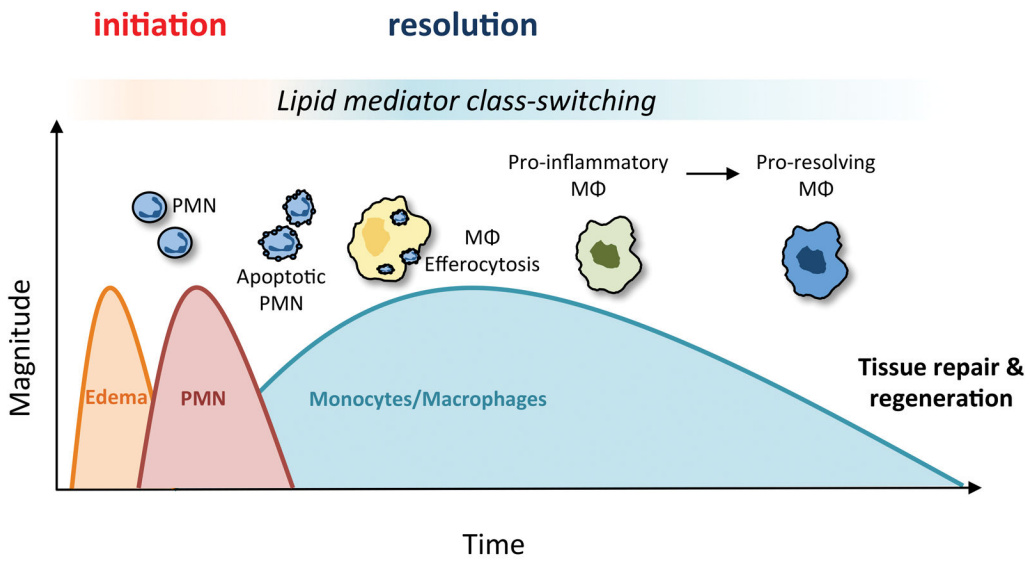

Figure 1. The coordinated temporal events of self-limited acute inflammation

The ideal outcome of an acute inflammatory response is complete resolution. The inflammatory response can be divided into two 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 due to increased blood flow and microvascular permeability and is mediated by the release of pro-inflammatory lipid mediators including the cysteinyl leukotrienes and prostaglandins. Polymorphonuclear neutrophils (PMN) infiltrate in response to lipid mediators including leukotriene B_4 and engulf and degrade pathogens. Subsequently, PMN undergo apoptosis and also switch from releasing pro-inflammatory mediators to pro-resolving mediators (e.g., resolvins) that signal the clearance of apoptotic cells by macrophages in an anti-inflammatory process termed efferocytosis. In addition to promoting efferocytosis, pro-resolving lipid mediators halt further PMN recruitment and stimulate a pro-resolving macrophage phenotype that is important for tissue repair.

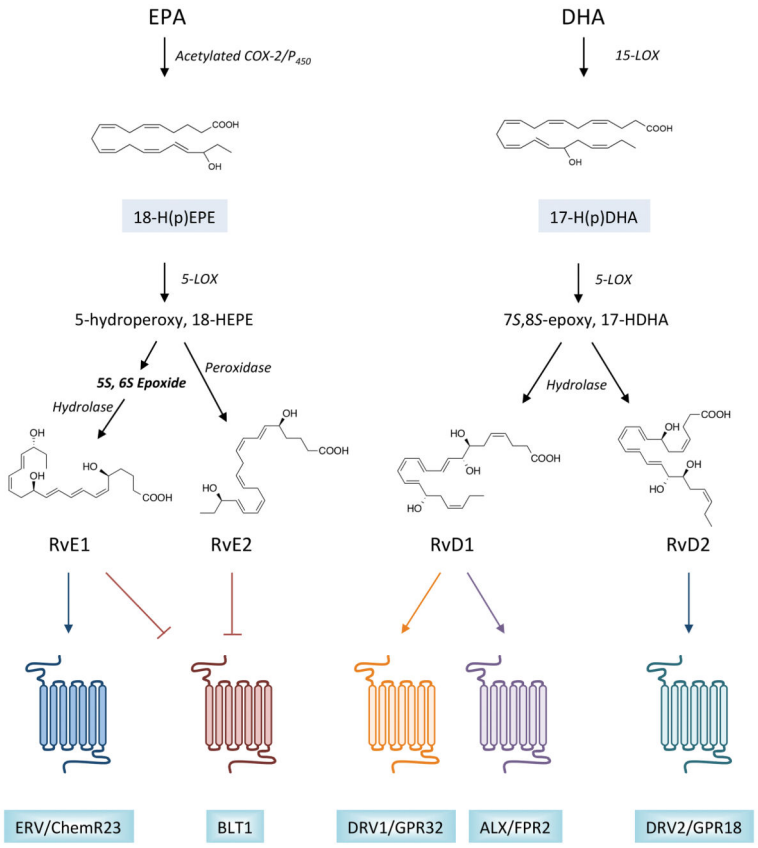

Figure 2. Biosynthesis and receptors for resolvins

The family of resolvins is divided primarily into two groups based on the omega-3 polyunsaturated fatty acid (PUFA) from which they are formed. For E-series resolvin biosynthesis, eicosapentaenoic acid (EPA) is utilized as a substrate for acetylated COX-2 or cytochrome P_{450} enzymes, giving rise to a 18-hydroperoxide intermediate that can be converted to either RvE1 or RvE2 by 5-LOX and additional enzymatic transformations, as indicated. Similarly, DHA serves as a substrate for 15-LOX, giving rise to a 17-hydroperoxide intermediate. This intermediate is subsequently converted to RvD1 or RvD2 via 5-LOX, the formation of an epoxide intermediate, and subsequent enzymatic hydrolysis. Monohydroxy fatty acids, 18-HEPE and 17-HDHA, serve as pathway markers for E-series resolvins and D-series resolvins, respectively. Once formed, resolvins elicit their potent biological actions by activating specific G-protein coupled receptors (GPCRs) in a stereoselective manner. RvE1 is an agonist for ERV/ChemR23, while both RvE1 and RvE2 block the actions of pro-inflammatory leukotriene B_4 by binding to its receptor, BLT1. D-series resolvins are agonists for distinct GPCRs, with RvD1 binding to DRV1/GPR32 and ALX/FPR2, and RvD2 binding to GPR18.

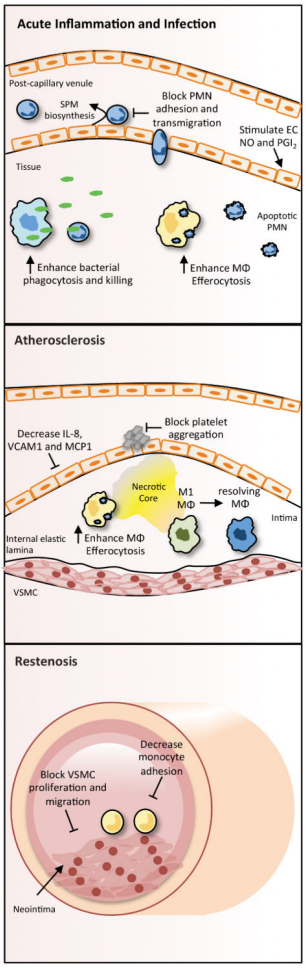

Figure 3. Biological actions of resolvins and other SPM related to cardiovascular inflammation

During acute inflammation or infection, SPM are generated during leukocyte:endothelial interactions and directly block PMN chemotaxis. By directly targeting the endothelial cells of the vasculature (e.g., post-capillary venules), SPM also prevent the capture, rolling and adhesion of PMN to the endothelial monolayer and block their extravasation across the endothelium. Additionally, they stimulate production of nitric oxide (NO) and prostacyclin (PGI_2) from endothelial cells. In the tissue, SPM promote the protective actions of leukocytes by enhancing the phagocytosis of bacteria and cellular debris and macrophage efferocytosis of apoptotic PMN. In the context of atherosclerosis, SPM decrease endothelial cell 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 efferocytosis and promotion of a resolving macrophage phenotype contributes to a more stable plaque with decreased necrosis. During pathologic inward remodeling (i.e. restenosis), SPM combat neointimal hyperplasia by inhibiting vascular smooth muscle cell (VSMC) proliferation and migration while simultaneously preventing monocyte adhesion and inflammatory signaling.