Mechanisms of Resolution of Inflammation
A Focus on Cardiovascular Disease

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Abstract—The inflammatory response is an integral part of the innate immune mechanism that is triggered in response to a real or perceived threat to tissue homeostasis, with a primary aim of neutralizing infectious agents and initiating repair to damaged tissue. By design, inflammation is a finite process that resolves as soon as the threat of infection abates and sufficient repair to the tissue is complete. Resolution of inflammation involves apoptosis and subsequent clearance of activated inflammatory cells—a tightly regulated event. Chronic inflammation is a characteristic feature in virtually all inflammatory diseases, including atherosclerosis, and it is becoming increasingly clear that derangement of the processes usually involved in resolution of inflammation is an underlying feature of chronic inflammatory conditions. This review will draw on evidence from a range of diseases in which dysregulated inflammation is important, with particular emphasis on cardiovascular disease.

Key Words: atherosclerosis ▪ eicosanoids ▪ lipids ▪ NO ▪ resolution of inflammation

Inflammation is a primary component of the immune system that is triggered by any stimulus that poses a real or perceived threat to tissue homeostasis.1 The acute inflammatory process is characterized by rapid recruitment of granulocytes (ie, neutrophils, eosinophils, and basophils) to the inflammatory site; the relative contribution of these cell types is dependent on the location of the inflammatory site in question. The migration of granulocytes to inflammatory loci is a necessary requirement for the neutralization and removal of deleterious agents; these cells play a key role in the defense against bacterial, fungal, and viral infections and in resistance to parasitic invasion and the allergic response. Resolution of inflammation is perceived to occur by elimination of granulocytes and the eventual return of tissue mononuclear cell (macrophage and lymphocyte) numbers to basal levels.2 For effective resolution to occur, cessation of proinflammatory signaling is a prerequisite that pre-empts removal of infiltrating granulocytes. During spontaneous resolution, neutrophils undergo apoptosis, a highly regulated cell death mechanism that prevents the release of histotoxic cellular contents.3 Alterations in neutrophil cell surface markers and morphological changes during apoptosis correlate with increased recognition by professional phagocytes, such as macrophages, that mediate effective clearance of dying cells.3,4 It is accepted that the resolution process is active, rather than passive, and is controlled by a range of tightly regulated biochemical and cellular mechanisms.2 The acute inflammatory response is self-limiting and normally results in tissue restoration and the return of tissue homeostasis. Persistent inflammatory stimuli or dysregulation of mechanisms of the resolution phase results in chronic inflammation,2 recognized to be a key underlying factor in the progression of a range of diseases, including atherosclerosis,5,6 arthritis,7 and chronic neurodegenerative diseases, such as Alzheimer’s disease.8 Atherogenesis is widely recognized to be an inflammatory process in the vascular wall, with early atherogenic events characterized by lipoprotein accumulation, leukocyte recruitment (especially monocytes and macrophages), and expression of proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF) α.5,9 Recent studies10–12 examining the mechanism of resolution in the context of atherosclerosis indicate that failure of resolution mechanisms may underlie the inflammatory processes involved. Atherosclerosis constitutes the underlying pathological features associated with many cardiovascular diseases, including coronary artery disease, myocardial infarction, stroke, and ischemic gangrene.9 Evidence is accumulating that, as well as being involved in the formation of atherosclerotic plaques, intensified inflammation can promote plaque rupture, with the resulting ischemic consequences.9,12 Therefore, because of the dysregulated inflammatory nature of atherosclerosis, an understanding of the mechanisms involved in the resolution of inflammation may reveal novel therapeutic targets with important clinical applications. In addition, it may lead to the identification of clinically relevant biomarkers of an aberrant resolution process, in a similar vein to the use of C-reactive
protein for the detection of cardiovascular disease. This review will discuss a range of mechanisms involved in the resolution of inflammation, with particular emphasis on atherosclerosis and cardiovascular diseases. The following list of mechanisms is by no means exhaustive; instead, we focused on a few topics undergoing research that we believe merit further investigation.

**Granulocyte Apoptosis: Inhibition of Cyclin-Dependent Kinases**

Apoptosis of inflammatory cells is a non-inflammatory mechanism of cell removal and plays a critical role in successful resolution of the inflammatory response. Recent studies have highlighted the importance of cyclin-dependent kinase (CDK) inhibitor drugs, such as R-roscovitine, as potential therapeutic agents with anti-inflammatory properties that promote resolution. The CDKs are traditionally viewed as cell cycle regulators and are differentially activated during the progression of the cell cycle. Given that neutrophils are terminally differentiated cells, inhibition of CDKs might be expected to have little impact in these cells. However, in addition to expressing functional CDKs, a range of pharmacological CDK inhibitor drugs promotes caspase-dependent neutrophil apoptosis (Figure 1). Furthermore, R-roscovitine overrides powerful TNF-α and lipopolysaccharide-induced prosurvival signals to promote neutrophil apoptosis. CDK inhibitor drug–induced apoptosis is triggered by downregulation of transcription of the prosurvival protein myeloid cell leukemia-1, with no effects observed on early parameters of neutrophil nuclear factor κB activation. Studies using in vivo mouse models of the resolution phase of inflammation have highlighted the therapeutic potential of these compounds through dramatic enhancement of the resolution of inflammation. In a resolving model of carrageenan-induced pleural inflammation, intraperitoneal administration of R-roscovitine enhanced the resolution of the established inflammation. Total inflammatory cell numbers were significantly decreased, with a dose of 100 mg/kg reducing inflammatory cell levels to near baseline at 24 hours after treatment. The powerful pro-resolution properties of R-roscovitine have been demonstrated in bleomycin-induced lung injury and in a model of passively induced arthritis; in these models, treatment administered after the inflammatory response was established resulted in successful reduction of inflammation and promotion of resolution, further highlighting the potential of R-roscovitine as a novel therapeutic agent in this field. In addition, R-roscovitine promoted human eosinophil apoptosis by loss of mitochondrial membrane potential and downregulation of McI-1. This ability of CDK inhibitors to promote eosinophil apoptosis, in addition to neutrophil apoptosis, has therapeutic potential for the resolution of eosinophilic-driven allergic diseases, such as asthma and eczema. However, the profound pro-resolution effects of CDK inhibitor drugs in acute inflammation models require further investigation in more chronic inflammation models, especially in cardiovascular disease models.

**NO Signaling in the Resolution of Inflammation**

The inorganic free radical, NO, is a key mediator in a wide range of biological processes, including regulation of inflammation. During the inflammatory response, activated inflammatory cells generate reactive oxygen and nitrogen species, including NO; of particular interest in the context of this review is the ability of NO to regulate apoptosis of a range of cells, including the inflammatory cells themselves. Depending on local concentrations and the cell type, NO can demonstrate both pro-apoptotic and anti-apoptotic properties (Figure 1). For example, in murine peritoneal macrophages, high concentrations of NO generated from L-arginine by NO synthase (NOS) induce apoptosis. Conversely, pre-treatment with low concentrations of NO protects a murine macrophage cell line (RAW 264) from cytotoxic concentrations of NO. Given the pro-apoptotic potential of NO, its presence might be of particular relevance during the resolution phase of inflammation. In the presence of NOS inhibitors, activated murine macrophages display a reduced ability to induce mesangial cell apoptosis. Treatment with the NOS inhibitor, N′-nitro-L-arginine methyl ester, reduces protein A–induced apoptosis of tumor cells, further supporting a role.
for NO in apoptosis regulation and promotion of the resolution phase of inflammation. In atherosclerosis, recruitment of inflammatory cells is a major contributing factor to plaque formation. Because of its proapoptotic properties, NO has the potential to promote the resolution of inflammation during the atherosclerotic process and might, therefore, be a promising target for therapeutic manipulation. Hypercholesterolemic rabbits treated with the NOS substrate, L-arginine, have elevated numbers of apoptotic cells, particularly macrophages, in the intimal lesions. Furthermore, regression of atheroma was also observed in this model, reinforcing its potential as a therapeutic target.

In addition to the generation of NO, activated inflammatory cells are likely to produce peroxynitrite, which is the product of the rapid reaction of NO with the superoxide anion. Like NO, peroxynitrite possesses a pro-apoptotic role in human neutrophils and induces rapid apoptosis via activation of caspases 2 and 3. However, apoptosis of monocyte-derived macrophages is only induced by peroxynitrite release, with NO proving ineffective in promoting apoptosis. The ability of macrophages to “resist” NO-induced apoptosis could indicate an ability of these cells to survive within the inflammatory milieu to affect phagocytosis of apoptotic cells and, therefore, contribute to the resolution of inflammation.

In addition to their apoptotic properties, reactive species derived from NO that are generated under inflammatory conditions are able to nitrate a range of molecules, including unsaturated fatty acids. The products of these reactions are highly electrophilic species that are able to interact with cysteine and histidine residues on proteins and, thus, induce post-translational modifications and alterations in protein activity and localization. These alterations can include induction of protein nuclear respiratory factor (Nrf2)/protein Kelch-like ECH-associated protein (Keap1), and peroxisome proliferator-activated receptor-γ signaling. Nitrated fatty acids demonstrate anti-inflammatory properties and inhibit endothelial TNF-α–induced vascular cell adhesion molecule 1 expression and monocyte rolling and adhesion, suggesting their role as a new class of lipid-derived anti-inflammatory mediators. Furthermore, subcutaneous administration of nitro-oleic acid to apolipoprotein E–deficient mice reduces atherosclerotic lesions and decreases infiltration of inflammatory cells into the vessel wall.

Bioactive Lipid Mediators in the Resolution of Inflammation

Lipid mediators derived from arachidonic acid (AA; 20:4, omega-6 (ω-6)) are known to regulate the inflammatory process and generate pro-inflammatory, anti-inflammatory, and proresolving mediators (Figure 2). Indeed, synthesis of the cyclooxygenase (COX)–generated eicosanoid, prostaglandin E2, regulates blood flow and vasodilatation. However, temporal analysis of eicosanoid generation in inflammatory exudates reveals a switch toward a lipid profile that actively promotes resolution after initial activity involving the proinflammatory mediator, prostaglandin E2. Exposure of human peripheral blood polymorphonuclear neutrophils (PMNs) to prostaglandin E2 results in the diversion of eicosanoid generation away from the proinflammatory 5-lipoxygenase (LO) product, leukotriene B4, to the 15-LO product, lipoxin A4 (LXA4), which promotes resolution. Treatment with the stable LXA4 analogue, 15-epi-LXA4, decreases PMN infiltration in the exudates. LXA4 and LXB4 are generated from AA by transeellular metabolism during inflammatory responses by sequential oxygenation from cell-specific LO enzymes present in the inflammatory milieu.

In addition, acetylation of COX-2 by aspirin modulates its structure and shifts its enzymatic activity away from its normal prostaglandin endoperoxide synthase activity to an LO-like process. This results in the generation of the LX epimer, 15-epi-LXA4, also known as aspirin-triggered lipoxin (ATL). Exposure of monocyte-derived macrophages to LXA4 or its stable synthetic analogues induces rapid phagocytosis of apoptotic PMNs in a concentration-dependent manner. In a murine model of thioglycollate-induced peritonitis, intraperitoneal injection of LXA4, LXB4, 15-epi-LXB4, or the stable synthetic analogue, 15(R/S)-methyl-LXA4, all stimulate monocyte-derived macrophage phagocytosis of PMNs within 15 minutes in a protein kinase C- and phosphatidylinositol 3-kinase-dependent manner, suggesting a role for these lipids as rapidly acting endogenous proresolving agents.

In healthy human patients after an acute dermal inflammatory response created by cantharidin, treatment with low-dose aspirin triggers ATL synthesis and reduces PMN and macrophage accumulation. Furthermore, LXA4 reduces microvascular permeability in inflammatory
rat mesenteric arteries, supporting its proresolution properties.\textsuperscript{44} In a randomized human trial\textsuperscript{45} of healthy patients who received aspirin or placebo, platelet reactivity, as assessed by plasma thromboxane B\textsubscript{2} levels, was inversely related to plasma ATL concentration, which might account for the anti-inflammatory and antineutrophil actions of aspirin. In neutrophils, myeloperoxidase suppresses the apoptotic process via modulation of Mac-1 signaling, thereby prolonging inflammation. In myeloperoxidase-treated neutrophils, the addition of ATL downregulated Mac-1 expression and promoted apoptosis via decreased expression of Mcl-1 and the promotion of mitochondrial dysfunction.\textsuperscript{46} Furthermore, the proresolution effects of ATL were demonstrated in vivo in models of myeloperoxidase-sustained acute lung injury, in which ATL administration accelerated resolution.

Recent studies\textsuperscript{47,48} have identified a new family of lipid mediators generated from the ω-3 fatty acids, eicosapentaenoic acid (20:5), and docosahexaenoic acid (DHA; 22:6) (Figure 2). These mediators, including the resolvins, protectins, and maresins, display potent, specialized, proresolving properties and were identified by lipidomic analysis of resolving self-limited inflammatory exudates.\textsuperscript{39,50} Resolvins are biosynthesized from either eicosapentaenoic acid or DHA (designated as E or D series, respectively) and bear similarity to lipoxins in that they can also be generated by the “alternative” acetylated COX-2 pathway in the presence of aspirin, thus producing “aspirin-triggered” forms.\textsuperscript{48} Like the D-series resolvins, protectins and maresins are generated from DHA. Protectins contain a conjugated-triene structure and possess potent anti-inflammatory properties.\textsuperscript{51} Protectin D1 inhibits TNF-α-induced leukocyte trafficking to a murine air pouch inflammatory site\textsuperscript{51} and regulates PMN infiltration in a murine model of peritonitis.\textsuperscript{52} In addition, maresin 1 is a recently identified macrophage-derived dihydroxy lipid mediator that demonstrates comparable phagocytosis-enhancing properties to both resolvin E1 and protectin D1.\textsuperscript{53} The generation of these compounds produces a range of specific member compounds that are stereospecific in their formation and action.\textsuperscript{49} Furthermore, these compounds act as specific receptors and demonstrate high potency. For example, resolvin E1 binds the G-protein couple receptor CMKLR1 (formerly termed ChemR23)\textsuperscript{54}; and the ip administration of 300 ng resolvin E1 or protectin D1 promotes phagocyte removal during a zymosan-induced model of peritonitis.\textsuperscript{55}

In an apolipoprotein E–deficient murine model of atherosclerosis, macrophage-specific overexpression of 12/15-LO, an essential enzyme in LX, resolin, and protectin generation, has been shown to protect against atherosclerotic lesion development.\textsuperscript{10} On further examination of the mechanism for protection, levels of plasma cholesterol or lipoprotein were unaltered, but expression of proinflammatory cytokines, such as IL-17 and chemokine (C-C motif) ligand-5, were down-regulated, indicating an inflammatory component in this disease. LXA\textsubscript{4}, resolvin D1, and protectin D1 reduce inflammatory cytokine expression and elevate macrophage uptake of apoptotic thymocytes. Furthermore, protectin D1 downregulates the expression of the adhesion molecules, vascular cell adhesion molecule 1 and membrane cofactor protein-1, which are known to regulate leukocyte recruitment during atherogenesis. In addition, patients with symptomatic peripheral artery disease demonstrate reduced plasma levels of ATL compared with healthy volunteers.\textsuperscript{11} The migration of vascular smooth muscle cells is an important feature of atherosclerotic lesion formation, and receptors for both ATL and resolvin E1 (LXA4 receptor and ChemR23, respectively) have been detected in human vascular smooth muscle cells. In addition, treatment with ATL and resolvin E1 inhibits platelet-derived growth factor–stimulated chemotaxis of vascular smooth muscle cells via decreased activation of platelet-derived growth factor receptor-β, further implicating the inflammatory aspect of this disease and identifying another potential therapeutic target.

A recent study\textsuperscript{56} has uncovered a novel range of bioactive lipid mediators generated by the action of COX-2 on the ω-3 polyunsaturated fatty acids, DHA, and docosapentaenoic acid (22:5) that possess anti-inflammatory properties in vitro. In activated macrophages, COX-2 generates a range of electrophilic oxo derivatives; this generation increases further after modulation of COX-2 activity by aspirin acetylation. Because of the highly electrophilic nature of these electrophilic oxo derivative compounds, they readily form adducts with cysteine and histidine protein residues in vivo, in a similar manner to nitrated fatty acids. Treatment of RAW 264 cells with a range of electrophilic oxo derivative compounds results in a concentration-dependent decrease in the expression of IL-6, membrane cofactor protein-1, IL-10, and inducible NOS, nitrite, and nitrate, suggesting a broad spectrum of anti-inflammatory effects. However, the generation and role of these compounds in inflammatory models have yet to be characterized.

**Summary**

Atherosclerosis is a chronic inflammatory disease, with dysregulation of the inflammatory process and failure of effective resolution being features throughout its life cycle, from initiation of lipoprotein accumulation and leukocyte recruitment to plaque formation and rupture. Although the formation of atherosclerotic plaques per se is not necessarily deleterious, plaque rupture is the trigger for a range of potentially fatal cardiovascular events. Because of the role of continual and intense local inflammatory processes in plaque rupture, an understanding of the mechanisms that control inflammation and promote its successful resolution in atherosclerosis will have significant importance in the development of new clinically relevant therapeutic targets, with the potential to reduce the prevalence of fatal cardiovascular events. Recent studies that have elucidated the structure and role of the novel proresolving ω-3–derived lipids in the resolution of inflammation are particularly exciting and highlight a fruitful area of research that has led to the development of novel therapeutic agents that are undergoing clinical evaluation.

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