REVIEW

Repair Mechanisms in Oxidant-Driven Chronic Inflammatory Disease

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The interplay that governs chronic diseases through pathways specifically associated with chronic inflammation remains undefined. Many metabolic events have been identified during the injury and repair process. Nonetheless, the cellular events that control the pathogenesis of inflammation-induced disease have not been fully characterized. We and others reason that chronic inflammatory diseases associated with a cascade of complex network mediators, such as nitric oxide, arachidonic acid metabolites, cytokines, and reactive oxygen species, play a significant role in the governance of alterations in homeostasis, oxidative stress, and thromboatherosclerosis. In this context, we discuss lipid mediators associated with the maintenance of health, including the specialized proresolving mediators that help drive cellular repair. Emphasis is placed on the pathophysiology of chronic metabolic insults involving both the airways and the cardiovascular system during oxidant-driven inflammatory disease. In this review, we highlight new pathways of inquiry that show promise for the identification of those metabolic targets that can improve therapy for chronic inflammation. (*Am J Pathol* 2016, 186: 1736–1749; <http://dx.doi.org/10.1016/j.ajpath.2016.03.001>)

Chronic inflammatory diseases, such as coronary heart disease and chronic obstructive pulmonary disease (COPD), are leading causes of death worldwide.^{1,2} In fact, investigation of molecular mechanisms that drive the acceleration of disease states in association with chronic inflammation remains quintessential. It is well known that inflammation in response to pathogenic insult is intertwined with repair processes at the site of injury. Accumulated evidence supports the concept that the onset of the inflammatory cascade can be initiated by infections and tissue injury. Accordingly, a highly regulated innate and adaptive immune response proceeds to protect the host. This acute inflammatory response is typically short-lived and self-limited in a healthy individual, resulting in the resolution of inflammation and complete healing of tissue. In some instances, permanent destruction of tissue can result from ineffective resolution of acute inflammation, which can progress to a variety of chronic disease states.³

Numerous diseases result from a defective inflammatory response, such as chronic inflammatory airway disease (eg, asthma and COPD)⁴ and cardiovascular disease (eg,

coronary heart disease and diabetes).^{5,6} We and others believe that chronic inflammatory diseases are associated with the biosynthesis of an altered complex network of inflammatory mediators by resident cells as well as inflammatory cells that infiltrate sites of injury.^{7,8} At these sites of injury, local and circulating mediators are released that include classic lipid mediators (prostaglandins and leukotrienes), reactive oxygen and nitrogen species, and others. During host defense, these mediators are often termed proinflammatory when their levels are amplified by further mobilization and activation of leukocytes at sites of damage.^{9,10} Thus, uncontrolled inflammation sets the stage for chronic inflammatory diseases, and severity is governed by signaling events as well as the inflammatory phenotype of cells. Such conditions determine disease progression or termination. Within this inflammatory setting, we describe multifaceted and distinct actions of newly discovered lipid mediators, termed specialized proresolving mediators

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(SPMs), with therapeutic potential. The source of these SPMs are free polyunsaturated fatty acids that are released from membrane phospholipids by the action of phospholipases and include arachidonic acid (AA; omega-6 fatty acids), eicosapentanoic acid (EPA; omega-3 fatty acid), and docosahexanoic acid (DHA, omega-3 fatty acid). SPMs possess anti-inflammatory actions that suppress the innate immune system, and proresolution actions that enhance the immune system and promote tissue restoration. Although some SPMs have dual anti-inflammatory and proresolution activities, both activities are not equivalent. Anti-inflammatory actions include the inhibition of neutrophil recruitment, platelet aggregation, vascular permeability, cytokine production, leukocyte-endothelial cell interactions, and reactive oxygen species (ROS) generation. Alternatively, proresolution actions include enhanced macrophage phagocytosis, enhanced clearance of microbes, inflammatory cells and apoptotic debris, and the stimulation of endothelial nitric oxide (NO) and prostacyclin (PGI₂) production.¹¹

Previous work by our group and others has demonstrated that there are metabolic interchanges between two pathways originating from the diet, the NO and lipid mediator pathways.⁸ These pathways are sources of biological mediators that temper inflammation and maintain tissue homeostasis. These findings are of great significance because they established the role of these pathways in oxidative stress as well as the development of atherosclerosis in humans.⁸ Owing to many new concepts that have been proposed regarding the mechanisms of action involving the NO and lipid mediator pathways, we have summarized in this review the current understanding of the promises and challenges involving the impact of these pathways in chronic inflammatory disease, including coronary heart disease and COPD.

Oxidative Stress and Chronic Inflammatory Disease

There is ample evidence that oxidative stress is intensified in inflammatory processes. Under normal physiological conditions, low levels of ROS are produced endogenously during cellular respiration and metabolism.¹² Balanced production of these oxidants is necessary for maintaining the reduction-oxidation (redox) status of cells, and for the regulation of cell signaling pathways and processes that include cell proliferation, aggregation, and apoptosis. However, certain conditions lead to an overproduction of pro-oxidant molecules that subsequently deplete the stores of antioxidant biological compounds that generally neutralize excessive ROS production. These conditions include the enhanced production of ROS and reactive nitrogen species (RNS) by phagocytic cells that are meant to destroy invading organisms, chronic exposure to environmental pollutants, and cigarette smoke (CS). As a result,

increased production of oxidants disrupts normal functions of biological molecules, including lipids, proteins, and nucleic acids, that lead to cellular transformation and tissue damage.¹³

Oxidative Stress in Vascular Injury

In normal blood vessels, a variety of cellular systems generate mediators that offer protection against disease by controlling blood flow, suppressing inflammation, and globally maintaining a healthy environment in the vessel wall (Figure 1). The inner lining of blood vessels consists of a single layer of endothelial cells that generate protective molecules and are essential for maintaining vascular health.^{14–16} However, studies have identified several risk conditions, including hypertension, high blood cholesterol, high blood glucose, and others, that damage blood vessels and disrupt homeostasis. Vascular damage is characterized by vasoconstriction, excessive inflammation, and potentially atherosclerosis that greatly increase the likelihood of stroke, heart attack, and ischemia.^{17–19} Therefore, investigation of the underlying mechanisms that alter the balance of protective mediators during disease progression becomes critical when developing effective therapies for treatment of cardiovascular disease.

Two powerful endothelial cell–derived mediators that protect against cardiovascular disease include NO produced from L-arginine via the action of three NO synthase (NOS) isoforms encoded by distinct genes, and PGI₂, a prostaglandin (PG) derived from the metabolism of the unsaturated fatty acid, arachidonic acid, by the cyclooxygenase (COX) enzymes. These molecules serve to promote vasodilation, inhibit the adherence of circulating inflammatory cells, and prevent lipid accumulation (ie, plaque formation).⁸ During inflammation, PGI₂ synthesis is reduced, and there is increased production of NO and ROS (Figure 1).

Disruption in NO Synthesis

As pathological conditions progress, a vicious cycle develops whereby increased production of ROS can disrupt the NO-producing enzymes in a blood vessel. Paradoxically, the normally beneficial NO becomes cytotoxic after chemical reactions with ROS that transform it to RNS. Like ROS, RNS can inhibit antioxidant defense mechanisms, and alter the functionality of proteins and enzymes that are essential for maintaining the balance of oxidants in a blood vessel.¹³ This disruption is referred to as endothelial dysfunction, a hallmark of many chronic blood vessel disorders that is characterized by the inability of the blood vessel to appropriately dilate in response to NO. This effect compromises the vascular equilibrium and breaks down the delicate balance of mediators that protect the vascular system.

Altered COX Metabolism

COX enzymes catalyze the conversion of the free fatty acid AA into PG precursors that are subsequently converted into

Figure 1 Under normal physiological conditions, the endothelium (EC) is responsible for local vascular tone through the synthesis of nitric oxide (NO) by endothelial NO synthase (eNOS) from arginine (Arg) and prostacyclin (PGI₂) by the cyclooxygenase (COX) pathway. NO freely diffuses across membranes and acts on guanylyl cyclase (sGC) in smooth muscle cells (SMCs), resulting in an increase in cyclic guanosine monophosphate (cGMP) levels, the activation of cGMP-dependent protein kinase G signaling, the reduction of calcium levels, and the induction of SMC relaxation. PGI₂ binds to its corresponding receptor (IP) that activates cyclic adenosine monophosphate (cAMP)-dependent protein kinase A signaling with consequent SMC relaxation. Alternatively, platelet-derived thromboxane A₂ (TxA₂) mediates vasoconstriction by binding to its receptor (TP) on SMCs, leading to phospholipase C activation and calcium release. Proinflammatory conditions catalyze the infiltration of inflammatory cells with a continuous influx of inflammatory mediators that amplify inflammation and disrupt the balance between vasodilation and vasoconstriction. Superoxide levels are elevated by metabolic reactions, and the overproduction of reactive oxygen species (ROS)/reactive nitrogen species (RNS) promote oxidative modifications that damage macromolecules and impair vascular function. AA, arachidonic acid; ATP, adenosine triphosphate; Cit, citrulline; EP, prostaglandin E₂ receptor; GTP, guanosine-5'-triphosphate; iNOS, inducible NOS; IP, prostacyclin receptor; NADPHox, nicotinamide adenine dinucleotide phosphate oxidase; oxLDL, oxidized low-density lipoprotein; PL, phospholipid; PLA₂, phospholipase A₂; PGIS, prostacyclin synthase; PKA, protein kinase A; PKG, protein kinase G; TNF- α , tumor necrosis factor- α ; TP, thromboxane receptor.

thromboxane A₂ (TxA₂), PGE₂ PGI₂, and other biologically active prostanoids that have a wide range of important regulatory roles in many organ systems. Although COX enzymes are structurally similar, they have marked physiological differences: COX-1 and COX-2 are found in almost all tissues, whereas COX-2 has enhanced protein expression levels at sites of inflammation.^{20,21} Despite their common origin, the three PG products listed above have markedly distinct effects on the body, a consequence that has been largely attributed to their site of synthesis and action. For example, TxA₂ is essential in preventing excessive bleeding,²² and PGE₂ provides critical protection to the stomach lining.²³ However, in the context of a blood vessel, TxA₂ is a potent vasoconstrictor associated with thrombus formation, whereas PGE₂ influences inflammation, atherosclerotic plaque rupture, and the formation of aneurysms.²⁴ On the other hand, a powerful vasodilator, such as PGI₂, is an inhibitor of thrombus formation and can decrease platelet adhesion to the blood vessel wall.²⁵ This difference between anti-inflammatory and proinflammatory PGs led to the development and use of selective COX-2 inhibitors that suppress inflammation with reduced gastrointestinal toxicity compared with traditional nonsteroidal anti-inflammatory drugs.²⁶ However, patients treated with COX-2 inhibitors experienced adverse cardiovascular consequences as a result of disturbing the PGI₂ and TxA₂ balance in blood vessels.²⁷ Studies have determined that targeted COX-2 inhibition can deplete vital sources of the vasodilator COX-2-derived PGI₂ in human blood vessels without restricting the

production of the vasoconstrictor COX-1-derived TxA₂. The benefit of low-dose aspirin in reducing cardiovascular disease stems from targeted COX-1 inhibition in platelets (the primary source of TxA₂ formation), without altering COX-2-derived PGI₂ production in the endothelium.²⁷ Thus, the selection of a drug for clinical use should be on the basis of the assessment of mechanisms by which PG synthesis can alter the function of the blood vessel at the local and systemic levels within the body. Cardiovascular disease can be triggered by NO deficiency or an imbalance between proinflammatory and anti-inflammatory PGs in the blood vessel wall. Although much of the research in this field has been conducted in isolated NO or COX systems, there is now substantial evidence that these pathways interact at the molecular level and should be studied together.^{28,29} Mechanisms and pathways established to date have elucidated functional changes in COX that are provoked by RNS (Figure 1). Indeed, the coexpression of COX-2 and the inducible form of NOS (iNOS) at a similar time during the course of disease, the unrestrained quantities of NO generation from iNOS, and the codistribution of COX-2, iNOS, and nitrated proteins (a RNS-induced post-translational protein modification) in blood vessel cells and at sites of inflammation in animal models and humans suggest that the two pathways interact.^{30–34} These findings have stimulated interest in the pharmacological manipulation of NO production to limit inflammatory PG synthesis.

To date, results strongly highlight the complex nature of NO chemistry, its related modifications of COX enzymes,

and associated responses in cells and tissues. We and others in the field have demonstrated that RNS interactions with COX-1 and COX-2 are divergent and can result in enzyme activation as well as inhibition.^{29,32} This can elicit two possible outcomes: maintenance of blood vessel tone via COX activation favoring PGI₂ production or vascular disease resulting from COX inhibition and disruption of PGI₂ production. These studies were extended to confirm the presence of RNS-induced COX-1 modification in human atherosclerotic tissue and its absence in nondiseased tissue. The modified COX-1 enzyme contained in atherosclerotic tissue is chemically altered by RNS and an Fe-porphyrin-driven mechanism that leads to targeted Tyr385 nitration and enzyme loss of function.^{35,36} Using a mouse model of atherosclerosis (*ApoE*^{-/-} mice on a high-fat diet), COX-1 nitration was observed in aortic atherosclerotic lesions that was absent in the *ApoE*^{-/-} mice, which were also missing the iNOS gene (*ApoE*^{-/-}/*iNOS*^{-/-} mice on a high-fat diet). This finding confirmed that iNOS-derived NO is responsible for COX nitration *in vivo*. Furthermore, nitration levels were associated with a reduction in PG production, indicating that iNOS-derived NO regulates PG biosynthesis in atherosclerotic blood vessels.³⁷ The detection of protein-incorporated tyrosine nitration (3-NT) in a variety of chronic inflammatory diseases is widely considered to be a hallmark of oxidative stress derived from NO.¹³ In an oxidizing environment, a transformation occurs in NOS activity whereby tetrahydrobiopterin, an essential cofactor of all mammalian NOS isoforms, becomes oxidized and disrupts NO production. In fact, in vascular conditions associated with tetrahydrobiopterin oxidation, endothelial NOS shifts from the production of NO to superoxide, a process that alters physiological NO availability and favors the formation of RNS. Thus, it is evident from *in vivo* studies that NOS disruption, coupled with tetrahydrobiopterin oxidation and 3-NT formation, can promote vascular injury.^{13,38,39}

Challenging the Classic Perception of Inflammation Beyond COX and NOX

There is a renewed appreciation for the actions of lipid mediators in an autocrine and paracrine manner. Eicosanoids are lipid mediators that are produced by the action of three enzymatic pathways on AA, once released from membrane phospholipids: i) COX enzymes metabolize AA to PGs, ii) lipoxygenase (LOX) enzymes metabolize AA to leukotrienes and lipoxins, and iii) cytochrome P450 (Cyt P450) enzymes metabolize AA to hydroxyeicosatetranoic and epoxyeicosatrienoic acids.⁹

Although many eicosanoids are dichotomous, they are typically associated with inflammation and disease progression. Although PGE₂ possesses anti-inflammatory and protective effects in the respiratory tract^{40–42} and in the gastrointestinal tract,²³ it is generally viewed as a proinflammatory molecule that is linked with enhanced COX-2 expression, the stimulation of matrix metalloproteinase expression, and atherosclerotic plaque rupture.⁴³ Numerous

studies have shown that reducing PGE₂ levels via enzymatic inhibition or receptor blockade may have a desirable outcome in reducing inflammation and cardiovascular disease progression.⁴⁴ However, it is now appreciated that factors that are classically known to stimulate inflammation and neutrophil influx into sites of injury may also limit the inflammatory response and signal neutrophil departure. Recent studies indicate that pathophysiology in association with lipid mediator profiles are dictated by several factors: i) a link between dietary lipid content, cell membrane composition, and lipid mediator patterns, ii) divergent biochemical properties for many lipid mediators, iii) certain cells lacking enzymatic components for synthesis of a complete panel of lipid mediators, iv) cell-cell communication and coordination of lipid mediator synthesis, and v) immune cells that display multiple functionalities during the inflammatory process.^{5,9,45–48}

During atherosclerosis, the accumulation of lipid-laden macrophages occurs in the arterial wall and this immune cell type plays a critical role during the active process of this inflammatory disease because it promotes both atherosclerosis progression and regression.^{5,49} Along with oxidant-driven endothelial dysfunction, disease progression is marked by monocyte adherence to activated endothelium, their infiltration into the vessel wall, and the subsequent formation of macrophage-derived foam cells because of the excessive uptake of oxidized low-density lipoprotein and cholesterol. This phagocytic macrophage phenotype is the prototypic host protection inflammatory response, also termed classic M1 macrophage activation.^{49–52} However, macrophages can adopt an alternate phenotype that participates in tissue repair and the restoration of homeostasis, termed M2 macrophage activation. Current advances in atherosclerosis research indicate that monocyte and macrophage subsets and their quantities influence mechanisms that contribute to the dynamics of macrophage recruitment, polarization, and their accumulation in plaque areas of the vessel wall. These studies have altered the perception that the plaque is solely derived from the continuous recruitment of monocytes; such studies have presented evidence that establishes local macrophage proliferation as an essential process that promotes inflammation and determines lesion expansion.^{53,54}

The factors listed above challenge the classic perception of oxidative inflammatory processes because they highlight adaptive responses that provide new therapeutic promises for reducing inflammation and halting disease progression and stimulation of tissue healing.

Oxidative Stress in Airway Disease

Owing to its large surface area, which is similar to blood vessels, the respiratory tract is a major target for the harmful effects of oxidant molecules. The pathogenesis of chronic airway inflammatory diseases, including COPD, is orchestrated by a variety of inflammatory mediators that include

lipid mediators, ROS, and RNS. The pathogenic mechanism of COPD is oxidative stress coupled with the infiltration of inflammatory cells into the respiratory tract. This results in airway epithelial dysfunction, airway mucous accumulation, impairment of host defense, and recurrent infection. In fact, >90% of COPD cases are caused by smoking, where patients have an accelerated loss of lung function compared with nonsmokers. Furthermore, oxidative stress from CS leads to biological changes in lung function long before clinical indications. Although CS contributes a significant burden of ROS that drives the pathogenesis of COPD, oxidative species are also generated from inflammatory and structural cells of the airways by NADPH oxidase, xanthine/xanthine oxidase system, and other enzyme systems. In fact, CS induces endothelial dysfunction that exacerbates oxidative stress and toxicity in endothelial and smooth muscle cells, which is pivotal in the pathogenesis of airway diseases.^{55–58}

The Role of NO in Airways

It is well known that NO biology is a key effector in multiple cell types within the airway, including basal cells, epithelial cells, endothelial cells, fibroblasts, macrophages, and smooth muscle cells. Endogenous NO synthesized by three NOS isoforms plays a critical role in regulating airway function and has both beneficial and detrimental effects, depending on the local formation of oxidant molecules.^{59,60} Although NOS isoforms are activated in response to either receptor-mediated calcium fluxes or phosphorylation of regulatory control sites, iNOS is a distinct isoform that is continuously active irrespective of calcium levels or phosphorylation; it is expressed in cells through transcriptional up-regulation with immunostimulants and oxidative stress.⁶¹ Endothelial NOS is expressed in endothelial cells of the bronchial and pulmonary circulation and plays a role in regulating vascular flow. Endothelial NOS is also expressed in alveolar endothelial cells, where it supplies peripheral NO, and in airway epithelial cells throughout the respiratory tract, where it regulates ciliary beating and mucociliary clearance.⁶² Elevated levels of exhaled NO have been observed in airways, with the source localized to the airway epithelium and infiltrating inflammatory cells.⁶³ Although increased expression of iNOS has been reported, the NOS isoform responsible for exhaled NO remains unconfirmed. Increased iNOS expression was also found in the bronchi of patients with COPD.^{64,65} Patients with COPD were also shown to express increased neuronal NOS protein and activity in lung tissue that correlates with disease severity and may be linked to oxidative stress.⁶⁶

As described above, endothelial dysfunction and diminished NO bioactivity occurs when the endothelium fails to serve normal physiological and protective roles. This can occur when the endothelium is damaged or when physiological responses are biochemically altered by oxidative stress settings such as CS, elevated levels of oxidized low-density lipoprotein, homocysteine, and others.^{67–69} NOS-derived NO is emerging as an important autocrine and

paracrine governor of cell proliferation. When produced at low levels, NO may accelerate cell proliferation, whereas larger pharmacological or pathophysiological levels of NO can often attenuate cell proliferation and trigger apoptosis. NO as a stimulator of cell proliferation was reported for lung epithelial cells where depletion of endogenous NO opposed proliferation by a cGMP-lowering mechanism.⁵⁹ Signal transduction mechanisms that may underlie NO-stimulated cell proliferation include the following: i) hyperactivity of the epidermal growth factor receptor, ii) up-regulation of COX-2, and iii) production of ROS that can alter NO bioavailability and favor RNS formation.^{64,65} Indeed, the pathogenesis of COPD involves numerous processes that include oxidative stress, inflammation, apoptosis, and altered cell proliferation.⁵⁵ The importance of oxidative stress and the role of oxidant species, such as superoxide, NO, and ONOO[−] (peroxynitrite), in tissue destruction is indicated in COPD by elevated protein 3-NT levels (a footprint of ONOO[−] exposure), and F_{2α}-isoprostanes (a biomarker of lipid peroxidation) in patient lungs.^{70,71} In fact, recent analysis of airway stem/progenitor basal cells from healthy smokers highlighted significant changes to molecules that are fundamental to cellular redox actions and metabolic activity. These metabolic changes are consistent with oxidant-driven disordering of airway biology before lung disease manifests clinically and offer a promise for identification of cell signaling abnormalities in early-stage disease.⁵⁸

Eicosanoid Metabolism in the Respiratory Tract

Eicosanoids play a pivotal role in both physiological and pathological processes in the lung.^{72,73} Eicosanoids with opposing actions are synthesized by many cell types in blood vessels as well as in airways. In the lung, leukotrienes (LTs) and PGs synthesized from AA can contribute to both healthy and disease states. Although altered eicosanoid synthesis involving PGs and LTs exerts proinflammatory and bronchoconstrictive effects in numerous airway pathologies, a few eicosanoids stand out for their anti-inflammatory and bronchoprotective roles. In the lung, COX-derived PGE₂ plays a crucial role as a potent vasodilator that induces its effects via its actions on two PGE₂ receptors, EP2 and EP4 (Figure 2).^{40–42} PGE₂ secretion also suppresses lung inflammation, immune responses, and fibrotic processes on the basis of its ability to limit continuous infiltration of inflammatory cells, their consequent activation, and proinflammatory mediator release of LTs from the 5-LOX pathway that can contribute to vascular remodeling and tissue damage.^{72,73} Thus, recent evidence supports a critical balance between PGs and LTs for maintaining homeostatic conditions in airways and all lung compartments (Figure 2). It can be concluded that dysregulation of PGE₂ signaling and receptor stimulation will promote lung injury. Oxidative stress exacerbates an existing imbalance in eicosanoid signaling in the lungs of COPD patients. In fact, a crucial pathological event in the rate of progression of airway obstruction in COPD is

Figure 2 The lung contains a large variety of structural and inflammatory cell types that can produce numerous lipid mediators, including prostaglandins (PGs) and leukotrienes (LTs). In the lung, as opposed to many other organ systems, prostaglandin E₂ (PGE₂) mediates lung homeostasis and has a protective role in tissue repair and limiting the immune inflammatory response (highlighted in blue). Through its actions on corresponding PGE₂ receptors (EP) in airway cells, PGE₂ stimulates wound closure in epithelial cells, induces bronchodilation in smooth muscle cells, inhibits the activation of phagocytic cells, and inhibits the proliferation of fibroblasts and lymphocytes. COX, cyclooxygenase; cPLA2, cytosolic phospholipase A2.

oxidant-driven enhanced recruitment of neutrophils, CD8⁺ T cells, and macrophages into the lung. Furthermore, a recent study by Hsiao et al.⁷⁴ reports a disruption in the metabolism and signaling pathways of SPMs in the human COPD lung as a result of cigarette smoking, a consequence that interferes with the resolution of inflammation. Although chronic CS is the main risk factor for COPD and its progression, it is now well documented that even acute exposure to CS can induce tissue damage and interfere with epithelial repair mechanisms, as evidenced by an increase in local inflammation, lipid peroxidation, and synthesis of matrix protein degradation products.⁵⁷ Detection of biomarkers during early-stage airway disease is crucial for understanding the pathobiology and for offering insight into potential therapies for improved treatment outcomes. Recently, it has been shown that serum metabolite biomarkers that correlate with lung function parameters offer a specific and sensitive prediction model for early-stage COPD.⁷⁵

Repair Mechanisms in Inflammatory Diseases and Their Clinical Relevance

The onset of an acute inflammatory response serves to protect the host from invading pathogens and harmful toxins, an

essential physiological process that is often successful at limiting injury and restoring tissue homeostasis. However, a lengthy and uncontrolled inflammatory process often results in unfavorable outcomes characterized by tissue destruction and loss of function. Therefore, understanding molecular switch points within the inflammatory process is anticipated to provide important insights into mechanisms that drive the progression and acceleration of disease and should reveal novel targets for therapeutic interventions. On the basis of recent evidence from the analysis of disease progression and regression, we can no longer assume that restoration to homeostatic levels is a passive process that is achieved by suppressing the fuels of inflammation, but rather, it is an active process where proinflammatory and anti-inflammatory pathways are linked.⁷⁶ In fact, disease fate is determined by communication and coordination between multiple cell types and their ability to shift through several phenotypes. Furthermore, new studies reveal that redox modifications of macromolecules can modulate the biosynthesis of inflammatory mediators. These findings have broad importance in the regulation of cell signaling events in response to oxidative stress and the cellular environment. Below, we discuss mechanisms with the potential to reverse oxidative stress, preserve cellular regulatory processes, and facilitate tissue repair in chronic blood vessel and airway diseases.

NO: A Regulator of Oxidative Stress

During the past decade, it has become evident that the intracellular redox environment plays a major role in the mechanisms underlying the actions of NO-dependent bioactivities (Figure 3). When produced at low physiological levels, NO is a modulator of numerous processes in the airway and vascular system (discussed above). Notably, the actions of NO are atypical in that they are not all direct. The vasodilating action of NO represents a direct mode of action and results from the covalent addition to heme-iron of soluble guanylate cyclase (Figure 1).⁷⁷ Indirect actions are determined by the chemical reactivity of NO and RNS and exert a variety of bioactivities. Cysteine S-nitrosylation (addition of NO to protein thiols) and tyrosine ring-nitration (addition of an NO₂ group) of signaling proteins are post-translational modifications that can positively or negatively regulate cellular processes.⁷⁸ NO-dependent modifications also extend to lipid groups that are capable of transducing a range of bioactivities that regulate cellular metabolism and the immune response.⁷⁹

Reversibility of NO-Induced Modifications

Many studies have confirmed that the impact of S-nitrosylation as a signal transducer of NO bioactivity stems from the fact that it is a site-specific short-lived modification that causes a change in protein activity.⁷⁸ Conversely, protein tyrosine nitration (3-NT formation) has been, for the most

part, associated with irreversible loss of protein function, and it has been established as a biomarker for oxidative damage and RNS production in chronic inflammatory conditions.¹³ However, a substantial body of evidence indicates the possibility of *in vivo* reversal of 3-NT, which was originally thought to be stable and irreversible. Denitrase activity was first observed in 1998 in Murad's laboratory, where coinubation of LPS-treated rat spleen homogenates with nitrated bovine serum albumin decreased 3-NT.⁸⁰ Since then, numerous reports demonstrated that denitrase activity is controlled, is target selective, and displays enzymatic activity.^{80–87} Thus, 3-NT formation may not solely serve as an inflammatory disease marker, but its reversibility allows for its consideration as a contributor to NO-regulated changes in protein activity in the setting of excess ROS (Figure 3).

To date, the loss of 3-NT has been evaluated and quantified by immunological and analytical techniques from many nitrated substrates in biological systems. In fact, our laboratory has used pure nitrated COX-1 (NO₂COX-1) as a denitrase substrate and a probe for evaluating the role of 3-NT solvent accessibility in determining the extent of denitration.⁸⁷ Notably, we extensively studied the structure and function of COX-1 and demonstrated that this enzyme is subjected to RNS-induced Tyr nitration in a targeted and site-selective manner.⁸⁸ Thus, it is possible that total 3-NT accumulation at a given time point represents an aggregate of two processes, nitration and denitration, which are

Figure 3 Nitric oxide (NO)-mediated and selective protein cysteine (Cys) S-nitrosylation, aromatic ring tyrosine (Tyr) nitration, and fatty acid Cys alkylation are among the reduction-oxidation-mediated modifications of macromolecules that can exert diverse bioactivities with the potential to reverse oxidative stress and preserve cellular regulatory processes. NO is derived from endothelial and neuronal NO synthase (eNOS and nNOS, respectively) under basal conditions, but large fluxes of NO are derived from increases in inducible NOS (iNOS) during inflammation. Inflammatory cells also produce excess superoxide (O₂⁻), which reacts with NO to produce reactive nitrogen species (RNS). Thus, NO and RNS induce NO-dependent modifications to biologically relevant molecules. Protein-3-NO₂ Tyr, protein 3-nitrotyrosine.

regulated by NOS metabolism. Perhaps the most pressing questions in any relevant pathogenic setting are as follows: i) which specific proteins become nitrated, ii) which specific Tyr residues are nitrated, and iii) does 3-NT reversibility contribute to disease regression? Numerous studies have set the stage for more careful determination of cellular denitration and whether this activity is a redox regulator that contributes to the restoration of impaired protein function during inflammation, where levels of ROS and RNS are elevated. However, despite the wealth of information on NO-dependent modifications and related signaling mechanisms, a persistent challenge is the availability of tools that enable the detection, identification, and quantification of these signals that are often unstable and present at low levels in cells and tissues. Additional research will open the door to a potentially important and novel area of translational research aimed at restoring redox homeostasis and cellular function.

Nitrated Fatty Acids

The interaction of NO and lipid mediator signaling pathways has been shown to exert biomodifications at the gene expression and protein levels that alter physiological quantities of many mediators that affect various inflammatory diseases. Another interaction that has manifested as a potential corrective/adaptive mechanism is the cross talk between NO and fatty acid metabolism.^{79,89} As with the oxidation of protein thiols and phenol rings by RNS, unsaturated fatty acids are also prone to oxidation by these species, resulting in the formation of nitroalkene moieties (Figure 3). In specific microenvironments, nitroalkenes act as effective electrophilic mediators that participate in rapid nonenzymatic reactions with nucleophilic groups, such as thiols. Through the generation of reversible protein adducts, nitroalkenes modulate the redox status of a cell and consequent signaling mechanisms that have been well documented to improve lipid profiles and reduce oxidative stress.^{90,91} Although the detection of nitrated fatty acids (NO₂-FA) is challenging because of their strong reactivity, the development of sensitive analytical tools has facilitated their characterization, quantification, and capture. In healthy individuals, basal levels of NO₂-FA were detected (0.7 nmol/L). However, it has been shown in experimental animal models of oxidative stress that these concentrations can increase 10-fold, which is necessary for NO₂-FA-mediated signaling events that attenuate inflammation.^{92,93}

The attenuation of cellular inflammation by the actions of NO₂-FAs is initiated for the most part by reversible protein thiol modifications. Examples include the following: i) modification of Kelch-like ECH-associated protein 1 and nuclear factor (erythroid-derived-2)-like 2 release and activation, key to up-regulation of the antioxidant response element-dependent gene products, ii) regulation of histone deacetylase activity and resultant activation of antioxidant and molecular chaperone genes heme oxygenase-1 and heat shock protein 70, iii) inhibition of the proinflammatory

NF-κB pathway via alkylation of its p65 subunit and downstream transcriptional inhibition of cytokines and their release, iv) Cys alkylation and activation of peroxisome proliferator-activated receptor-γ and the suppression of inflammatory pathways, particularly those related to lipid metabolism and obesity, v) interaction with the redox-sensitive mitochondrial peroxiredoxin system that is a crucial antioxidant defense mechanism, and vi) inhibition of the proinflammatory signal transducer and activator, STAT-1.⁹¹

Convergence of lipid and NO-mediated signaling raises important questions: Does the nutrient status of host alter cytokine and mediator production? Does NO play a role in energy balance? Can NO₂-FAs exert an adaptive anti-inflammatory signaling reaction in response to oxidative stress? Are NO₂-FAs detectable and quantifiable in a changing lipid environment during disease progression? Recent studies demonstrate that steady-state levels of NO₂-FAs and their downstream actions are modulated by prostaglandin reductase-1, a COX pathway enzyme that also mediates the conversion of 15-oxo-PGE₂ to 13,14-dihydro-15-oxo-PGE₂. Thus, it is possible that cross talk between NO₂-FAs, PGE₂, and its downstream product, 15-oxo-PGE₂, can temper COX-driven inflammation.⁸⁹ Although NO₂-FAs may emerge as NO-derived signaling mediators that coordinate both physiological and pathological events by inducing an adaptive anti-inflammatory response, it is necessary to focus on accessing the wanted target molecule via total organic synthesis. This approach will provide the framework for establishing whether NO₂-FAs and related pathways and products are operational *in vivo* in humans.

Lipid Mediators in Health

Cardiovascular disease, including atherosclerosis, is traditionally viewed as a lipid-based disorder exacerbated by Western diets that are laden with saturated fats. Moreover, substantial evidence from clinical and experimental studies indicates that lipid molecules perform crucial functions in biological processes with consequences to both the maintenance of healthy cells and the progression of disease. Lipids confer structural integrity to cell membranes, serve as an energy source, and serve as signaling molecules. Notably, lipid mediators are produced from multiple membrane lipid categories that include fatty acids, glycerophospholipids, sphingolipids, and others. Herein, we address the dysregulation of lipid mediators, their actions in inflammatory settings, and impact on repair mechanisms in this oxidative environment.

In an inflammatory milieu, the activated macrophage stands out as a producer of numerous lipid mediators and a crucial modulator of the immune response. Although classic macrophage activation (M1) is linked with the enhanced release of proinflammatory cytokines aimed at pathogen destruction, alternative macrophage activation (M2) has

been shown to promote tissue healing. Notably, studies by Robbins et al⁵³ identify a novel dynamic process in a murine model of atherosclerosis whereby the scavenger receptor A is implicated in promoting lesional macrophage proliferation and self-renewal processes. These processes likely promote inflammation via macrophage polarization to the M1 phenotype. Activation signatures and programs of M1 and M2 macrophages vary, and their functions are dependent on membrane lipid composition that are directly linked to dietary fat intake. In the context of lipid mediator biosynthesis, secretion of mediators is facilitated by the capacity for this cell type to recognize a broad range of danger signals via a wide variety of membrane pattern recognition receptors.^{94–96} Until recently, studies of lipid mediator biology were limited because methods necessitated a change in the biological markers to observe the product (ie, the use of tracers, exogenous substrates, derivatization, and/or radioimmunoassays), and compounds were measured one at a time because the technology did not permit a comprehensive analysis of the action of all lipid molecules simultaneously. Advances in the field of lipidomics have provided a means of investigating how lipid mediators interact with one another, and in different cells. They have allowed the distinction between AA-, EPA-, and DHA-derived lipid mediators, facilitated the discovery of SPMs, and enabled investigators to broadly quantify changes in expression of lipid species with diverse properties in complex biological mixtures. Thus, it is now feasible to identify and quantify these compounds with great precision and to accurately describe changes during cell metabolism.

Numerous studies have linked macrophage activation and lipid mediator signaling with proinflammatory events in pathogenesis and disease progression. For example, the COX pathway is implicated in vascular disease settings via the activity of COX-2–derived PGE₂ production and EP-4 receptor activation in macrophages as a pivotal modulator of matrix metalloproteinase expression and consequent plaque destabilization and rupture.⁹⁷ Another lipid mediator pathway is associated with airway inflammation and pathogenesis through LOX activity, which results in LT formation. In particular, M1 activation results in leukotriene B₄ (LTB₄) release among other inflammatory molecules that further attract and activate several inflammatory cell types required in host defense.⁹⁸ Notably, the COX products prostaglandins D₂ and F₂α can constrict bronchial airways (Figure 2). The net outcome is airway constriction and an amplification of the immune response that leads to airway remodeling and compromised lung function.^{99,100}

Recent studies of lipid mediator signaling have focused on *in vivo* evidence of transcellular biosynthesis and lipid mediator class switching that revealed two important cellular mechanisms: unstable intermediates can transfer between multiple cell types; it is not a requirement for a given cell type to carry the comprehensive cassette of enzymes for complete lipid mediator biosynthesis, and during inflammation, certain lipid mediators can possess a

dual role. First, they initiate host defense responses. Second, they initiate the synthesis of alternate lipid mediators that facilitate neutrophil exit, the resolution of inflammation, and the restoration of tissue homeostasis. During the 1970s, Moncada et al¹⁰¹ observed that lipid mediator products from one cell type were used by another cell type.¹⁰² They recognized that the platelet-derived intermediate PGH₂ was further converted to PGI₂ by arterial endothelium that was pretreated with pharmacological inhibitors to suppress PGH₂ formation via COX inhibition. Thus, PG intermediates from platelets transferred to endothelial cells for further downstream metabolism. In the 1990s, studies by Capra et al⁴⁸ demonstrated that neutrophils were the primary generators and donors of leukotriene A₄ (LTA₄) to neighboring cell types that did not possess 5-LOX activity. Indeed, endothelial cells and vascular smooth muscle cells lacking 5-LOX could further metabolize LTA₄ into downstream products. The literature now supports evidence for transcellular biosynthesis of lipid mediators and provides us with a new perspective on the broad range of activities that can modulate the inflammatory response (Figure 4). By studying clinical and experimental models of self-limited inflammation during the past 10 years, Serhan and colleagues^{98,103} documented an alteration in lipid mediator signaling during acute inflammation that allowed for the synthesis of nonclassical lipid products (SPMs) that oppose inflammation and facilitate tissue restoration. They showed that the inflammatory mediators PGE₂ and PGD₂ from human polymorphonuclear neutrophils also served as inducers of anti-inflammatory pathways by facilitating a switch from 5-LOX synthesis of LTB₄ to lipoxin A₄ (LXA₄) synthesis by 15-LOX. As mentioned earlier, although not all SPMs have dual anti-inflammatory and proresolution actions, lipoxins and resolvins have been shown to display both activities.¹¹ Notably, LXA₄ impedes chemotaxis and signals macrophages to clear apoptotic cellular debris. This is a pivotal protective response that controls the extent of inflammation and related outcomes. Furthermore, both dietary fatty acid content and polyunsaturated fatty acids within membrane phospholipids can influence numerous enzymatic activities, particularly in the inflammatory process. Mechanisms by which omega-3-fatty acids exert their anti-inflammatory and proresolution effects are a subject of intense research. Toward this end, we and others have shown that omega-3 fatty acids can serve as alternative COX substrates that alter enzymatic activities and consequent proinflammatory lipid mediator output.^{88,104} Newly discovered polyunsaturated fatty acid–derived SPMs include the AA-derived lipoxins as well as the EPA- and DHA-derived resolvins, protectins/neuroprotectins, and maresins (Figure 4).⁴⁶ The structural assignments and complete stereochemistry of these SPMs have been confirmed via total organic synthesis.¹⁰³ More important, their potent stereospecific actions have been demonstrated *in vivo* to be in the low nanogram to microgram dose range.¹⁰⁵ Lipoxins produced by the consecutive action of

Figure 4 Polyunsaturated fatty acid (PUFA)-driven lipid mediator biosynthesis is classically associated with the production of proinflammatory mediators, such as arachidonic acid (AA)-derived prostaglandins and leukotrienes that stimulate an inflammatory response. During inflammation, novel PUFA-derived lipid mediators are generated via class-switching that promotes anti-inflammatory and tissue healing processes after host defense activities. These dual anti-inflammatory and proresolution activities are governed by potent specialized proresolving mediators (SPMs) that are derived from AA, eicosapentanoic acid (EPA), and docosahexanoic acid (DHA). SPMs synthesized during resolution include lipoxins, resolvins, maresins, protectins, aspirin-triggered (AT) lipoxins, and AT-resolvins. COX, cyclooxygenase; LOX, lipoxygenase; oxPL, oxidized phospholipid; PGI₂, prostacyclin.

LOX from multiple cell types were the first identified proresolution lipid mediators that act on G-protein-coupled receptors.⁴⁵ Lipoxins can also be synthesized by the Cyt P450 pathway and by aspirin acetylation of COX-2 to generate aspirin-triggered lipoxins.¹⁰⁶ Thus, although aspirin is appreciated for its cardiovascular benefits via the blockade of PGE₂ and TxA₂ from COX-1, the acetylation of COX-2 and production of aspirin-triggered lipoxins in inflammatory cells provide an additional conduit by which beneficial mediators are generated.

LXA₄ was shown to inhibit LTB₄-induced leukocyte transmigration and interaction with resident cells and for signaling the clearance of apoptotic cellular debris by macrophages at sites of inflammation. During lung inflammation, this role was demonstrated in airway epithelial cells. In COPD, interference with LXA₄ levels intensifies inflammation, which highlights its corresponding receptor ALX/FPR2 as a potential therapeutic target for COPD treatment.¹⁰⁷ Notably, lipoxins and aspirin-triggered lipoxins have been shown to improve endothelial function by preventing leukocyte adhesion to vascular endothelial cells, stimulating NO production and suppressing ROS formation in endothelial cells, and by inducing the expression and release of heme-oxygenase 1, which has a protective role against oxidative injury.^{108–110} As is the case with lipoxins, the orchestrated activity between leukocytes

and COX-2 pathways in inflammatory cells can convert EPA and DHA to E-series and D-series resolvins via the intermediates 18R-hydroxyeicosapentanoic acid and 17R-hydroxydocosahexanoic acid. COX-2 acetylation by aspirin, particularly during antiplatelet therapy, incorporates these intermediates into EPA and DHA, which are then transported to neighboring leukocytes, where they exert their biological activities.

E-resolvins and their precursors were detected in healthy humans and in the vasculature from the interaction of leukocytes with endothelial cells. Their corresponding receptor ChemR23 has been detected in numerous cells and tissues, including heart, aorta, and lung. An important resolvins-relevant bioaction includes the promotion of a macrophage phenotype that actively ingests apoptotic cellular remnants. Similarly, D-series resolvins and their aspirin-triggered isomers significantly inhibit neutrophil infiltration and dampen classic macrophage activation in favor of enhanced macrophage phagocytosis *in vivo*.¹⁰³ Interestingly, D-resolvins have been shown to stimulate the production of vasoactive PGI₂ as well as NO generation and release in the vasculature.⁴⁴ This vasoprotective and anti-inflammatory cross talk between the NO and LOX pathways resembles the cross talk between NO and COX pathways discussed earlier. A recent study by Hsiao et al⁷⁴ reports a disruption in SPM metabolism in the human COPD lung induced by

long-term cigarette smoking, a consequence that interferes with the resolution of inflammation. Toward this end, although supplementation with resolvin D1 did not alter macrophage activation and polarization, this treatment significantly suppressed inflammation and it attenuated oxidative stress and cell death in a murine model of COPD. Thus, SPMs can be considered to be therapeutic mediators in chronic lung disease.⁷⁴ Collectively, SPMs play important roles in tissue regeneration, wound healing, and improvement of clinical symptoms that are associated with many inflammatory disorders.

Finally, it has been observed that altered phospholipid metabolism is associated with the pathophysiology of cardiovascular diseases and lung injury. Recently, Deeb et al⁵⁸ demonstrated that the burden of oxidants from CS significantly altered the metabolome of human airway basal cells, the stem progenitors that differentiate into other cellular components that replenish the airway epithelium under normal conditions. As expected, redox pathways were significantly perturbed in basal cells from cigarette smokers. Interestingly, liquid chromatography–mass spectrometry metabolomics indicated diminished phosphatidylcholine and lysophosphatidylcholine levels in basal cells of smokers. These phospholipids constitute a large percentage of pulmonary surfactants and are major components of cell membranes that mediate cell-signaling events. It is known that membrane phospholipids are targets of ROS, and that oxidative stress is associated with elevated levels of lipid peroxidation products that are commonly associated with inflammation and adverse biological effects. Interestingly, a recent study reported a novel class of phospholipid oxidation products that mimic SPMs in their anti-inflammatory and proresolving activity. These oxidized phospholipids are generated from glycerophosphocholine and structurally resemble 15-deoxy-prostaglandin J2, which is a known anti-inflammatory mediator.¹¹¹ Clearly, the field is moving in a direction to address and develop novel treatments for the attenuation of inflammatory processes in several organ systems.

Conclusion

In this review, we attempted to identify major repair mechanisms involved in oxidant-driven inflammatory diseases during thrombotic, atherosclerotic, and COPD states. Many of the biological response modifiers have been identified during the past 10 to 15 years that are common to all of the major organ systems. They clearly interact with one another, and participate in transcellular metabolic processes involved in repair mechanisms after an inflammatory event. The identification of novel lipid mediators and oxidative stress precursors that efficiently suppress the inflammatory response, attenuate the oxidant burden, facilitate the clearance of apoptotic cell debris, and promote the restoration of tissue integrity, highlight the potential for new

targets and treatments in a wide range of diseases that are associated with uncontrolled inflammation.

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