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Resolution, the Grail for Healthy Ocular Inflammation

Karsten Gronert

Vision Science Program, School of Optometry, University of California, Berkeley, Berkeley, CA 94720, U.S.A.

Abstract

Acute inflammation is a frequent, essential and beneficial response to maintain normal tissue function. PMN are the primary effector cells of acute inflammatory responses and their timely resolution by macrophages from an injured, stressed or infected tissues are required for the successful execution of this routine tissue response. Dysregulation of this fundamental program is a major factor in the global disease burden and contributes to many ocular diseases. Counter-regulatory signals are critical to the controlled activation of innate and adaptive immune responses in the eye and recent studies have identified two circuits in the cornea, uvea and/or retina, namely 15-lipoxygenase and heme-oxygenase, which control inflammation, promote resolution of PMN and afford neuroprotection. The role of these counter-regulator and pro-resolution circuits may provide insight into ocular inflammatory diseases and opportunities to restore stressed ocular tissue to a pre-inflammatory state, namely homeostasis, rather than limiting therapeutic options to palliative inhibition of pro-inflammatory circuits.

Keywords

Neutrophil; Macrophage; Acute Inflammation; Resolution; Heme-oxygenase; 15-Lipoxygenase; Retina; Cornea; ω -3 PUFA; Neuroprotection

1. Acute and Self-Resolving Inflammation: A Beneficial and Routine Tissue Response

Acute inflammation is an essential response to tissue injury, stress and infection. By design inflammation is a self-limiting and self-resolving response that evolved to provide constant protection and to maintain tissue integrity and homeostasis. Inflammation is a reoccurring and frequent response, especially for tissues that are in intimate and continuous contact with the external environment such as the gastrointestinal and pulmonary tract, skin, cornea and conjunctiva. Indeed in view of the constant exposure of these tissues to pathogens, irritants, toxins and allergens mucosal tissues are likely in a continual state of mild subclinical inflammation. Hence, it is essential that this routine tissue response is tightly regulated and controlled by highly evolved and overlapping circuits. This review will focus on emerging evidence for protective pathways in the eye that have been identified as central mediators of inflammatory resolution.

Correspondence: Karsten Gronert, Ph.D., Vision Science Program, School of Optometry, University of California, Berkeley, 594 Minor Hall, MC 2020, Berkeley, CA 94720-2020, Tel.: 510-642-1076, Fax: 510-643-5109, kgronert@berkeley.edu.

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A hallmark of most inflammatory responses is early PMN recruitment from the vasculature via rolling and adhesion to the activated vascular endothelium. A notable exception is lymphocyte driven auto-immune or allergic responses, however even lymphocyte or macrophage initiated inflammatory responses (e.g., asthma, rheumatoid arthritis) often trigger pronounced recruitment of vascular PMN or eosinophils to the injured tissue. Even though many tissues in the eye exhibit privileged or highly evolved immune responses to protect the delicate visual axis the successful and essential execution of acute inflammation is governed by the same general pathways, mediators and effector cells that direct inflammatory responses in other organs (Streilein, 2003; Kumar, 2004; McDermott *et al.*, 2005; Niederkorn & Stein-Streilein, 2010).

PMN are the primary line of defense against potential infection and are the most abundant leukocyte with a relative short half live and a remarkable turn over on the order of 10^{11} cells per day in humans (Kennedy & DeLeo, 2009). PMN tissue levels in humans can range from a few thousand that accumulate in healthy human tears overnight to over a billion per day in the synovial fluid of patients with rheumatoid arthritis (Sack *et al.*, 1992; Nathan & Ding, 2010). These primary effector cells of the innate immune response contain a potent arsenal of bactericidal agents, reactive oxygen species (ROS), proteolytic enzymes and chemical mediators whose deployment has to be carefully controlled to minimize collateral PMN mediated tissue injury (Kumar, 2004). Indeed exuberant or dysregulated activation of PMN can cause significant tissue and organ damage that has been linked to numerous inflammatory diseases such gout, ischemia-reperfusion injury, fibrosis, corneal melts and keratitis. More importantly, this early and transient event in the inflammatory response (i.e., PMN infiltration) triggers macrophage recruitment for the efficient removal of dead and spent PMN, a critical step in resolution of acute inflammation (Figure 1). Macrophages are important immune effector cells, master regulators and scavenger cells with a remarkable plasticity to differentiate into at least three distinct macrophage populations that drive host defense, wound healing and immune regulation (Mosser & Edwards, 2008). Nonphlogistic activation, controlled differentiation and recruitment of scavenging macrophages is especially critical for maintenance of the retina where scavenging macrophages play a critical role in the non-inflammatory removal of cell waste beneath the retinal pigment epithelial cell to prevent drusen deposition (Mosser & Edwards, 2008; Raisler *et al.*, 2008). Differentiation of macrophages is controlled by transient signals from innate immune cells and longer acting signals from antigen-specific immune cells. Hence, prolonged recruitment and activation of PMN and macrophages is precarious as it may stall inflammatory resolution and lead to PMN mediated tissue injury and protein modifications, which may promote antigen-antibody reactions and transition to chronic inflammation (Nathan & Ding, 2010).

Throughout our life the vast majority of healthy inflammatory responses, especially triggered by injury, result in the rapid recruitment of PMN to ingest and kill potential microbes and a subsequent wave of macrophages that removes apoptotic PMN and promotes wound healing (Figure 1). The resolution of the inflammatory response marks the successful protection of the compromised tissue, removal of dead cells and debris, and the return of the injured tissue to homeostasis and normal function. Unfortunately, the inflammatory program is regulated by a complex array of effector cells as well as inflammatory circuits and mediators in the epithelium, vasculature and mesenchymal cells, all of which are affected by environmental and genetic factors.

It is now recognized that non-resolving or dysregulated inflammation is a major factor in the global disease burden. Chronic inflammation and prolonged inflammation is a major feature of diseases such as atherosclerosis, obesity, diabetes, rheumatoid arthritis, asthma and some cancers. Many of these prominent diseases have well-established ocular manifestation such diabetic retinopathy, and scleritis, uveitis and dry eye syndrome in patients with rheumatoid

arthritis. More importantly, it is now appreciated that inflammation also contributes significantly to most ocular diseases including the leading cause of blindness, age-related macular degeneration (AMD). In human's polymorphism in the complement system, namely complement factor H, CFH), a major and obligatory circuit in the innate immune system of all organism has been identified as a risk factor for AMD (Edwards *et al.*, 2005; Edwards & Malek, 2007; Raisler *et al.*, 2008). Moreover, animal studies using knockout mice that are deficient in macrophage recruitment (CC-cytokine ligand 2 (Ccl2)^{-/-} and CC-cytokine receptor 2 (Ccr2)^{-/-}) recapitulate key pathologies of human AMD, namely bilateral drusen-like accumulation and progression to spontaneous choroidal neovascularization (CNV) (Ambati *et al.*, 2003; Nozaki *et al.*, 2006; Raisler *et al.*, 2008), which underscores the essential role of controlled leukocyte recruitment and activation for normal tissue function. In addition, recent reports have demonstrated that oxidative damage to the outer retina (photoreceptors), which is highly enriched in DHA, leads to the formation of a unique reactive oxidation fragment of DHA, carboxyethylpyrrole (CEP) (Hollyfield *et al.*, 2008). CEP modified proteins are more abundant in the drusen of eyes from patients with AMD and auto-antibodies against the CEP protein adducts are elevated in the blood of AMD patients. Mice immunized with CEP adducts generate CEP antibodies and develop AMD-like lesion as well as deposition of complement in the Bruch's membrane. These clinical and experimental data provide an elegant model that links the slow accumulation of oxidative damage in the retina and dysregulation of the complement system, which may initiate the pathogenesis or the pathological progression of AMD (Hollyfield, 2010; Hollyfield *et al.*, 2010).

Unraveling the cause of misdirected and dysregulated inflammation is the focus of long-standing and intense global research efforts that encompass virtually every organ system in the body. Traditionally, it was assumed that resolution of inflammation is a consequence of the winding down of pro-inflammatory pathways and the eventual removal of apoptotic PMN by macrophages. However, it is now clear that resolution of inflammation requires a delicate balance of counter-regulatory signals. These protective signals are resident in the tissue to maintain an anti-inflammatory tone and by activating pathways, which raise the threshold for activation of signal transduction and expression of pro-inflammatory circuits. Moreover, they regulate macrophage and lymphocyte differentiation and directly stimulate macrophages to phagocytose apoptotic PMN; a new paradigm that has been the subjective of recent reviews (Lawrence *et al.*, 2002; Gilroy *et al.*, 2004; Serhan & Savill, 2005; Serhan *et al.*, 2007; Gronert, 2008; Kennedy & DeLeo, 2009; Nathan & Ding, 2010). It is also clear that current treatment options for inflammatory diseases, which are primarily aimed at suppressing or inhibiting pro-inflammatory pathways, do not treat the underlying cause of the disease but merely provide palliative and temporary treatment. Specifically, if the pre-inflammatory state of the epithelium, parenchyma, extracellular matrix and vasculature are not restored as well as a resident anti-inflammatory tone, then stimulation of inflammation persists leading to continual reinitiating of the inflammatory response (Nathan & Ding, 2010) and potential autoimmune reactions.

2. Keeping Ocular Immune and Inflammatory Responses in Check: Counter-Regulatory Signals

The notion that endogenous suppression of immune response is an active process was recognized early in the field of ocular research. Spurred by observations that date back 130 years, which described the immune privilege of the anterior chamber of the eye, the anterior segment has been a pioneering model to study immune regulation (Nieder Korn & Stein-Streilein, 2010). It was initially thought that immune privilege was a feature of the anatomical isolation of the cornea and hence antigen ignorance endowed the tissue with a privileged immune response. Seminal research by Streilein and Kaplan discovered that immune privileged in the anterior segment of the eye is an active process (Kaplan *et al.*, 1975; Kaplan & Streilein,

1977; Streilein & Niederkorn, 1981; Niederkorn & Stein-Streilein, 2010). The dynamic immuno-regulation and active suppression of antigen-specific inflammation has been termed anterior chamber-associated immune deviation (ACAID) and extensively studied (Streilein, 1995, 2003; Niederkorn, 2007; Niederkorn & Kaplan, 2007; Stein-Streilein & Taylor, 2007; Niederkorn, 2009; Niederkorn & Stein-Streilein, 2010), it involves a complex regulatory circuits that connects the eye, thymus, spleen and sympathetic nervous system and depends on both T regulatory lymphocytes (Treg) and natural killer cells (NKT). This impressive body of work also uncovered what has been called a “pot-pourri” of soluble anti-inflammatory and immunosuppressive factors (Niederkorn, 2009) that endow the anterior chamber with an unusual tone of suppressive signal to inhibit initiation of immune-mediated inflammation such as antigen induced delayed-type hypersensitivity. This large array of diverse factors include: TGF- β , α -melanocyte stimulating hormone (α MSH), soluble Fas ligand (sFasL), FasL and TRAIL many of which inhibit activation or induce apoptosis of leukocytes and/or lymphocytes (Niederkorn, 2009).

However, our understanding of how acute inflammation, infection and recruited PMN resolve from an injured or stressed ocular tissue, a frequent and essential innate response, is still evolving. More importantly, only limited studies have investigated if a deficiency in counter-regulatory signals rather than hyperactivity of pro-inflammatory circuits (the current target for anti-inflammatory therapy) leads to the vicious cycle of inflammatory disease. The relevance of counter-regulator signals is underscored by the fact that highly evolved, conserved and essential components of the innate immune system, namely the complement and Toll-like receptor system, require redundant regulatory proteins as checkpoints to control activation (Liew *et al.*, 2005; Serhan *et al.*, 2007; Bora *et al.*, 2008). Soluble decoy receptors, soluble regulatory proteins, negative intracellular regulator and/or transmembrane protein regulators are critical to restrain the cytotoxic innate immune response and control regulatory T cells activation; thus they are an indispensable component of healthy self-resolving inflammation. Loss of function mutations in humans or mice have identified at least 81 genes that are required to maintain a basal anti-inflammatory tone in tissues and loss of their activity leads to spontaneous inflammation without infection, injury or antigen stimulation (Nathan & Ding, 2010), which underscores that active and resident anti-inflammatory circuits are critical checkpoints for the initiation and amplification of inflammation.

3. Pathways for Inflammatory Resolution in the Eye

Several recent studies using gene knockout mice as loss of function models have established an essential role of two prominent early response circuits in the resolution of acute inflammation in the eye. These circuits are 15-lipoxygenase (Humans: ALOX15, 15-LOX; mouse: Alox15, 12/15-LOX), which generates protective and pro-resolving lipid signals (Bazan, 2006; Kuhn & O'Donnell, 2006; Gronert, 2008; Serhan *et al.*, 2008a; Calandria *et al.*, 2009) and heme-oxygenase an essential enzyme that generates potent antioxidants and the bioactive gas carbon monoxide (Otterbein *et al.*, 2003; Bach, 2005; Ryter *et al.*, 2006; Abraham & Kappas, 2008; Gronert, 2008).

3.1. Resident Protective Lipid Mediator Circuits

Lipid autacoids are of particular interest as virtually every cell can generate specific and highly conserved bioactive lipid signals such as arachidonic acid (AA, ω -6 C20:4, eicosatetraenoic acid) derived eicosanoids. More importantly, these short-lived lipid signals are some of the earliest cellular responses to injury, infection or stress. It is thus not surprising that primary treatment options for inflammation, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, target biosynthetic enzymes (cyclooxygenase and phospholipase A₂) for the formation of eicosanoids. However, it is now recognized that several members in the eicosanoid family do not amplify or initiate inflammation but are key counter-regulatory

signals that inhibit inflammatory circuits and promote inflammatory resolution (Lawrence *et al.*, 2002; Gilroy *et al.*, 2004; Serhan *et al.*, 2007; Gronert, 2008; Serhan *et al.*, 2008a). In this regard a key enzyme in the formation of protective lipid mediators 15-LOX is the focus of ongoing research efforts. 15-LOX is one of the most inducible genes in macrophages and its expression is induced by interleukin-13 and interleukin-4 (Kuhn & O'Donnell, 2006), cytokines that induce the wound healing macrophages (M2) phenotype (Mosser & Edwards, 2008). Its essential role in maintaining tissue function is emphasized by the fact that 15-LOX is highly expressed in mucosal epithelial cells, corneal epithelium and retinal pigment epithelium. Moreover, it is intriguing that virtually all human epithelial cancers lose expression of 15-LOX indicating that the enzyme has an essential role in regulating epithelial proliferation and apoptosis (Shureiqi *et al.*, 2005; Wu *et al.*, 2008).

Even though, 15-LOX is a resident enzyme the cornea and retina its tissue levels and activity is highly upregulated by recruited 15-LOX expressing macrophages during acute inflammation or normal housekeeping activity of scavenging macrophages. 15-LOX is a key enzyme for the formation of the anti-inflammatory and pro-resolving eicosanoid, lipoxin A₄ (LXA₄), and the recently identified family of ω -3 PUFA derived lipid signals, docosahexaenoic acid (ω -3 C22:6, DHA) derived resolvins (RvD1-4) and protectins (NPD1). 15-LOX can either directly generate protective autacoids like NPD1 or it generates pivotal metabolic intermediates that are further transformed by 5-LOX (Figure 2), a prominent enzyme in macrophages and PMN, to LXA₄ or RvD1-4 (Serhan *et al.*, 2008b). These prominent endogenous lipid autacoids restrain PMN and lymphocyte activation, dendritic cell function, formation of pro-inflammatory chemokines by epithelial cells, promote clearance of apoptotic PMN by macrophages and regulate epithelial wound healing; hence, this lipid circuit is considered critical to the resolution of acute inflammation (Lawrence *et al.*, 2002; Gilroy *et al.*, 2004; Serhan *et al.*, 2007; Gronert, 2008; Serhan *et al.*, 2008b; Kenchgowda & Bazan, 2009). Enzymatic formation and structures of these oxygenated PUFA are highly conserved in most vertebrates including fish and frogs (Gronert *et al.*, 1995; Hong *et al.*, 2005; Serhan *et al.*, 2008a; Serhan *et al.*, 2008b), and it is remarkable that human pathogens such as *Pseudomonas aeruginosa* and *Toxoplasma gondii* exploit this anti-inflammatory lipid circuit to redirect host immune responses (Aliberti *et al.*, 2002a; Aliberti *et al.*, 2002b; Bannenberg *et al.*, 2004; Vance *et al.*, 2004).

3.2. The Lipoxin A₄ Circuit

A rapidly evolving field of research now established the endogenous formation and/or protective bioactions of a 15-LOX-initiated circuit in the cornea, retina and uvea (Gronert *et al.*, 2005; Bazan, 2006; Biteman *et al.*, 2007; Qin *et al.*, 2008; Bazan, 2009; Calandria *et al.*, 2009; Kenchgowda & Bazan, 2009; Cortina *et al.*, 2010; Leedom *et al.*, 2010). Direct evidence for a central role of 15-LOX in corneal inflammatory response was obtained with 12/15-LOX knockout mice, which exhibit a phenotype of impaired wound healing and amplified inflammatory responses and inflammatory neovascularization, which correlate with impaired endogenous LXA₄ formation (Gronert *et al.*, 2005; Biteman *et al.*, 2007; Leedom *et al.*, 2010). A loss of function phenotype that is rescued by topical treatment with LXA₄. In addition to LXA₄, other mouse 12/15-LOX-derived metabolites, namely, 12(S)-HETE and 15(S)-HETE, can exert beneficial actions in the cornea. 12(S)-HETE and 15(S)-HETE can function as intracellular second messengers for growth factors and modulate the proliferation of corneal epithelial cells, thereby promoting wound closure (Kenchgowda & Bazan, 2009). 15-LOX in the healthy cornea is predominantly expressed in epithelial cells (Chang *et al.*, 2005; Gronert *et al.*, 2005) as expression and activity of 15-LOX is abrogated by de-epithelialization and restored during epithelial wound healing in mice (Gronert *et al.*, 2005). More importantly, G-protein coupled receptors for LXA₄ (ALX) are expressed in both human and mouse corneas (Gronert *et al.*, 1998; Gronert *et al.*, 2005; Jin *et al.*, 2009; Leedom *et al.*, 2010). ALX has been

established as an anti-inflammatory receptor that transduces the counter-regulatory and pro-resolving bioactions of LXA₄ and DHA-derived RvD1 and the receptor is expressed in epithelial cells, PMN, macrophages and peripheral blood T lymphocytes (Chiang *et al.*, 2006; Krishnamoorthy *et al.*, 2010). Its role in inflammatory resolution was established in a gain of function model employing mice that were engineered to express the human ALX receptor in leukocytes (Devchand *et al.*, 2003). These transgenic mice in response to yeast antigen-induced peritonitis demonstrate reduced amplitude of acute inflammation and accelerated PMN resolution. It is thus of interest that 15-LOX and ALX receptor expression and activity are dynamically regulated during inflammatory responses in the cornea, which points towards a fundamental role of the LXA₄ circuit in corneal inflammatory responses (Gronert *et al.*, 2005; Jin *et al.*, 2009; Leedom *et al.*, 2010). In addition to corneal inflammatory responses the LXA₄ circuit may have a role in intraocular inflammatory diseases as recent reports demonstrate that LXA₄ treatment attenuates endotoxin-induced uveitis (EIU) (Medeiros *et al.*, 2008; Karim *et al.*, 2009). EIU is a common animal model that exhibits many of the key features of human uveitis, including dilation of vessels, macromolecule leakage, edema, and infiltration of leukocytes and lymphocytes. Topical treatment with LXA₄ greatly attenuated EIU reducing protein leakage, PMN infiltration and pro-inflammatory circuits (i.e. TNF- α , COX-2, VEGF and NF- κ B activation). The role of the 15-LOX/LXA₄ circuit in retina or conjunctiva remains to be explored.

3.3. DHA an Essential ω -3 PUFA: Precursor for Protective Mediators

In addition to AA, cells release other essential PUFAs that are substrates for 15-LOX, especially DHA. Like AA, DHA is found in all human tissues, plasma and milk at concentrations 1-20% of total fatty acids (Arterburn *et al.*, 2006). Moreover, in the cerebral cortex, sperm and retina, DHA is present at higher concentrations than AA. In particular, retinal photoreceptor outer segments have the highest DHA content of any cell and an unusual DHA-retention ability and numerous lines of evidence establish a critical role for DHA in human physiology, namely, neuronal and retinal photoreceptor functions (Bazan, 2006). Indeed, experimental evidence demonstrates decreased blood levels of DHA in various forms of retinitis pigmentosa and in animal models of inherited retinal degeneration (Bazan, 2006). Moreover, current clinical trials (i.e. NEI Age-Related Eye Disease Study) have demonstrated that dietary ω -3 PUFA intake is inversely associated with neovascular age-related macular degeneration (AMD) (SanGiovanni *et al.*, 2000; SanGiovanni & Chew, 2005; SanGiovanni *et al.*, 2007; SanGiovanni *et al.*, 2008; SanGiovanni *et al.*, 2009; Sangiovanni *et al.*, 2009), namely consumption of fish oils (eicosapentaenoic acid (EPA) and DHA) lowers progression of bilateral drusen to central geographic atrophy and neovascularization. Increased dietary intake of ω -3 PUFAs also correlated with a decreased incidence of dry eye syndrome in a well-characterized population of women participating in the Women's Health Study (Miljanovic *et al.*, 2005). These clinical findings are supported by animal studies, which show that topical treatment with α -linolenic acid (ω -3, C18:3), a precursor for essential ω -3 PUFA (EPA and DHA), reversed leukocyte infiltration and expression of TNF- α and IL-1 α in a murine model of dry eye (Rashid *et al.*, 2008). Moreover, in animal models of macular degeneration and retinopathy of prematurity diets enriched in DHA and EPA reduced the development of retinal lesions, inflammation and pathological neovascularization (Connor *et al.*, 2007; Tuo *et al.*, 2009). These and numerous other reports have identified dietary ω -3 PUFA, especially DHA, as an important factor for ocular health and inflammatory diseases (Bazan, 2006).

The anti-inflammatory, cardioprotective and immunosuppressive actions of dietary ω -3 PUFA are well established based on long standing research efforts; but the molecular mechanism for this striking actions was only recently uncovered (Bazan, 2006; Gronert & Hassan, 2007; Gronert, 2008; Serhan *et al.*, 2008a; Serhan *et al.*, 2008b). Specifically, EPA and DHA are transformed in a fashion analogous to eicosanoid biosynthesis to novel classes of ω -3 PUFA-

derived mediators. These mediators have been named resolvins and protectins as they are formed during the resolution phase of inflammation and exhibit potent anti-inflammatory, pro-resolving and neuroprotective properties. Reports from several laboratories provide compelling evidence that these protective ω -3 PUFA-derived mediators provide the elusive molecular mechanism for the remarkable beneficial and essential effects of dietary ω -3 PUFA (Lukiw *et al.*, 2005; Gonzalez-Periz *et al.*, 2006; Connor *et al.*, 2007; Gronert & Hassan, 2007; Levy *et al.*, 2007; Schwab *et al.*, 2007; Serhan *et al.*, 2008a; Bazan, 2009; Gonzalez-Periz *et al.*, 2009; Hassan & Gronert, 2009; Kenchgowda & Bazan, 2009).

In view of the abundance of DHA, especially in the retina and brain, considerable research interest has focused on novel protective DHA-derived autacoids, in particular NPD1, which regulates critical events in the acute inflammatory/reparative response, its resolution and protects neurons and retinal pigment epithelial cells against oxidative stress induced apoptosis (Gronert, 2008; Bazan, 2009; Kenchgowda & Bazan, 2009; Zhang & Bazan, 2010). It is of particular interest that this protective mediator is generated by a single enzyme, namely 15-LOX, which is highly expressed in retinal pigmented and corneal epithelial cells and wound healing macrophages. 15-LOX directly generates NPD1 (10,17-dihydroxy-DHA) and 17-hydroxy-DHA (17-HDHA) (Figure 2); the complete structure of NPD1 has been assigned (Serhan *et al.*, 2006). 17-HDHA is further metabolized by leukocyte/epithelial 5-LOX (Figure 2), in mechanism analogous to lipoxin biosynthesis, to several distinct 17S-resolvins (RvD1-4), which like NPD1 exhibit anti-inflammatory actions (Serhan *et al.*, 2008b). Hence, during acute inflammation interactions of leukocytes with epithelial cells leads to the formation of a temporally defined arrays of protective DHA-derived mediators, which are an integral component of the resolution pathway. Significant endogenous formation of these protective DHA-derived mediators (NPD1, RvD1, 17-HDHA) has been reported by several laboratories, including our group, in both human and mouse tissues (Lukiw *et al.*, 2005; Gonzalez-Periz *et al.*, 2006; Levy *et al.*, 2007; Serhan *et al.*, 2008b; Gonzalez-Periz *et al.*, 2009; Hassan & Gronert, 2009), which includes reports in the retina and cornea (Gronert *et al.*, 2005; Bazan, 2006; Connor *et al.*, 2007; Qin *et al.*, 2008). The protective DHA-derived signals have distinct but overlapping bioactions and both *in vitro* and *in vivo* experiments have demonstrated that similar to LXA₄, resolvins and protectins reduce leukocyte infiltration, regulate cytokines/chemokines and reactive oxygen species, and more importantly increase macrophage phagocytosis of apoptotic PMN (Bazan, 2006; Gronert & Hassan, 2007; Schwab *et al.*, 2007; Gronert, 2008; Zhang & Bazan, 2010). Hence, 15-LOX sets in place a resident lipid circuit that counter-regulates inflammatory circuits, contributes to the anti-inflammatory tone of the tissue and during inflammation is amplified by recruited effector cells to promote resolution of PMN.

The important role of DHA derived signals and the resident epithelial 15-LOX circuit is underscored in the retina where retinal pigment epithelial cells are essential to maintain and phagocytose photoreceptors, recycle DHA and form the critical retinal-blood barrier. Failure of RPE cells to function properly leads to photoreceptor damage or death, and consequently, impaired vision or blindness (Bazan, 2006; Zhang & Bazan, 2010). The retina is particularly prone to oxidative stress due to the high oxygen demand and blood flow in the tissue. Oxidative stress, trauma and vascular inflammation induce RPE dysfunction, which contributes to retinopathies, including pathological neovascularization and AMD. Hence, it is striking that the 15-LOX circuit is emerging as an RPE resident pathway that counteract these challenges (Bazan, 2006, 2009; Zhang & Bazan, 2010). Oxidative stress increases production of DHA-derived NPD1 in RPE cells, which inhibits oxidative stress-mediated pro-inflammatory gene induction and apoptosis, and ultimately promotes RPE cell survival. More importantly, human RPE cells deficient in 15-LOX exhibit increased sensitivity to oxidative stress-induced apoptosis, an impaired protective function that is restored selectively by treatment with NPD1. NPD1 has also been shown to inhibit retinal ganglion cell apoptosis following optic nerve

transaction, pathological retinal angiogenesis and inflammation, and promote nerve regeneration in the innervated cornea (Bazan, 2006; Connor *et al.*, 2007; Qin *et al.*, 2008; Bazan, 2009; Cortina *et al.*, 2010; Zhang & Bazan, 2010). Taken together, these recent studies demonstrate a role for 15-LOX-triggered DHA signals in maintaining and protecting the neural retina.

3.4. The Cytoprotective Heme-Oxygenase System

Heme-oxygenases (HO-1 and HO-2) constitute an essential cytoprotective system that is the rate-limiting enzyme for metabolizing essential heme and recycling iron. In the process, HO-1 generates the antioxidant biliverdin, which is converted to bilirubin, and the bioactive gas carbon monoxide. The heme-oxygenase system contains two principle enzymes: 1) HO-2, which is expressed at basal level in many healthy uninjured tissues and 2) HO-1, which is a highly inducible cytoprotective and early response gene. HO-1 amplification by genetic or pharmacological approaches causes striking anti-proliferative, anti-inflammatory and anti-apoptotic actions in many disease models (Otterbein *et al.*, 2003; Ryter *et al.*, 2006; Abraham & Kappas, 2008). HO-1 essential role in health and inflammation are underscored by the fact that human deficiencies in HO-1 and genetic deletion of HO-1 in mice leads to spontaneous chronic and non-resolving inflammation without injury or infection. Moreover, HO-1 expression is induced in macrophages specifically during the resolution phase and HO-1 mediates the anti-inflammatory actions of the key anti-inflammatory and immune regulatory cytokine IL-10 (Willis *et al.*, 2000; Willoughby *et al.*, 2000; Bach, 2005).

The HO system is constitutively expressed in the healthy cornea (HO-2) and is highly upregulated during acute and self-resolving injury responses (HO-1). Deletion of HO-2 in mice results not only in a defect of basal HO activity but also failed upregulation of HO-1 in acute inflammatory/reparative response of the cornea. In these HO deficient mice normal self-resolving inflammation and epithelial injury shifts to sustained and amplified leukocyte infiltration, failed wound healing and pronounced pathological neovascularization, hallmarks of chronic inflammation (Abraham *et al.*, 1995; Seta *et al.*, 2006; Biteman *et al.*, 2007; Patil *et al.*, 2008; Bellner *et al.*, 2009). Strikingly, the phenotype of chronic inflammation correlates with impaired formation of LXA₄ and in 12/15-LOX deficient mice (Alox15 KO) induction of HO-1 is impaired and correlates with amplified PMN infiltration, chemokine formation and delayed wound healing. The 15-LOX KO phenotype of dysregulated inflammation is rescued by adding back LXA₄, which restores normal HO-1 expression, wound healing and inflammation. Experiments with human corneal epithelial cells have confirmed that specific 15-LOX derived mediators, LXA₄, NPD1 and 17-HDHA, amplify expression of cytoprotective HO-1 (Biteman *et al.*, 2007). In addition, systemic pharmacological upregulation of HO-1 in endotoxin-induced uveitis affords effective protection against acute inflammation by reducing leukocytes in the aqueous humor and down-regulating pro-inflammatory cytokines and inducible nitric oxide (iNOS) (Ohta *et al.*, 2003). The role of the HO system in the retina and conjunctiva remains to be defined.

Taken together, these findings suggest an essential role of the HO system in ocular inflammation, which is consistent with an impressive body of work that has demonstrated that HO is a fundamental protective response to inflammation and oxidative stress as well as a key feature of inflammatory resolution (Willoughby *et al.*, 2000; Ohta *et al.*, 2003; Otterbein *et al.*, 2003; Bach, 2005; Abraham & Kappas, 2008; Gronert, 2008). Moreover, these studies as well as reports from other inflammatory models (Willis *et al.*, 2000; Willoughby *et al.*, 2000; Nascimento-Silva *et al.*, 2005) suggest an interdependence of the HO system with lipid mediators circuits that have been identified as key component of the resolution program (Lawrence *et al.*, 2002; Gilroy *et al.*, 2004; Serhan *et al.*, 2007), such as the 15-LOX/LXA₄

circuit. Dysregulation of these ocular resolution circuits may provide insight and therapeutic targets for inflammatory diseases in the visual axis.

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References

- Abraham NG, da Silva JL, Lavrovsky Y, Stoltz RA, Kappas A, Dunn MW, Schwartzman ML. Adenovirus-mediated heme oxygenase-1 gene transfer into rabbit ocular tissues. *Invest Ophthalmol Vis Sci* 1995;36:2202–2210. [PubMed: 7558713]
- Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacol Rev* 2008;60:79–127. [PubMed: 18323402]
- Aliberti J, Hieny S, Reis e Sousa C, Serhan CN, Sher A. Lipoxin-mediated inhibition of IL-12 production by DCs: a mechanism for regulation of microbial immunity. *Nature Immunology* 2002a;3:76–82. [PubMed: 11743584]
- Aliberti J, Serhan C, Sher A. Parasite-induced lipoxin A4 is an endogenous regulator of IL-12 production and immunopathology in *Toxoplasma gondii* infection. *Journal of Experimental Medicine* 2002b; 196:1253–1262. [PubMed: 12417634]
- Ambati J, Anand A, Fernandez S, Sakurai E, Lynn BC, Kuziel WA, Rollins BJ, Ambati BK. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med* 2003;9:1390–1397. [PubMed: 14566334]
- Arterburn LM, Hall EB, Oken H. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr* 2006;83:1467S–1476S. [PubMed: 16841856]
- Bach FH. Heme oxygenase-1: a therapeutic amplification funnel. *FASEB J* 2005;19:1216–1219. [PubMed: 16051687]
- Bannenberg G, Moussignac RL, Gronert K, Devchand PR, Schmidt BA, Guilford WJ, Bauman JG, Subramanyam B, Perez HD, Parkinson JF, Serhan CN. Lipoxins and novel 15-epi-lipoxin analogs display potent anti-inflammatory actions after oral administration. *British Journal of Pharmacology* 2004;143:43–52. [PubMed: 15302682]
- Bazan NG. Cell survival matters: docosahexaenoic acid signaling, neuroprotection and photoreceptors. *Trends Neurosci* 2006;29:263–271. [PubMed: 16580739]
- Bazan NG. Neuroprotectin D1-mediated anti-inflammatory and survival signaling in stroke, retinal degenerations, and Alzheimer's disease. *J Lipid Res* 2009 50:S400–405. [PubMed: 19018037]
- Bellner L, Martinelli L, Halilovic A, Patil K, Puri N, Dunn MW, Regan RF, Schwartzman ML. Heme oxygenase-2 deletion causes endothelial cell activation marked by oxidative stress, inflammation, and angiogenesis. *J Pharmacol Exp Ther* 2009;331:925–932. [PubMed: 19773531]
- Biteman B, Hassan IR, Walker E, Leedom AJ, Dunn M, Seta F, Laniado-Schwartzman M, Gronert K. Interdependence of lipoxin A4 and heme-oxygenase in counter-regulating inflammation during corneal wound healing. *FASEB J* 2007;21:2257–2266. [PubMed: 17384141]
- Bora NS, Jha P, Bora PS. The role of complement in ocular pathology. *Semin Immunopathol* 2008;30:85–95. [PubMed: 18299835]
- Calandria JM, Marcheselli VL, Mukherjee PK, Uddin J, Winkler JW, Petasis NA, Bazan NG. Selective survival rescue in 15-lipoxygenase-1 deficient retinal pigment epithelial cells by the novel docosahexaenoic acid-derived mediator, neuroprotectin D1. *J Biol Chem* 2009;284:17877–17882. [PubMed: 19403949]
- Chang MS, Schneider C, Roberts RL, Shappell SB, Haselton FR, Boeglin WE, Brash AR. Detection and Subcellular Localization of Two 15S-Lipoxygenases in Human Cornea. *Invest Ophthalmol Vis Sci* 2005;46:849–856. [PubMed: 15728540]
- Chiang N, Serhan CN, Dahlen SE, Drazen JM, Hay DW, Rovati GE, Shimizu T, Yokomizo T, Brink C. The lipoxin receptor ALX: potent ligand-specific and stereoselective actions in vivo. *Pharmacol Rev* 2006;58:463–487. [PubMed: 16968948]

- 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;13:868–873. [PubMed: 17589522]
- Cortina MS, He J, Li N, Bazan NG, Bazan HE. Neuroprotectin D1 synthesis and corneal nerve regeneration after experimental surgery and treatment with PEDF plus DHA. *Invest Ophthalmol Vis Sci* 2010;51:804–810. [PubMed: 19797230]
- 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 Journal* 2003;17:652–659. [PubMed: 12665478]
- Edwards AO, Malek G. Molecular genetics of AMD and current animal models. *Angiogenesis* 2007;10:119–132. [PubMed: 17372852]
- Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science* 2005;308:421–424. [PubMed: 15761121]
- Gilroy DW, Lawrence T, Perretti M, Rossi AG. Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov* 2004;3:401–416. [PubMed: 15136788]
- Gonzalez-Periz A, Horrillo R, Ferre N, Gronert K, Dong B, Moran-Salvador E, Titos E, Martinez-Clemente M, Lopez-Parra M, Arroyo V, Claria J. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J* 2009;23:1946–1957. [PubMed: 19211925]
- Gonzalez-Periz A, Planaguma A, Gronert K, Miquel R, Lopez-Parra M, Titos E, Horrillo R, Ferre N, Deulofeu R, Arroyo V, Rodes J, Claria J. Docosahexaenoic acid (DHA) blunts liver injury by conversion to protective lipid mediators: protectin D1 and 17S-hydroxy-DHA. *FASEB J* 2006;20:2537–2539. [PubMed: 17056761]
- Gronert K. Lipid autacoids in inflammation and injury responses: A matter of privilege. *Molecular Interventions* 2008;8:28–35. [PubMed: 18332482]
- Gronert K, Gewirtz A, Madara JL, Serhan CN. Identification of a human enterocyte lipoxin A₄ receptor that is regulated by interleukin (IL)-13 and interferon γ and inhibits tumor necrosis factor α -induced IL-8 release. *Journal of Experimental Medicine* 1998;187:1285–1294. [PubMed: 9547339]
- Gronert K, Hassan IR. Reaping the benefits of renal protective lipid autacoids. *Drug Discovery Today: Disease Mechanisms* 2007;4:3–10.
- Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado Schwartzman M. A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. *J Biol Chem* 2005;280:15267–15278. [PubMed: 15708862]
- Gronert K, Virk SM, Herman CA. Thrombocytes are the predominant source of endogenous sulfidopeptide leukotrienes in the American bullfrog (*Rana catesbeiana*). *Biochim Biophys Acta* 1995;1259:203–210. [PubMed: 8541326]
- Hassan IR, Gronert K. Acute changes in dietary omega-3 and omega-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury and formation of renoprotective docosahexaenoic acid-derived protectin D1. *J Immunol* 2009;182:3223–3232. [PubMed: 19234220]
- Hollyfield JG. Age-related macular degeneration: the molecular link between oxidative damage, tissue-specific inflammation and outer retinal disease: the Proctor lecture. *Invest Ophthalmol Vis Sci* 2010;51:1275–1281. [PubMed: 20185837]
- Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med* 2008;14:194–198. [PubMed: 18223656]
- Hollyfield JG, Perez VL, Salomon RG. A hapten generated from an oxidation fragment of docosahexaenoic acid is sufficient to initiate age-related macular degeneration. *Mol Neurobiol* 2010;41:290–298. [PubMed: 20221855]
- Hong S, Tjonahen E, Morgan EL, Lu Y, Serhan CN, Rowley AF. Rainbow trout (*Oncorhynchus mykiss*) brain cells biosynthesize novel docosahexaenoic acid-derived resolvins and protectins-Mediator lipidomic analysis. *Prostaglandins Other Lipid Mediat* 2005;78:107–116. [PubMed: 16303609]

- Jin Y, Arita M, Zhang Q, Saban DR, Chauhan SK, Chiang N, Serhan CN, Dana R. Novel Anti-inflammatory and Pro-resolving Lipid Mediators Block Inflammatory Angiogenesis. *Invest Ophthalmol Vis Sci* 2009;50:4743–4752. [PubMed: 19407006]
- Kaplan HJ, Streilein JW. Immune response to immunization via the anterior chamber of the eye. I. F. lymphocyte-induced immune deviation. *J Immunol* 1977;118:809–814. [PubMed: 321682]
- Kaplan HJ, Streilein JW, Stevens TR. Transplantation immunology of the anterior chamber of the eye. II. Immune response to allogeneic cells. *J Immunol* 1975;115:805–810. [PubMed: 239065]
- Karim MJ, Bhattacharjee P, Biswas S, Paterson CA. Anti-inflammatory effects of lipoxins on lipopolysaccharide-induced uveitis in rats. *J Ocul Pharmacol Ther* 2009;25:483–486. [PubMed: 20021273]
- Kenchgowda S, Bazan HE. Significance of lipid mediators in corneal injury and repair. *J Lipid Res* 2009;5:879–891.
- Kennedy AD, DeLeo FR. Neutrophil apoptosis and the resolution of infection. *Immunol Res* 2009;43:25–61. [PubMed: 19066741]
- 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;107:1660–1665. [PubMed: 20080636]
- Kuhn H, O'Donnell VB. Inflammation and immune regulation by 12/15-lipoxygenases. *Progress in Lipid Research* 2006;45:334–356. [PubMed: 16678271]
- Kumar, V.; Fausto, N.; Abbas, A. Robbins and Cotran Pathological Basis of Disease. W.B. Saunders Company; Philadelphia: 2004.
- Lawrence T, Willoughby DA, Gilroy DW. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nature Reviews Immunology* 2002;2:787–795.
- 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]
- Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, Israel E, Haley KJ, Serhan CN. Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J Immunol* 2007;178:496–502. [PubMed: 17182589]
- Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of toll-like receptor-mediated immune responses. *Nat Rev Immunol* 2005;5:446–458. [PubMed: 15928677]
- Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J Clin Invest* 2005;115:2774–2783. [PubMed: 16151530]
- McDermott AM, Perez V, Huang AJ, Pflugfelder SC, Stern ME, Baudouin C, Beuerman RW, Burns AR, Calder VL, Calonge M, Chodosh J, Coster DJ, Dana R, Hazlett LD, Jones DB, Kim SK, Knop E, Li DQ, Mitchell BM, Niederkorn JY, Pearlman E, Wilhelmus KR, Kurie E. Pathways of corneal and ocular surface inflammation: a perspective from the cullen symposium. *Ocul Surf* 2005;3:S131–138. [PubMed: 17216102]
- Medeiros R, Rodrigues GB, Figueiredo CP, Rodrigues EB, Grumman A Jr, Menezes-de-Lima O Jr, Passos GF, Calixto JB. Molecular mechanisms of topical antiinflammatory effects of lipoxin A(4) in endotoxin-induced uveitis. *Mol Pharmacol* 2008;74:154–161. [PubMed: 18413658]
- Miljanovic B, Trivedi KA, Dana MR, Gilbard JP, Buring JE, Schaumberg DA. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. *Am J Clin Nutr* 2005;82:887–893. [PubMed: 16210721]
- Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* 2008;8:958–969. [PubMed: 19029990]
- 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:C557–563. [PubMed: 15901601]
- Nathan C, Ding A. Nonresolving Inflammation. *Cell* 2010;140:871–882. [PubMed: 20303877]
- Niederkorn JY. Regulatory T cells and the eye. *Chem Immunol Allergy* 2007;92:131–139. [PubMed: 17264489]

- Nieder Korn JY. Role of NKT cells in anterior chamber-associated immune deviation. *Expert Rev Clin Immunol* 2009;5:137–144. [PubMed: 20046994]
- Nieder Korn JY, Kaplan HJ. Rationale for immune response and the eye. *Chem Immunol Allergy* 2007;92:1–3. [PubMed: 17264477]
- Nieder Korn JY, Stein-Streilein J. History and physiology of immune privilege. *Ocul Immunol Inflamm* 2010;18:19–23. [PubMed: 20128645]
- Nozaki M, Raisler BJ, Sakurai E, Sarma JV, Barnum SR, Lambris JD, Chen Y, Zhang K, Ambati BK, Baffi JZ, Ambati J. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci U S A* 2006;103:2328–2333. [PubMed: 16452172]
- Ohta K, Kikuchi T, Arai S, Yoshida N, Sato A, Yoshimura N. Protective role of heme oxygenase-1 against endotoxin-induced uveitis in rats. *Exp Eye Res* 2003;77:665–673. [PubMed: 14609554]
- Otterbein LE, Soares MP, Yamashita K, Bach FH. Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol* 2003;24:449–455. [PubMed: 12909459]
- Patil K, Bellner L, Cullaro G, Gotlinger KH, Dunn MW, Schwartzman ML. Heme oxygenase-1 induction attenuates corneal inflammation and accelerates wound healing after epithelial injury. *Invest Ophthalmol Vis Sci* 2008;49:3379–3386. [PubMed: 18441305]
- Qin Q, Patil KA, Gronert K, Sharma SC. Neuroprotectin D1 inhibits retinal ganglion cell death following axotomy. *Prostaglandins Leukot Essent Fatty Acids* 2008;79:201–207. [PubMed: 19019647]
- Raisler BJ, Nozaki M, Baffi J, Hauswirth WW, Ambati J. Toward a higher fidelity model of AMD. *Adv Exp Med Biol* 2008;613:185–192. [PubMed: 18188944]
- Rashid S, Jin Y, Ecoiffier T, Barabino S, Schaumberg DA, Dana MR. Topical omega-3 and omega-6 fatty acids for treatment of dry eye. *Arch Ophthalmol* 2008;126:219–225. [PubMed: 18268213]
- Ryter SW, Alam J, Choi AM. Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications. *Physiol Rev* 2006;86:583–650. [PubMed: 16601269]
- Sack RA, Tan KO, Tan A. Diurnal tear cycle: evidence for a nocturnal inflammatory constitutive tear fluid. *Investigative Ophthalmology & Visual Science* 1992;33:626–640. [PubMed: 1544788]
- SanGiovanni JP, Agron E, Clemons TE, Chew EY. Omega-3 long-chain polyunsaturated fatty acid intake inversely associated with 12-year progression to advanced age-related macular degeneration. *Arch Ophthalmol* 2009;127:110–112. [PubMed: 19139352]
- SanGiovanni JP, Agron E, Meleth AD, Reed GF, Sperduto RD, Clemons TE, Chew EY. omega-3 Long-chain polyunsaturated fatty acid intake and 12-y incidence of neovascular age-related macular degeneration and central geographic atrophy: AREDS report 30, a prospective cohort study from the Age-Related Eye Disease Study. *Am J Clin Nutr* 2009;90:1601–1607. [PubMed: 19812176]
- SanGiovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog Retin Eye Res* 2005;24:87–138. [PubMed: 15555528]
- SanGiovanni JP, Chew EY, Agron E, Clemons TE, Ferris FL 3rd, Gensler G, Lindblad AS, Milton RC, Seddon JM, Klein R, Sperduto RD. The relationship of dietary omega-3 long-chain polyunsaturated fatty acid intake with incident age-related macular degeneration: AREDS report no. 23. *Arch Ophthalmol* 2008;126:1274–1279. [PubMed: 18779490]
- SanGiovanni JP, Chew EY, Clemons TE, Davis MD, Ferris FL 3rd, Gensler GR, Kurinij N, Lindblad AS, Milton RC, Seddon JM, Sperduto RD. The relationship of dietary lipid intake and age-related macular degeneration in a case-control study: AREDS Report No. 20. *Arch Ophthalmol* 2007;125:671–679. [PubMed: 17502507]
- SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. *Pediatrics* 2000;105:1292–1298. [PubMed: 10835071]
- Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* 2007;447:869–874. [PubMed: 17568749]
- 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;21:325–332. [PubMed: 17267386]
- Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual antiinflammatory and pro-resolution lipid mediators. *Nat Rev Immunol* 2008a;8:349–361. [PubMed: 18437155]

- Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J Immunol* 2006;176:1848–1859. [PubMed: 16424216]
- Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol* 2005;6:1191–1197. [PubMed: 16369558]
- Serhan CN, Yacoubian S, Yang R. Anti-inflammatory and proresolving lipid mediators. *Annu Rev Pathol* 2008b;3:279–312. [PubMed: 18233953]
- Seta F, Bellner L, Rezzani R, Regan RF, Dunn MW, Abraham NG, Gronert K, Laniado-Schwartzman M. Heme Oxygenase-2 Is a Critical Determinant for Execution of an Acute Inflammatory and Reparative Response. *Am J Pathol* 2006;169:1612–1623. [PubMed: 17071585]
- Shureiqi I, Wu Y, Chen D, Yang XL, Guan B, Morris JS, Yang P, Newman RA, Broaddus R, Hamilton SR, Lynch P, Levin B, Fischer SM, Lippman SM. The critical role of 15-lipoxygenase-1 in colorectal epithelial cell terminal differentiation and tumorigenesis. *Cancer Res* 2005;65:11486–11492. [PubMed: 16357157]
- Stein-Streilein J, Taylor AW. An eye's view of T regulatory cells. *J Leukoc Biol* 2007;81:593–598. [PubMed: 17194735]
- Streilein JW. Unraveling immune privilege. *Science* 1995;270:1158–1159. [PubMed: 7502038]
- Streilein JW. Ocular immune privilege: therapeutic opportunities from an experiment of nature. *Nature Reviews Immunology* 2003;3:879–889.
- Streilein JW, Niederkorn JY. Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. *J Exp Med* 1981;153:1058–1067. [PubMed: 6788883]
- Tuo J, Ross RJ, Herzlich AA, Shen D, Ding X, Zhou M, Coon SL, Hussein N, Salem N Jr, Chan CC. A high omega-3 fatty acid diet reduces retinal lesions in a murine model of macular degeneration. *Am J Pathol* 2009;175:799–807. [PubMed: 19608872]
- Vance RE, Hong S, Gronert K, Serhan CN, Mekalanos JJ. The opportunistic pathogen *Pseudomonas aeruginosa* carries a secretable arachidonate 15-lipoxygenase. *Proceedings of the National Academy of Sciences of the United States of America* 2004;101:2135–2139. [PubMed: 14766977]
- Willis D, Moore AR, Willoughby DA. Heme oxygenase isoform expression in cellular and antibody-mediated models of acute inflammation in the rat. *J Pathol* 2000;190:627–634. [PubMed: 10727990]
- Willoughby DA, Moore AR, Colville-Nash PR, Gilroy D. Resolution of inflammation. *Int J Immunopharmacol* 2000;22:1131–1135. [PubMed: 11137620]
- Wu Y, Fang B, Yang XQ, Wang L, Chen D, Krasnykh V, Carter BZ, Morris JS, Shureiqi I. Therapeutic molecular targeting of 15-lipoxygenase-1 in colon cancer. *Mol Ther* 2008;16:886–892. [PubMed: 18388920]
- Zhang C, Bazan NG. Lipid-Mediated Cell Signaling Protects against Injury and Neurodegeneration. *J Nutr* 2010;140:858–863. [PubMed: 20181788]

Abbreviations

PUFA	polyunsaturated fatty acid
DHA	Docosahexaenoic Acid, ω -3 PUFA C22:4
AA	eicosatetraenoic acid, ω -6 PUFA C20:4
ALX	Lipoxin A ₄ receptor, fPRL1
HO	Heme-oxygenase
COX	cyclooxygenase, prostaglandin H synthase
LOX	lipoxygenase
PMN	polymorphonuclear leukocytes, neutrophils
CO	carbon monoxide
LXA ₄	Lipoxin A ₄

NPD1	neuroprotectin D1, protectin D1, 10R, 17S-dihydroxy-DHA
RvD	resolvin D
VEGF	vascular endothelial growth factor
TNF	tumor necrosis factor

Figure 1. Beneficial inflammation requires active resolution

Tissues experience frequent acute inflammation in response to injury, stress or infection. Recruitment of vascular PMN is initiated and amplified by carefully regulated pro-inflammatory circuits. Successful execution of acute inflammation requires phagocytosis of spent PMN by macrophages, a non-inflammatory response. Phagocytosis of PMN induces formation of anti-inflammatory mediators and differentiation to wound healing macrophages, which are essential to restore normal tissue function. Resident circuits in the cornea, uvea and retina, namely 15-LOX and/or HO produce autacoids that counter-regulate pro-inflammatory circuits, reduce PMN infiltration and activate macrophages to remove PMN. Dysregulation of this fundamental process leads to failed PMN resolution, activation of inflammatory macrophages and T-cells, setting the stage for an inflammatory disease.

Scheme illustrating the potential cellular and multi-cellular biosynthetic routes for NPD1 (10*R*,17*S*-dihydroxy-DHA), RvD1 (7*S*,8*R*, 17*S*-trihydroxy-DHA) and LXA₄ (5*S*,6*R*,15*S*-trihydroxy-LXA₄) during an acute inflammatory response in the human eye. Interaction of the cytoprotective hemoxygenase system (HO-1) with 15-lipoxygenase (15-LOX) and regulation by the LXA₄ receptor (ALX), which is also a receptor for RvD1, are indicated. Signaling by a potential NPD-1 G-protein coupled receptor is also shown. Docosahexaenoic acid (DHA, ω-3 PUFA) metabolic pathways are shown in blue and arachidonic acid (ω-6 PUFA) pathways are shown in black. Relative expression levels of 15-LOX and 5-LOX in epithelial cells, macrophages and PMN are indicated by font size.