Resolvins and protectins: mediating solutions to inflammation

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Resolution of inflammation has historically been viewed as a passive process, occurring as a result of the withdrawal of pro-inflammatory signals, including lipid mediators such as leukotrienes and prostaglandins. Thus, most anti-inflammatory drugs have traditionally targeted primarily mediator pathways that are engaged at the onset of inflammation. Only recently has it been established that inflammation resolution is an active process with a distinct set of chemical mediators. Several clinical and epidemiological studies have identified beneficial effects of polyunsaturated fatty acids (PUFAs) for a variety of inflammatory diseases, yet without mechanistic explanations for these beneficial effects. Resolvins and protectins are recently identified molecules that are generated from \( \omega-3 \) PUFA precursors and can orchestrate the timely resolution of inflammation in model systems. Dysregulation of pro-resolving mediators is associated with diseases of prolonged inflammation, so designing pharmacological mimetics of naturally occurring pro-resolving mediators offers exciting new targets for drug design. This review describes the discovery and synthesis of these novel lipid mediators, their receptors and mechanisms of action, and summarizes the studies to date that have uncovered roles for resolvins and protectins in disease states.


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Abbreviations: ATL, aspirin-triggered lipoxins; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; MS, mass spectrometry; PD, protectin; PG, prostaglandin; PMN, polymorphonuclear leukocyte; PUFA, polyunsaturated fatty acid; Rv, resolvin

Introduction

Inflammation is an essential biological process for maintenance of homeostasis and recovery from tissue injury or foreign pathogens. Prolonged inflammation, however, can be destructive and maladaptive, leading to disease and tissue destruction (Nathan, 2002). Despite many recent advances in the treatment of inflammatory disorders, mechanisms for the resolution of inflammation are still poorly understood and provide many new potential therapeutic targets in addressing diseases associated with unresolved inflammation (Gilroy et al., 2004; Serhan et al., 2008). Resolution has been well appreciated to be one of the four major outcomes for acute inflammation, along with progression to chronic inflammation, abscess development or scar formation (Cotran and Collins, 1999) (Figure 1). It was traditionally believed that resolution of inflammation was a passive process (Cotran and Collins, 1999), driven primarily by the declining levels of pro-inflammatory mediators over time and ‘fizzling out’ of the acute inflammatory response. Recent studies have demonstrated, however, that effective resolution of inflammation, including timely clearance of leukocytes and return of host stromal/parenchymal cells to a ‘non-inflammatory’ state, a process known as catabasis, is indeed an active process and is similar in complexity to the onset of inflammation (as reviewed in Serhan et al., 2004). Teleologically, the host can better control the onset and resolution of inflammatory
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Figure 1 Illustration of the potential fates for acute inflammation. Tissue injury activates the release and formation of arachidonate-derived prostaglandins and leukotrienes, which regulate early events in the inflammatory response, such as changes in blood flow, oedema and leukocyte recruitment. Specialized counter-regulatory lipid mediators, such as lipoxins, resolvins and protectins, are generated at a later time and act in a tissue-specific manner to initiate the resolution of inflammation.

Rv and PD biosynthesis – lessons learned from the lipoxins and their 15-epimers

Distinct series of pro-resolving lipid mediators are generated, depending on the parent substrate and the presence or absence of aspirin. Lxs and aspirin-triggered lipoxins (ATLs), for example, are generated in a transcellular fashion from arachidonic acid (Fiore and Serhan, 1990; Claria and Serhan, 2005). While LX biosynthesis occurs via interactions between 5-lipoxygenase (5-LOX) and either 12-LOX or 15-LOX, the generation of closely related 15-epimer-LXs occurs via interactions between 5-LOX and aspirin-modified cyclooxygenase-2 (COX-2) (Claria and Serhan, 1995). Expression of COX-2 is increased during acute inflammation (Fukunaga et al., 2005) and the ingestion of aspirin leads to acetylation of COX-2, which blocks PG formation (Samelson, 1982). Acetylated COX-2 is not catalytically inactive, converting arachidonic acid to 15R(H-EET rather than PGs (Claria and Serhan, 1995). 15R-HETE can serve as a substrate for 5-LOX for transformation to 15R-LXA4. Aspirin-triggered 15-epi-LXs are ~twofold more potent than 15R-LXs (Takano et al., 1998; Serhan and Chiang, 2008 ). Of interest, 15-epi-LXs can also be generated in the absence of aspirin by cytochrome p450-mediated generation of 15R-HETE (Claria et al., 1996).

Similar to arachidonic acid-derived LXs and 15-epi-LXs, a series of novel compounds derived from 5Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid (EPA) and 4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid (DHA), the most abundant omega-3 polyunsaturated fatty acids (PUFAs) in cold water marine fish oils, have recently been discovered in resolving marine inflammatory exudates (Serhan et al., 2000; 2002; and as reviewed in Serhan et al., 2008). These naturally occurring bioactive lipid mediators are termed Rvs (derived from...
‘resolution phase interaction products’) (Figures 2 and 3) and PDs (Figure 4). Rvs are categorized as either E-series (from EPA) or D-series (from DHA) and aspirin-triggered epimers have been identified for each family (as reviewed in Serhan and Chiang, 2008). Similar to LXs, protectin D1 (PD1) is a product of 15-LOX-mediated conversion of a PUFA substrate, in this case DHA (Hong et al., 2003; Serhan et al., 2006). Rvs and PDs possess stereospecific and potent immunoregulatory actions that are protective in vitro and in vivo (Serhan et al., 2008). Further evidence has demonstrated roles for these compounds, in particular PD1, in protection of retinal epithelial cells (Mukherjee et al., 2004; Mukherjee et al., 2007b), experimental stroke-related ischaemia-reperfusion injury (Marcheselli et al., 2003) and animal models of Alzheimer’s disease (Lukiw et al., 2005). Careful structure-function analyses for each Rv and PD1 have been determined using a murine experimental model of peritonitis (reviewed in Serhan and Chiang, 2008). Cellular and molecular actions for Rvs and PD1 are reviewed in Table 1.

Using the same COX and LOX enzymes operative during the initiation of acute inflammation in converting arachidonic acid (ω-6) to PGs and LTs, the host is able to shift the chemical mediator profile from pro-inflammatory to pro-resolving by modulating biosynthetic pathways to induce 15-LOX expression and activity to generate LXs, RVs and PD1. Disruption of this class switching process by COX-2 or LOX enzyme inhibitors or deficiency blocks the timely resolution of leukocyte clearance (Fukunaga et al., 2005; Schwab et al., 2007); demonstrating critical roles for pro-resolving COX-2 and LOX derived mediators in inflammation resolution. This experimentally derived resolution deficit can be rescued with exogenous administration of pro-resolving mediators at doses that are less than the inhibitor dose required to interrupt COX-2 and LOX enzymatic cascades (Schwab et al., 2007). In addition, animals can be protected from inflammatory diseases by overexpression of 15-LOX (Shen et al., 1996; Serhan et al., 2003) or expression of human anti-inflammatory receptors, such as the human lipoxin A4 receptor (ALX) (Levy et al., 2002; Devchand et al., 2003). These findings emphasize that the biochemical synthesis of these protective compounds is enzymatically mediated and leads to stereospecific molecules that interact with specific high affinity receptors. In this way, Rvs and PDs are distinct from auto-oxidation products of DHA and EPA, which are non-specific and can also be present during inflammation (Lee et al., 1984). The relationship between endogenous anti-inflammatory mediators and timely resolution is depicted in Figure 1.

**Identification of novel compounds via lipidomic profiling**

Rvs and PDs were initially identified by harvesting in vivo exudates during the resolution phase of acute inflammation,
defined by the time period of rapidly declining PMN cell numbers (Cotran and Collins, 1999). The mouse dorsal air pouch model was selected for isolation of these mediators because it allows for the cellular and biochemical analysis of limited, self-resolving acute inflammatory responses, facilitating the isolation and discovery of molecules involved in this spontaneous resolution of inflammation (Winyard, 2003). Mice were injected with TNF-α and exudates were collected

Figure 3  Biosynthesis of D-series resolvins (Rvs). DHA can be enzymatically converted to several distinct Rvs. (A) Similar to EPA, aspirin(ASA)-acetylated cyclooxygenase-2 or cytochrome p450 can convert docosahexaenoic acid (DHA) to a stereoselective intermediate, 17R-hydroperoxy-DHA, for subsequent enzymatic conversion to the 17R-RV D series (AT-RvD1-4). (B) Generation of RvD1-4 from DHA proceeds via a 15-lipoxygenase-mediated conversion to 17S-H(p)-DHA.

4 h later, when PMN numbers were decreasing (Serhan et al., 2000; 2002). These exudates were taken to liquid-chromatography-ultraviolet-mass spectrometry (LC-UV-MS)-based lipidomic analyses (as reviewed in Serhan et al., 2007). In tandem, multiple lipid mediator libraries with physical properties, such as M& and M/MS spectra, elution times and UV spectra were constructed to compare mediators isolated from exudates for matching purposes (Lu et al., 2005). As novel compounds were isolated, retrograde analysis was carried out using biogenic as well as total organic synthesis to identify structures and characterize stereochemistry (Serhan et al., 2000; 2002; 2006; Hong et al., 2003; Arita et al., 2005a; Sun et al., 2007). Using these methods, several classes of Rvs and PDs were identified.

**EPA-derived E-series Rvs**

Resolvin E1 (RvE1) and Resolvin E2 (RvE2), two major products in the family of EPA-derived resolvins, were originally isolated in vivo from murine dorsal air pouches treated with aspirin and EPA and were also generated in vitro from co-incubation of human endothelial cells with PMNs (Serhan et al., 2000). RvE1 is spontaneously produced in healthy subjects and levels are increased in individuals taking aspirin and/or EPA (Arita et al., 2005a). Transcellular formation of RvE1 can occur with the conversion of C20:5 to 18R-HEPE (18R-hydroxyeicosapentaenoic acid) by endothelial cells expressing COX-2 and treated with aspirin. Similar to 15(R)-HETE in 15-epi-LX formation, 18R-HEPE can be released from endothelial cells to neighboring leukocytes for subsequent conversion by 5-LOX to RvE1 via a 5(6) epoxide-containing intermediate (Serhan et al., 2000; Arita et al., 2005a) (Figure 2). This interaction is blocked by selective COX-2 inhibitors but not by indomethacin or acetaminophen (Serhan et al., 2000). Using gas chromatography-MS and liquid chromatography-tandem-MS-MS-based lipidomic analysis, the basic structure of this compound was elucidated as 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-EPA and complete stereochemistry was confirmed using total organic synthesis (Serhan et al., 2000; Arita et al., 2005a). This compound was found to be highly stereoselective both in vivo and in vitro. Synthesis of RvE1 is summarized in Figure 2.

RvE1 decreases PMN tissue accumulation by blocking human PMN transendothelial migration (Serhan et al., 2000) and facilitating apical PMN clearance from mucosal epithelial cells (Campbell et al., 2007). Other bioactions of RvE1 include attenuation of LTb2-LTBP1 pro-inflammatory signaling (NF-kB activation) (Arita et al., 2007), inhibition of PMN superoxide anion generation in response to TNFα or the bacterial surrogate peptide N-formyl-methionyl-leucyl-phenylalanine (Gronert et al., 2004), stimulation of macrophage phagocytosis of apoptotic PMNs (Schwab et al., 2007), inhibition of dendritic cell migration and cytokine release (Arita et al., 2005a; Haworth et al., 2008), and upregulation of CCR5 expression on leukocytes (Ariel et al., 2006). In addition to potent anti-inflammatory properties in acute inflammation, administration of RvE1 in a rabbit model of periodontitis also resulted in complete regeneration of damaged tissues, including bone, and normalization of systemic markers of inflammation, including C-reactive protein and IL-1β (Hasturk et al., 2007). Moreover, pre-treatment with RvE1, in contrast to molecules of the LX series, also conferred dramatic protection from inflammation-induced tissue and bone loss in periodontitis (Hasturk et al., 2006). In the murine dorsal air pouch model, RvE1 proved to be log-orders more potent than its dexamethasone or aspirin counterparts. Only nanogram amounts of RvE1 reduced leukocyte infiltration by 50–70% as compared with microgram amounts of dexamethasone or milligram amounts of aspirin (Serhan et al., 2000; Arita et al., 2005a). Recent studies have revealed that RvE1 is a potent modulator of pro-inflammatory leukocyte expression molecules, such as L-selectin, and selectively disrupts thromboxane-mediated platelet aggregation (Dona et al., 2008), adding additional...
Table 1 Molecular and cellular effects of resolvins and protectins

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<th>Parent PUFA</th>
<th>Mediator</th>
<th>Cell target</th>
<th>Action</th>
<th>Receptor</th>
<th>Reference</th>
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<tr>
<td>EPA</td>
<td>Resolvin E1</td>
<td>PMNs</td>
<td>Decreases transendothelial and transepithelial migration; attenuates BLT1 dependent pro-inflammatory signalling (NF-κB activation); reduces infiltration; protects from gastrointestinal inflammation in colitis</td>
<td>BLT1</td>
<td>(Serhan et al., 2000; Arita et al., 2007; Campbell et al., 2007) (Bannenberg et al., 2005) (Arita et al., 2005b)</td>
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<td></td>
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<td>Platelets</td>
<td>Disrupts thromboxane-mediated platelet aggregation</td>
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<td>Upregulates CCR5 expression</td>
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<td>Th17 cells</td>
<td>Decreases recruitment in response to allergen</td>
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<td>Dendritic cells</td>
<td>Inhibits migration and IL-12 production</td>
<td>ChemR23</td>
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<td>Macrophages</td>
<td>Stimulates phagocytosis of apoptotic PMNs</td>
<td>ChemR23</td>
<td>(Schwab et al., 2007)</td>
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<td>Eosinophils</td>
<td>Decreases recruitment in response to allergen</td>
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<td>EPA</td>
<td>Resolvin E2</td>
<td>PMNs</td>
<td>Decreases infiltration</td>
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<td></td>
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<td>Macrophages</td>
<td>Stimulates phagocytosis of apoptotic PMNs</td>
<td>ChemR23</td>
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<td>DHA</td>
<td>Resolvin D1</td>
<td>PMNs</td>
<td>Decreases infiltration in murine skin air pouch model; Limits infiltration in renal ischaemic injury</td>
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<td>Microglial cells</td>
<td>Inhibits IL-1β expression in vitro</td>
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<td>Promotes apoptosis in vitro</td>
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<td>Glial cells</td>
<td>Reduces cytokine production</td>
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<td>Epithelial cells</td>
<td>Protects from oxidative-stress induced apoptosis (retinal pigment epithelium)</td>
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<td>Eosinophils</td>
<td>Decreases recruitment in response to allergen</td>
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<td>DHA + ASA</td>
<td>AT-Resolvin D1</td>
<td>PMNs</td>
<td>Dose-dependent decrease in tissue infiltration</td>
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ASA, aspirin; CCR5, CC chemokine receptor 5; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PMNs, polymorphonuclear leukocytes; PUFA, polyunsaturated fatty acid.
mechanistic explanations to its anti-inflammatory and pro-resolving actions. LXA₄ and RvE1 prevent pathological inflammation that can be detrimental, but also regulate physiological inflammation that is beneficial to the host, such as in wound healing. For example, when mouse cornea undergoes thermal injury, topically applied LXA₄ or RvE1 increases the rate of re-epithelialization by 75% and decreases levels of the pro-inflammatory chemokine CXCL1 by 60% (Gronert et al., 2005). Overall, RvE1 initiates resolution of inflammation and causes decreased numbers of PMNs in exudates at earlier times than spontaneous resolution (as reviewed in Serhan et al., 2008).

Bioactive eicosanoids, such as PGs, LTs and LXs, transduce their signals via interactions with specific cognate receptors (Brink et al., 2003). Similarly, structure-activity assays suggested the presence of receptors for EPA-derived RvE1, and recently RvE1 cognate receptors were identified by screening G-protein-coupled receptors with assays of RvE1-mediated inhibition of TNF-α induced NF-κB activation as an endpoint (Arita et al., 2005a). [3H]-labeled RvE1 specifically binds to the receptor ChemR23, which shares a 36.4% homology of its amino acid sequence with ALX and is expressed on dendritic cells and monocytes (Arita et al., 2005a). In addition to blocking cytotoxic-induced NF-κB activation, RvE1 binding to leukocytes initiates specific arrays of intracellular signals, including activation of mitogen-activated protein (MAP) kinases (Arita et al., 2005a). ChemR23 was previously identified as a receptor for the peptide chemerin that also transduces anti-inflammatory signals (Cash et al., 2008). Thus, like ALX (Perretti et al., 2002), ChemR23 can interact with either lipid or peptide ligands. The specific binding of RvE1 to human PMN membrane occurs with high affinity with a Ki of 48.3 nM at 4°C. This [3H]-labeled RvE1 binding to PMN membranes can be displaced by unlabelled RvE1 (Ki = 34.3 nM), LTB₄ (Ki = 0.08 nM), as well as LTβ receptor 1 (BLT1) selective antagonist U-75302 (Ki = 1.5 nM). Interestingly, [3H]-RvE1 binding to human ChemR23 competed with homologand RvE1 (Ki = 330 nM) or chemerin peptide (Ki = 429 nM), but not with LTB₄. These findings suggest binding sites on human PMNs distinct from ChemR23 (Arita et al., 2007). RvE1 interactions at BLT1 lead to partial agonist/antagonist effects to locally dampen LTB₄-BLT1 signals on PMNs (Arita et al., 2007). Therefore, it can be concluded that RvE1 is acting via at least two distinct receptors, exerting agonist effects on LTβ-BLT1 signals on PMN activation and as a ChemR23 agonist to decrease cytokine production by dendritic cells and enhance clearance of apoptotic PMNs by macrophages.

Metabolic pathways for RvE1 inactivation have recently been uncovered and found to include enzymatic NAD-dependent dehydrogenation that is regiospecific at carbon 18 to generate 18-oxo-RvE1 (Arita et al., 2006). These RvE1 inactivation pathways are species-, tissue- and cell-type specific with different metabolites generated in the lung compared with human PMNs in peripheral blood (Arita et al., 2006). Metabolomic profiles of RvE1 have identified additional products, including 10,11-dihydro-RvE1 and 20-carboxy-RvE1. Direct comparisons of 10,11-dihydro-RvE1, 18-oxo-RvE1 and 20-carboxy-RvE1 with RvE1 indicated decreased bioactivity in vivo for the metabolites relative to RvE1, which was active at concentrations as low as 1 nM, for increasing macrophage phagocytosis (Hong et al., 2008). Regulation of this pro-resolving activity by metabolic inactivation provides tissue and cell-localized control and mechanisms for restoration of homeostasis.

RvE2 is a second member of the EPA-derived family of E-series Rvs, and shares homology to RvE1 (Tjonahen et al., 2006). The full stereochemical structure for RvE2 is 5S,18(R)-dihydroxy-eicosapentaenoic acid (Figure 2). RvE2 is synthesized by human PMNs in amounts greater than RvE1, equipotent to RvE1 when given intravenously and additive to RvE1 at low doses when given intraperitoneally (Tjonahen et al., 2006). The RvE2 receptor is yet to be molecularly characterized. RvE2 stops zymosan-induced PMN infiltration and displays potent anti-inflammatory properties in murine peritonitis (Tjonahen et al., 2006).

D-series Rvs

D-series Rvs were also initially discovered in resolving exudates of mice given DHA plus aspirin. The aspirin-triggered Rv D1 (AT-RvD1) and RvD1 are derived from DHA, involving a pathway with sequential oxygenations, initiated by 15-LOX or aspirin-acetylated COX-2, respectively, followed by 5-LOX with an epoxide containing intermediate (Figure 3). For AT-RvDs, DHA is initially converted to 17R-hydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid (17R-DHA). The stereochemistry at carbon 17 is conserved, so AT-RvDs each carry a 17R alcohol group configuration, resulting in a distinct series of AT-RVs – AT-RvD1 to AT-RvD4 (Figure 3A). In the absence of aspirin, endogenous DHA substrate is enzymatically converted in vivo to 17S alcohol containing series of Rvs (RvD1-RvD4) (Figure 3B), and triene-containing structures via a LOX-mediated mechanism (Hong et al., 2003). The complete stereochemistry of RvD1 (7S,8R,17S-trihydroxy-4Z,7Z,10Z,13Z,15E,19Z-docosahexaenoic acid) and AT-RvD1 (7S,8R,17S-trihydroxy-4Z,9E,13Z,15E,19Z-docosahexaenoic acid) was recently confirmed (Serhan et al., 2002; Sun et al., 2007). RvD1 synthesis requires a two-step enzymatic process in vivo, yet it can be generated in vitro with a one-pot single enzyme incubation (Sun et al., 2007). These products’ counter-regulatory bioactivities were determined in structure-function assays for control of peritoneal PMN infiltration (Serhan et al., 2002; Sun et al., 2007). Bioactive compounds were structurally characterized using retrograde analysis to determine the route of synthesis and origin of these compounds (Serhan et al., 2002). In murine peritonitis, RvD1 is equipotent to AT-RvD1, limiting PMN infiltration in a dose-dependent fashion (Sun et al., 2007). RvD1 is also more resistant to metabolic inactivation than LXA₄, but it is eventually converted to 17-oxo-RvD1, which is biologically inactive (Sun et al., 2007).

PDs

DHA also serves as a precursor for the biosynthesis of additional bioactive counter-regulatory lipid mediators. For (neuro)protectin D1 (N)PD1 formation, DHA is rapidly released for conversion to 17S-hydroxy-DHA that serves as a biosynthetic
precursor (Serhan et al., 2006) (Figure 4). The complete stereochemistry of PD1, the lead member of the PD family, was characterized by total organic synthesis of related isomers and matching studies with biologically derived compounds to show that PD1 (NPDI, when generated in neural tissues) is 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid (Serhan et al., 2006). The geometry of the double bonds and intermediates confirmed that, in fact, PD1 is generated enzymatically by 15-LOX via an epoxide intermediate at the 16(17) position (Hong et al., 2003; Serhan et al., 2006).

PD1 is synthesized by human peripheral blood mononuclear cells and in TH2 CD4+ T-cells in a LOX-dependent manner (Hong et al., 2003; Serhan et al., 2006). This naturally occurring autacoid has been isolated from murine exudates, murine brain cells, human microglial cells (Serhan et al., 2002) and in peripheral human blood (Hong et al., 2003). PD1 is log orders of magnitude more potent than its parent compound DHA (Hong et al., 2003; Duffield et al., 2006; Serhan et al., 2006; Levy et al., 2007a) and demonstrates tissue-specific bioactivity. These effects are in contrast to the less potent and non-selective effects of positional isomers of PD1, including 4S,17S-diHDHA or 7S,17S-diHDHA (Hong et al., 2002) and in peripheral human blood (Hong et al., 2003). In Alzheimer's disease, NPDI biosynthesis is activated by soluble amyloid protein-α (Lukiw et al., 2005). In this disorder, levels of DHA, NPDI and 15-LOX are selectively decreased in the hippocampus, providing a plausible mechanism for decreased neuroprotection in Alzheimer's disease; that being less inhibition of apoptosis and subsequently, increased neuronal cell death (Marcheselli et al., 2003; Mukherjee et al., 2004; Lukiw et al., 2005). In a placebo-controlled randomized trial, Alzheimer's disease patients treated with DHA-rich dietary supplements had reduced release of IL-1β, IL-6 and granulocyte colony-stimulating factor from peripheral blood mononuclear cells (Vedin et al., 2008). Retinal pigment epithelium cells generate NPDI when undergoing oxidative stress, allowing for up-regulation of anti-apoptotic proteins (BCL-2, BCL-XL), down-regulation of pro-apoptotic proteins (BAD and BAX) and toxic metabolite A2E-mediated apoptosis, protecting them from oxidative-stress-induced apoptosis and cell aging (Mukherjee et al., 2004; Mukherjee et al., 2007a,b). Following an ischaemic stroke, 17-R D-series Rvs are generated (in the presence of aspirin) and leukocyte infiltration and pro-inflammatory gene expression are countered by the generation of NPDI (Marcheselli et al., 2003).

Interestingly, airway mucosa is also enriched with DHA, and intriguing bioactivity for PD1 has recently been demonstrated in an experimental model of asthma (Levy et al., 2007a). Administration of PD1 (2-200 ng) intravenously to allergen-sensitized mice just prior to aerosol allergen challenge, protected the animals from the development of airway hyperresponsiveness and eosinophilic and T-cell-mediated inflammation. In this model, PD1 also dampened levels of TH2 inflammatory cytokines, pro-inflammatory lipid mediators and airway mucus (Levy et al., 2007a). These protective actions were also observed with PD1 when it was administered after airway inflammation was already established. In this setting, intravenous PD1 (20 ng) potently accelerated resolution of the allergic airway inflammation (Levy et al., 2007a). PD1’s observed in vivo effects also include decreased T-cell migration, TNF and interferon-γ signalling and promotion of T-cell apoptosis (Ariel et al., 2005).

For purposes of comparison, LX-mediated resolution of lung injury and inflammation has been investigated in several animal models (Levy et al., 2002; Fukunaga et al., 2005; Levy et al., 2007b). With murine allergic airway responses, LXS and PD1 share similar protective actions, yet several differences also exist, supporting the notion that these counter-regulatory autacoids have distinct signalling pathways. While both LXS and PD1 block IL-13 and CysLT (cysteinyl leukotriene) generation without significant effects on IL-12 concentrations in bronchoalveolar lavage fluids (BALFs), the –IC50 dose for PD1 was approximately 1 log lower than for bioactive analogs of LXA4 (Levy et al., 2002; 2007a,b). IL-5 production was reduced in BALFs by LX-stable analogs, but not by PD1 (Levy et al., 2002; 2007a,b). Of note, administration of PD1 led to decreased levels of LXA4, indicative of a LX-independent signalling pathway. In addition, PD1 decreases levels of PGD2, a key prostanooid implicated in airway responses as well as the induction of 15-LOX, and consequently, LX biosynthesis (Levy et al., 2001; 2007a). Together, these findings point to distinct pro-resolving signalling pathways for PDs and LXS. Relative to the prominent clinical asthma therapeutic montelukast, PD1, LXS, LX stable analogs and RvE1 are at least as potent and all display broader immunomodulatory actions (Levy et al., 2002; 2007a,b; Aoki et al., 2008; Haworth et al., 2008).

In addition to its role in protecting from tissue injury and inflammation in the lung (Levy et al., 2007a), similar actions for PD1 are observed in renal tissues. In response to ischaemia-reperfusion injury, murine kidneys spontaneously produce both D series Rvs and PD1 (Duffield et al., 2006), demonstrating that these compounds are endogenously generated in response to inflammation. Used as a rescue treatment when given 10 min following injury, RvD1 provides renoprotection, as evidenced by a lower peak serum creatinine value at 24 and 48 h following injury. At a cellular level, tissue leukocytes are reduced and there is inhibition of toll-like receptor-mediated macrophage activation by both RvD1 and PD1 in this model system (Duffield et al., 2006), indicating that RvD1 and PD1 can serve as ‘braking’ signals to prevent a runaway inflammatory response. Similarly to RvE1, PD1 also shifts the onset of resolution to an earlier time point than spontaneous resolution and decreases the RI for PMNs. Both RvE1 and PD1 also up-regulate CCR5 on PMNs, which acts as a ‘stop signal’ to chemokine signalling by clearing pro-inflammatory CCR5 ligands from the inflammatory milieu (Ariel et al., 2006) (Table 1).

Molecular insights to clinical observations

Since 1929, it has been established that omega-3 fatty acids are essential to health, and dietary deficiency can lead to disease (Burr and Burr, 1929). Multiple epidemiological studies that followed this initial discovery have further demonstrated that omega-3 PUFAs are dysregulated in many disease states, including atherosclerosis, cystic fibrosis, asthma, cardiovascular disease, cancer and liver injury.
In a separate analysis of these subjects’ BALFs, decreased levels of stroke in higher amounts with aspirin and dietary boxane pathways. If generated during myocardial infarction PUFA supplementation, RvE1 serves tissue-specific protective roles for catabasis.

One study of dietary supplementation with omega-3 PUFA serves to highlight the regulation of pathological inflammation as a potential point for pharmacological intervention. Of particular note, a clinically significant difference between the rate of death, myocardial infarction and stroke (Iso et al., 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these investigators, 1999).

These studies of dietary supplementation with omega-3 PUFA serves to highlight the regulation of pathological inflammation as a potential point for pharmacological intervention. Of particular note, a clinically significant difference between the rate of death, myocardial infarction and stroke among patients receiving both aspirin and omega-3 PUFA versus controls was reported in a randomized controlled study conducted by the Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto miocardico (GISSI group (GISSI investigators, 1999). There was no mechanistic explanation for the beneficial effect of aspirin in the presence of these PUFA in this study. Given recent insights, the findings of the GISSI trial point to aspirin-triggered formation of RvE1 from EPA and DHA in addition to its inhibition of PG and thromboxane pathways. If generated during myocardial infarction or stroke in higher amounts with aspirin and dietary omega-3 PUFA supplementation, RvE1 would serve tissue-specific protective roles for catabasis.

In addition to cardiovascular, renal, hepatic and neural tissues, EPA and DHA may also serve important protective functions in injured and inflamed lung. A recent trial of enteric supplementation with a low-carbohydrate, high-fat diet enriched with EPA led to substantial improvement in clinical outcomes in the acute respiratory distress syndrome (ARDS) (Gadek et al., 1999). Patients receiving the intervention diet had significant decreases (approximately 2.5-fold) in total cells and PMNs per ml BALF, and significant improvements in oxygenation, mechanical ventilator support and length of stay in the intensive care unit (Gadek et al., 1999). In a separate analysis of these subjects’ BALFs, decreased levels of IL-8 and LTB4 were identified and related to the decreased BALF PMNs and alveolar membrane protein permeability with the EPA-enriched enteral diet (Pacht et al., 2003). These exciting findings from a single centre are now being investigated in multiple sites as part of the EDEN-Omega ARDSnet trial. EPA-derived RvE1 have not been studied in these patients, but RvE1 displays potent protective actions in airway inflammation (Aoki et al., 2008; Haworth et al., 2008) and RvE2 is a potent regulator of PMN functional responses (Tjonahen et al., 2006). Airway tissue concentrations of DHA are decreased in absolute mass and relative to arachidonic acid in other diseases of pathologic airway inflammation, including asthma and cystic fibrosis (Freedman et al., 2004). Recently, PD1 and its biosynthetic precursor 17S-hydroxy-DHA were identified in exhaled breath condensates from human subjects with decreased levels of PD1 produced during asthma exacerbations (Levy et al., 2007a). Together with results for RvE1 and PD1 in murine models of asthma, these findings support roles for EPA- and DHA-derived compounds in protection from excessive airway inflammation in several lung diseases.

If RvE1 and PD1 are the missing link to explain the salutary effects of dietary supplementation with EPA and DHA, then individual metabolism of these fatty acids could account for the variability observed in clinical studies that have had difficulty establishing benefit in oral supplementation with omega-3 fish oils (Van Biervliet et al., 2008). Gene by diet interactions has been established for omega-6 supplementation and risk for atherosclerosis (Dwyer et al., 2004). The important role that genetic variation can play in PUFA metabolism is emphasized in recent studies with FAT-1 transgenic mice. Compared with control animals, mice that are engineered to overexpress fatty acid desaturase produce higher levels of EPA and DHA plus increased amounts of RvE1 and PD1 when challenged with a pro-inflammatory stimulus (Hudert et al., 2006). These mice are relatively protected from gastrointestinal inflammation in colitis (Hudert et al., 2006) and demonstrate less tumour metastasis from melanoma (Xia et al., 2006).

**Mimetics as emerging therapeutic targets for drug design in inflammatory diseases**

Because they are endogenously generated compounds with protective actions, RvE1 and PD1 serve as rational templates for the design of molecular mimetics as potential therapeutic agents. Historically, the emphasis in development of anti-inflammatory drugs has been largely on interfering with the pro-inflammatory signalling cascade. Only recently has the focus shifted to augmenting naturally occurring pro-resolution responses in the host. As more counter-regulatory chemical mediators are isolated and characterized, newer pharmacological targets emerge for drug design of stable pro-resolving mimetics. Similar to the development of aspirin-triggered LXA4, stable analogs (ATLa) (Takano et al., 1997; Schottelius et al., 2002), an RvE1 stable analog has been designed and synthesized by chemical modification at the omega-end of RvE1 (Arita et al., 2006). The analog, 19-(p-fluorophenoxy)-RvE1 methyl ester, carries bioequivalence to native RvE1 in reducing pro-inflammatory cytokines and leukocyte numbers in murine periodontitis, and is highly resistant to omega-oxidation or rapid dehydrogenation (Arita et al., 2006).

These and other bioactive mimetics that resist metabolic inactivation are currently in development. The exciting possibility exists that this approach to drug development will

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lead to a new genus of pharmacological agents that serve as mimetics of natural resolution pathways (Serhan and Chiang, 2008). At this juncture, more research is needed on these new pro-resolving chemical mediators and their mechanisms of action to learn whether RVs and PDs will ultimately provide long sought-after solutions to resolving pathological inflammation.

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Statement of conflict of interest

BDL is an inventor on patents for the lipoxins, resolvins and protectins and their analogs that are assigned to Brigham and Women’s Hospital and have been licensed for clinical development. PK has no conflict of interest to disclose.

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