

## REVIEW

# Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus

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Complete resolution of an acute inflammatory response and its return to homeostasis are essential for healthy tissues. Here, we overview ongoing efforts to characterize cellular and molecular mechanisms that govern the resolution of self-limited inflammation. Systematic temporal analyses of evolving inflammatory exudates using mediator lipidomics-informatics, proteomics, and cellular trafficking with murine resolving exudates demonstrate novel endogenous pathways of local-acting mediators that share both anti-inflammatory and pro-resolving properties. In murine systems, resolving-exudate leukocytes switch their phenotype to actively generate new families of mediators from major omega-3 fatty acids EPA and DHA termed resolvins and protectins. Recent advances on their biosynthesis and actions are reviewed with a focus on the E-series resolvins (RvE1, RvE2), D series resolvins (RvD1, RvD2) and the protectins including *neuroprotectin* D1/protectin D1 (NPD1/PD1) as well as their aspirin-triggered epimeric forms. Members of each new family demonstrate potent stereo-specific actions, joining the lipoxins as endogenous local signals that govern resolution and endogenous anti-inflammation mechanisms. In addition to their origins and roles in resolution biology in the immune system, recent findings indicate that these new mediator families also display potent protective actions in lung, kidney, and eye as well as enhance microbial clearance. Thus, these endogenous agonists of resolution pathways constitute a novel genus of chemical mediators that possess pro-resolving, anti-inflammatory, and antifibrotic as well as host-directed antimicrobial actions. These may be useful in the design of new therapeutics and treatments for diseases with the underlying trait of uncontrolled inflammation and redox organ stress.

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**Keywords:** leukocytes; eicosanoids; resolvins; acute inflammation;  $\omega$ -3 fatty acids; protectins

**Abbreviations:** 10S,17S-diHDHA, double dioxygenase product of DHA; AA, arachidonic acid; ATL, aspirin-triggered lipoxin A<sub>4</sub> (5S,6R,15R-trihydroxyl-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid); DHA, docosahexaenoic acid; EPA, eicosa-pentaenoic acid; LC-MS-MS, liquid chromatography-tandem mass spectrometry; LOX, lipoxygenase; LXA<sub>4</sub>, lipoxin A<sub>4</sub> (5S,6R,15S-trihydroxyl-7,9,13-*trans*-11-*cis*-eicosatetraenoic acid); NPD1, neuroprotectin D1; PD1 generated in neural systems; PD, Protectins, the family of DHA-derived mediators possessing a conjugated triene structure as a distinguishing feature; NPD1/PD1, protectin D1 (10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid); PMN, polymorphonuclear leukocyte; PUFA, polyunsaturated fatty acids; Rv, resolvins, resolution phase interaction products carrying bioactivity; RvD1, resolvin D1 (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid); RvD2, resolvin D2 (7S,16R,17S-trihydroxy-docosa-4Z,8E,10Z,12E,14E,19Z-hexaenoic acid); RvE1, resolvin E1 (5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid); RvE2, resolvin E2 (5S,18-dihydroxyeicosapentaenoic acid)

## Introduction

Acute inflammation has several outcomes that include progression to chronic inflammation, scarring and fibrosis or complete resolution (Cotran *et al.*, 1999). Resolution was defined at the histologic/tissue level and was thought of as a

passive process *in vivo* as, for example, with the dwindling with time of chemotactic stimuli at the site of inflammation. With the isolation of endogenous anti-inflammatory and pro-resolving mediators and their characterization, it became clear that resolution is an active process involving biochemical circuits that actively biosynthesize local mediators within the resolution phase, such as the resolvins (Serhan, 1997; Serhan *et al.*, 2000, 2002). The resolution phase has emerged as a new terrain for drug design and resolution-directed therapeutics (Gilroy *et al.*, 2004; Lawrence

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*et al.*, 2005). Resolution by precise definition is not the same as endogenous anti-inflammation. For example, a pro-resolving small molecule can, in addition to serving as an agonist of anti-inflammation, also promote the uptake and clearance of apoptotic neutrophils (polymorphonuclear leukocyte, PMN) from the site of inflammation by macrophages (Maderna and Godson, 2005; Serhan, 2005). A recent consensus report from investigators at the forefront of this emerging area has addressed these definitions to help delineate this new terrain (Serhan *et al.*, 2007). Moreover, because many currently used drugs were developed without an appreciation of their potential impact in resolution, some agents such as the widely used COX-2 inhibitors proved to be resolution toxic (Gilroy *et al.*, 1999; Bannenberg *et al.*, 2005; Serhan *et al.*, 2007), whereas others can possess pro-resolving actions, such as glucocorticoids (Rossi and Sawatzky, 2007), cyclin-dependent kinase inhibitors (Rossi *et al.*, 2006) and aspirin (Serhan *et al.*, 1995; Serhan, 2007).

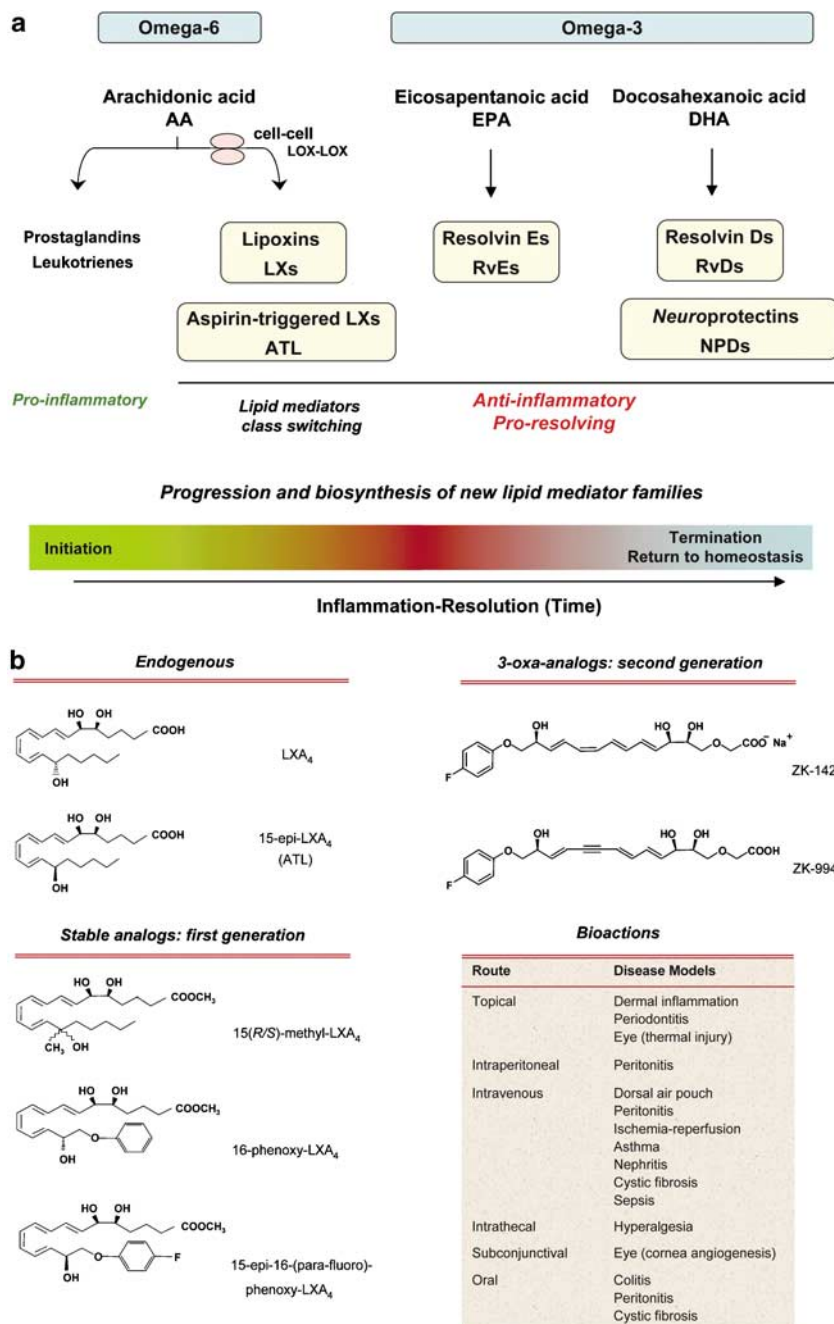
Interest in natural resolving mechanisms has been heightened in recent years (Henson, 2005; Luster *et al.*, 2005; Serhan and Savill, 2005) because inflammation (characterized by the cardinal symptoms dolor, calor, rubor and loss of function) is now recognized as a central feature in the pathogenesis of many prevalent diseases in modern Western civilization, such as atherosclerosis (Libby, 2002), cancer (Coussens and Werb, 2002), asthma (Barnes, 1999), autoimmune disease, and various neuropathological disorders including stroke, Alzheimer's and Parkinson's diseases (Majno and Joris, 1996; Nathan, 2002; Erlinger *et al.*, 2004; Hansson *et al.*, 2006). Resolution of inflammation is required for the return from inflammatory disease to health, that is, catabasis (Bannenberg *et al.*, 2005). New evidence from this laboratory and others indicates that the catabasis from inflammation to the 'normal' noninflamed state is not merely passive termination of inflammation but rather an actively regulated program of resolution (Serhan *et al.*, 2007). This event is accompanied by lipid mediator class switching from pro-inflammatory prostaglandins (PGs) and leukotrienes (LT) to the biosynthesis of anti-inflammatory mediators, such as lipoxins (LXs) (Levy *et al.*, 2001), as well as the appearance of new families of pro-resolving mediators biosynthesized in exudates from  $\omega$ -3 polyunsaturated fatty acid (PUFA) precursors (Serhan *et al.*, 2000, 2002; Hong *et al.*, 2003) (Figure 1a).

The essential roles of omega-3 PUFAs in preventing disease in rodents were established in 1929 (Burr and Burr, 1929). In humans, the mechanism of action underlying the many decades of reports since Burr and Burr (1929) showing beneficial actions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the major omega-3 PUFA, remains a topic of interest and considerable discussion (Lands, 1987; Bazan, 1990; Simopoulos *et al.*, 1999; Salem *et al.*, 2001) because structure-activity relationships remained to be established. Hence, elucidating the molecular mechanisms of DHA- and EPA-reported beneficial actions is an important challenge for molecular and translational medicine. One prevailing and popular theory suggests that the omega-3 PUFA compete with the storage of arachidonic acid (AA), replacing it and blocking the production of pro-

inflammatory eicosanoids (Lands, 1987). However, none of the new mediators reviewed here and their actions were known at the time. Along with the pro-inflammatory PGs and LT, the  $n$ -6 essential fatty acid AA is precursor to LX and aspirin-triggered LX (Figure 1), which possess potent anti-inflammatory and pro-resolving actions (Table 1). These findings emphasize that the popular view of essential  $n$ -6 and  $n$ -3 PUFA actions in inflammation and homeostasis was incomplete.

In this review, we summarize the evidence available to date that indicates that  $\omega$ -3 PUFAs are precursors to potent novel mediators that are enzymatically generated and identified in the resolving inflammatory exudates (Serhan *et al.*, 2000, 2002; Hong *et al.*, 2003) and are also neuroprotective (Marcheselli *et al.*, 2003; Mukherjee *et al.*, 2004). The term resolvins (resolution-phase interaction products) was first introduced to signify that the new structures were endogenous mediators possessing potent anti-inflammatory and immunomodulatory actions demonstrated in the nanogram dose range *in vivo* (Serhan *et al.*, 2002). These include reducing neutrophil traffic and pro-inflammatory cytokines, as well as lowering the magnitude of the inflammatory response *in vivo* (Serhan *et al.*, 2000, 2002). The terms protectin and neuroprotectin (when generated in neural tissues) (Serhan *et al.*, 2006a) were introduced given the anti-inflammatory (Hong *et al.*, 2003) as well as the protective actions of the DHA-derived mediator NPD1/PD1 in neural systems (Mukherjee *et al.*, 2004), stroke (Marcheselli *et al.*, 2003) and Alzheimer's disease (Lukiw *et al.*, 2005).

The earlier oxygenated products characterized as being formed from  $\omega$ -3 PUFA are similar in structure to known AA-derived eicosanoids, but either devoid of bioactions or far less potent than their AA counterparts (for examples, see Lee *et al.*, 1984). This sharply contrasts the biosynthesis and actions of specific members of the resolvins and protectin families that carry potent biological actions demonstrable in the nanomolar and picomolar ranges *in vitro* and *in vivo* (Serhan *et al.*, 2000, 2002; Hong *et al.*, 2003; Marcheselli *et al.*, 2003; Mukherjee *et al.*, 2004). In this review, we summarize the bioactivities of pro-resolving mediators and underlying biochemical pathways of the  $\omega$ -3 PUFA-derived mediators. These include (1) resolvins of the E series (resolvin E1 or RvE1) from EPA, (2) resolvins of the D series (resolvin D1 or RvD1) and their aspirin-triggered (AT) epimeric forms from DHA and (3) the neuroprotectins/protectins (PDs) from DHA. These novel mediators are endogenous agonists controlling inflammation via stimulating resolution. These novel endogenous anti-inflammatory and pro-resolving mediators provide the biotemplates to produce stable analogs and mimetics as in Serhan *et al.* (1995); together with the LXs they constitute a pharmacologic genus of pro-resolving and anti-inflammatory molecules. Armed with a quantitative set of resolution indices (Bannenberg *et al.*, 2005) and a firm consensus of the cellular, molecular and tissue events and precise terms defining resolution from leading scholars in the area (Serhan *et al.*, 2007), we may find this new genus of pro-resolving agonists of resolution to be a useful therapeutic approach to treat many diseases characterized by uncontrolled inflammation in their pathogenesis and progression.



**Figure 1** New families of PUFA-derived lipid mediators and their roles in the progression of acute Inflammation. (a) Specialized chemical mediators and signals appear to be programmed at the tissue level to actively participate in leukocyte responses (PMN, monocytes and macrophages) required for resolution. The chemical mediators are involved in the initiation of acute inflammation, such as PGs and LT. With time, a class switching occurs toward pro-resolving lipid mediators. These mediators include (1)  $\omega$ -6 PUFA arachidonic acid (AA)-derived lipoxins (LXs) and aspirin-triggered LXs (ATL); (2)  $\omega$ -3 PUFA eicosapentaenoic acid (EPA)-derived resolvin E-series (RvEs); (3) docosahexaenoic acid (DHA)-derived resolvin D-series (RvDs) and protectins (PDs). PDs are also termed neuroprotectins when generated in neural tissues. LXA<sub>4</sub>, ATL, resolvins and protectins share anti-inflammatory and pro-resolving properties, yet have distinct actions within the resolution orchestra. (b) Structures of LXA<sub>4</sub>, ATL and their representative stable analogs (Bannenberg *et al.*, 2004; Chiang *et al.*, 2006). For detailed bioactions in disease models, see Table 1.

## Endogenous mediators in initiating inflammation and its resolution

The role of chemical mediators in inflammation became apparent with the discovery of the PGs, named by the

Swedish laureate von Euler (for recent review, see Flower, 2006), and LT, from seminal studies of Borgeat *et al.* (1976). Bergström (1982), Samuelsson and colleagues established in earlier studies that AA is transformed into many potent bioactive compounds such as PGs, LT, and the more recent

**Table 1** *In vivo* actions of LXA<sub>4</sub> and aspirin-triggered ATL

Disease model	Action(s)	Dosage (route)	Duration of postexposure	References
Dermal inflammation	Inhibit neutrophil recruitment into ear skin and prevent vascular permeability	240 nmol (topical) 20 µg cm <sup>-2</sup>	8 h	Takano <i>et al.</i> (1997, 1998) Bannenberg <i>et al.</i> (2004) Schottelius <i>et al.</i> (2002)
Skin (dorsal air pouch)	Block TNF-α induced leukocyte recruitment Block P gingivalis-elicited leukocyte infiltration and lower PGE <sub>2</sub> levels within exudates	10 µg (i.v.) 10 µg (intrapouch)	4 h 4 h	Clish <i>et al.</i> (1999) Pouliot <i>et al.</i> (2000)
I/R injury	Attenuate hindlimb I/R-induced lung injury	10 µg (i.v.) 500 µg kg <sup>-1</sup> (i.v.)	3 h	Chiang <i>et al.</i> (1999) Bannenberg <i>et al.</i> (2004)
	Detachment of adherent leukocytes in mesenteric I/R	10 nmol l <sup>-1</sup> (superfusion)	2 h	Scalia <i>et al.</i> (1997)
Peritonitis	Inhibit neutrophil recruitment and regulate chemokine/cytokine production	300 ng (i.p.) 50 ng–50 µg kg <sup>-1</sup> (oral)	4–24 h	Bannenberg <i>et al.</i> (2005) Bannenberg <i>et al.</i> (2004)
	Inhibit vascular leakage	60 µg kg <sup>-1</sup> (i.p.)	4 h	Chiang <i>et al.</i> (2003)
	Promote phagocytosis of neutrophil by macrophage	1–2 µg (i.p.)	<1 h	Mitchell <i>et al.</i> (2002)
	Promote lymphatic removal of microbial particles (zymosan)	300 ng (i.p.)	24 h	Schwab <i>et al.</i> (2007)
Colitis (IBD)	Attenuate pro-inflammatory gene expression and reduce severity of colitis	10 µg ml <sup>-1</sup> (oral)	0–20 days	Gewirtz <i>et al.</i> (2002)
	Inhibit weight loss, inflammation and immune dysfunction	300–1000 µg kg <sup>-1</sup> (oral, daily)	7 days	Fiorucci <i>et al.</i> (2004)
Mesangioproliferative nephritis	Reduce proteinuria, IL-1β, IL-6 and proliferation score of mesangial cells	i.v., every 8 h	4 days	Wu <i>et al.</i> (2007)
Kidney	Antagonizes LTD4-induced falls in glomerular filtration rate	1–2 µg kg <sup>-1</sup> min <sup>-1</sup> (infused into renal arteries)	20 min	Katoh <i>et al.</i> (1992)
	Protects from ischemic acute renal failure	15 µg per mouse (perfusion)	24 h	Leonard <i>et al.</i> (2002)
Pleuritis	Shorten the duration of pleural exudation	0.5–5 µg (local)	24 h	Bandeira-Melo <i>et al.</i> (2000)
Asthma	Inhibit airway hyperresponsiveness and pulmonary inflammation	10 µg per mouse per day (i.v.)	5 days	Levy <i>et al.</i> (2002)
	Modulates airway obstruction in humans	100 µM (inhalation)		Christie <i>et al.</i> (1992)
Cystic fibrosis	Decrease neutrophilic inflammation, pulmonary bacterial burden and disease severity	10 µg ml <sup>-1</sup> (oral) plus 1 µg (i.v.)	6 days	Karp <i>et al.</i> (2004)
Sepsis	Attenuate LPS-induced acute lung injury	0.7 mg kg <sup>-1</sup> (i.v.)		Jin S-W <i>et al.</i> (2007)
Angiogenesis	Reduce angiogenic phenotype: endothelial cell proliferation and migration	10 µg per pouch (local)	6 days	Fierro <i>et al.</i> (2002)
Periodontitis (oral inflammation and bone loss)*	Reduce microbe-initiated, neutrophil-mediated tissue damage and bone destruction	6 µg twice per week (topical application around the second premolar)	6 weeks	Serhan <i>et al.</i> (2003)
Eye	Accelerate cornea reepithelialization, limit sequelae of thermal injury (i.e., neovascularization, opacity) and promote host defense	1 µg three times per day (topical application on eye)	2–4 days	Gronert <i>et al.</i> (2005)
	Suppress corneal angiogenesis, inhibiting inflammatory cytokines and VEGF/VEGFR-2 expression	100 ng per 10 µl (subconjunctivally, once daily)	3 days	Jin Y <i>et al.</i> (2007)
BMT	Protect against BMT-induced GvHD	50 µg kg <sup>-1</sup> (host), 100 ng ml <sup>-1</sup> (donor)	9 days	Devchand <i>et al.</i> (2005)
Carrageenan-induced hyperalgesia	Prolong paw withdraw latency, reducing hyperalgesic index; reduce pain Reduce paw edema	24 nmol kg <sup>-1</sup> (i.v.) 0.3 nmol (intrathecal)	0–8 h	Svensson <i>et al.</i> (2007)

Abbreviations: BMT, bone marrow transplant; GvHD, graft-vs-host diseases; IBD, inflammatory bowel disease; IL, interleukin; I/R, ischemia/reperfusion; LPS, lipopolysaccharide; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.

\*All animal models were designed and carried out in either mice or rats, except for periodontitis, which was carried out in rabbits.

addition, the LXs (Samuelsson, 1982; Samuelsson *et al.*, 1987). This departure of PUFA from playing structural roles in cell membranes or as energy stores came with recognition that AA is transformed by both cyclooxygenase (COX) and lipoxygenase (LOX) mechanisms to potent products, which

led to a Nobel prize in 1982 (Bergström, 1982; Samuelsson, 1982; Vane, 1982). Peripheral blood neutrophils release mediators derived from AA via COX and LOXs that are mostly pro-inflammatory (Samuelsson, 1983). Recent results, however, have heightened our awareness that neutrophils

(PMN), which are first to arrive on the scene, in addition to initially releasing pro-inflammatory mediators, can with time switch their product profile of lipid mediators to produce 'protective' lipid mediators that serve as agonists to actively limit inflammation and promote resolution (Serhan, 2004).

Within this framework, LXs were the first mediators biosynthesized from AA identified to possess both anti-inflammatory and pro-resolving actions (Serhan, 2005). In general, this is a departure from the well-appreciated pro-inflammatory roles of lipid mediators (Samuelsson, 1983). LXs are generated from endogenous sources of the  $n-6$  AA (Figure 1) and are potent agonists of resolution *in vivo* in many disease models (Table 1), thus serving as checkpoint controllers of inflammation (Chiang *et al.*, 2006). Of interest, an early human trial with lipoxin A<sub>4</sub> (LXA<sub>4</sub>) conducted by Professor TH Lee and colleagues in London demonstrated that LXA<sub>4</sub> was safe and blocked airway challenge and hyperreactivity (Christie *et al.*, 1992). Increasing evidence demonstrating that resolution of inflammation is actually an active biochemical process came with the identification of specialized chemical mediators biosynthesized during the resolution phase such as the resolvins and protectins, which actively promote resolution (Serhan *et al.*, 2000; Levy *et al.*, 2001; Gilroy *et al.*, 2004) and the return to homeostasis (Serhan, 2002) (*vide infra*).

### Molecular links to $\omega$ -3 PUFA and resolution of inflammation

It has often been questioned whether essential fatty acids, such as the  $\omega$ -3 EPA or DHA, are converted to potent lipid mediators, as is the case with AA. In view of the compelling results from the GISSI study, which showed improvements in >11 000 cardiovascular patients, that is, reduction in the incidents of sudden death to ~45% in those taking almost 1 g of  $\omega$ -3 per day (GISSI-Prevenzione Investigators, 1999; Marchioli *et al.*, 2002). However, the mechanism of this  $\omega$ -3 action in humans with vascular disease was not established, and the impact of ongoing aspirin therapy was not accounted for in the analyses. Inspection of their methods indicated that all the patients also took daily aspirin; the contribution of aspirin to the beneficial outcomes was not taken into account (GISSI-Prevenzione Investigators, 1999). Also, an abundant literature with  $\omega$ -3 PUFA given at doses of milligrams to grams per day pointed to beneficial actions in many diseases including inflammatory diseases as well as cancer (Lands, 1987; Bazan, 1992). Each of the three major human LOXs (5-LOX, 12-LOX and 15-LOX) can convert  $\omega$ -3 PUFA to various monohydroxy-containing products. The biological importance of these products, if any, was not known (Hamberg and Samuelsson, 1967; Lee *et al.*, 1984; Sawazaki *et al.*, 1994). DHA is also subject to nonenzymatic oxygenation to isoprostane-like compounds termed neuroprostanes that reflect oxidative stress in the brain (Reich *et al.*, 2000) or, when mishandled, can rapidly auto-oxidize to monohydroxy racemates (VanRollins *et al.*, 1984). However, despite the many decades of research with  $\omega$ -3 PUFA, the cellular and molecular mechanisms accounting for their

reported *in vivo* immunoprotective actions remained to be established, and their direct connection to human disease and treatment potential is still an important biomedical challenge.

### Aspirin initiates biosynthesis of endogenous pro-resolving and anti-inflammatory lipid mediators

Although it is clear that aspirin inhibits PG formation and hence a key mechanism in anti-inflammatory therapy (Vane, 1982), and that aspirin has well-appreciated clinical uses and ability to limit leukocyte traffic into sites of inflammation, the mechanism remained unknown. In this context, aspirin impinges on the endogenous LX-generating system during cell-cell interactions in the vasculature, triggering formation of epimeric forms of LXs, termed aspirin-triggered LXs (ATL) (Clària and Serhan, 1995; Chiang *et al.*, 2006).

As is the case for other autacoids, both LXA<sub>4</sub> and ATL are rapidly generated in response to stimuli, act locally and then are rapidly inactivated by metabolic enzymes, namely 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and eicosanoid oxidoreductase (EOR) (also known as LXA<sub>4</sub>/PGE 13,14-reductase/LTB<sub>4</sub> 12-hydroxydehydrogenase (PGR/LTB<sub>4</sub>DH)). To this end, stable analogs were constructed with specific modifications of the native structures of LXA<sub>4</sub> and ATL (Serhan *et al.*, 1995) to resist enzymatic metabolism. These analogs not only display prolonged half-life in blood but also enhanced bioavailabilities as well as potency *in vivo* (Clish *et al.*, 1999). As a class, LXs, ATL and their stable analogs share potent protective actions in controlling inflammation (Table 1).

In light of these results, attention was focused next on understanding the biochemical events activated during healthy resolution of acute inflammatory responses. The LX and ATL stable analogs are potent and are effective in many experimental disease models (Table 1). Of interest, the first-generation LX/ATL analogs proved to be active when administered *i.v.*, *i.p.*, topically and orally available (Schottelius *et al.*, 2002; Serhan *et al.*, 2003; Bannenberg *et al.*, 2004). The second generation of LX/ATL analogs (Guilford *et al.*, 2004) are also bioavailable and have proven effective in murine models of colitis (Wallace and Fiorucci, 2003; Fiorucci *et al.*, 2004) and asthma (Levy *et al.*, 2007b). In asthma, the ZK-994 LX/ATL analog (see Figure 1b for structures) was more effective than Singulair in reducing airway inflammation and airway bronchoconstriction (Levy *et al.*, 2007b). In view of these compelling findings in experimental animal systems demonstrating anti-inflammatory and pro-resolving actions *in vivo* (Mitchell *et al.*, 2002; Kieran *et al.*, 2004), a proof of concept was established, namely, that treatment with a pro-resolving and anti-inflammatory lipid mediator or its stable analog mimetic can reduce inflammation and disease symptoms in animals (Table 1). Of interest, recent results demonstrate that LX and their ATL analogs block pain signals (Svensson *et al.*, 2007) and regulate neural stem cells (Wada *et al.*, 2006). Hence, it will be of interest to learn whether these or related agonists of resolution can reduce disease symptoms in humans and promote the return of the tissues to homeostasis in a human trial.

## Impact of anti-inflammatory agents on PUFA-derived mediators

Aspirin, in addition to its well-appreciated ability to inhibit the pro-inflammatory PG, can also 'switch on' the body's own anti-inflammatory lipid mediators, namely aspirin-triggered lipid mediators. This new action of aspirin involves COX-2-bearing cells such as vascular endothelial cells or epithelial cells and their coactivation with PMN (Clària and Serhan, 1995; Chiang *et al.*, 2006). Briefly, inflammatory stimuli (for example, tumor necrosis factor (TNF)- $\alpha$ , lipopolysaccharide (LPS)) induce COX-2 to generate 15R-HETE when aspirin is administered. Aspirin irreversibly acetylates COX-2 and changes the enzyme's products from intermediates for PG and thromboxane to precursors for ATL, namely 15R-HETE. This precursor carries a carbon-15 alcohol in the *R* configuration and is rapidly converted by 5-LOX in activated PMN to 15 epimeric-LXs, or ATL, that carry their 15 position alcohol in the *R* configuration rather than 15S (native LX). This biosynthetic mechanism is also operative with EPA-derived RvEs and DHA-derived RvDs (Serhan *et al.*, 2000, 2002) in that aspirin initiates formation of these  $\omega$ -3-derived mediators by acetylating vascular COX-2. Therefore, these aspirin-triggered (AT) protective mediators provide a novel mechanism underlying aspirin's clinical benefits.

Other commonly used nonsteroidal anti-inflammatory drugs (NSAIDs) do not acetylate COX-2. Some do, however, with recombinant COX-2 and EPA, permit formation of 18R-hydroxyeicosapentaenoic acid (18R-HEPE), a precursor for EPA-derived RvEs (see below), even though 18R-HEPE levels are lower than those in the absence of these NSAIDs (Serhan *et al.*, 2000). In comparison, when incubated with recombinant COX-2 and DHA, these NSAIDs did not give

appreciable amounts of 17R-HDHA, a precursor for DHA-derived RvDs (Serhan *et al.*, 2002). Of interest, some NSAIDs inhibit enzymes involved in the inactivation of PUFA-derived mediators, namely 15-PGDH and EOR, thus likely leading to accumulation of LXs and Rvs (Table 2). The widely used anti-inflammatory glucocorticoids may also facilitate the formation and action of PUFA-derived mediators via regulating not only the biosynthetic and metabolic enzymes, but also one of the receptors for LXA<sub>4</sub>/ATL, namely ALX (Sawmynaden and Perretti, 2006; Hashimoto *et al.*, 2007). Along these lines, both thiazolidinediones and statins have anti-inflammatory actions; however, the underlying mechanisms are not clear. Of interest, a recent report by Birnbaum *et al.* (2006) demonstrated that both pioglitazone and atorvastatin increase the myocardial content of LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub>, which in turn can likely contribute to the anti-inflammatory properties of thiazolidinediones and statins.

## Mediator lipidomics: profiling and complete structure elucidation of novel endogenous mediators

We developed mediator informatics and lipidomics employing liquid chromatography-ultraviolet-tandem mass spectrometry (LC-UV-MS-MS)-based analyses of inflammatory exudates. We also constructed lipid mediator libraries with physical properties, such as MS and MS/MS spectra, elution times and UV spectra for matching studies (Lu *et al.*, 2005, 2006). These databases and informatics were geared to evaluate whether novel lipid mediators are indeed generated during the resolution phase of inflammation (Serhan *et al.*,

**Table 2** Impact of anti-inflammatory agents on LX, RV and PD: biosynthesis, inactivation and receptors

Anti-inflammatory agent/Impact	
<b>NSAID</b>	
Aspirin	Switches COX-2 activity to produce 15R-HETE (from AA; Clària and Serhan, 1995), 18R-HEPE (from EPA; Serhan <i>et al.</i> , 2000), and 17R-HDHA (from DHA; Serhan <i>et al.</i> , 2002) Increases ATL formation <i>in vivo</i> in experimental animals (reviewed in Chiang <i>et al.</i> , 2006) and in human (Chiang <i>et al.</i> , 2004) Initiates RvE1 formation <i>in vivo</i> in experimental animals (Serhan <i>et al.</i> , 2000) and in human (Arita <i>et al.</i> , 2005a) Initiates RvD formation <i>in vivo</i> in experimental animals (Serhan <i>et al.</i> , 2002) Inhibits 15-PGDH (Hansen, 1974)
Indomethacin	Permits 18R-HEPE generation with recombinant COX-2, yet reduces its levels (Serhan <i>et al.</i> , 2000) Inhibits 15-PGDH (Cho and Tai, 2002; Hansen, 1974) and EOR (Clish <i>et al.</i> , 2001)
Acetaminophen Diclofenac	Permits 18R-HEPE generation with recombinant COX-2, yet reduces its levels (Serhan <i>et al.</i> , 2000) Inhibits EOR (Clish <i>et al.</i> , 2001)
<b>Steroid</b>	
Dexamethasone	Upregulates 5-LOX expression (Colamorea <i>et al.</i> , 1999; Uz <i>et al.</i> , 2001) Inhibits 15-PGDH (cell-type dependent) (Tong and Tai, 2000) Upregulates ALX receptor (Hashimoto <i>et al.</i> , 2007; Sawmynaden and Perretti, 2006)
<b>Thiazolidinedione</b>	
Pioglitazone Ciglitazone	Increases LXA <sub>4</sub> and 15-epi-LXA <sub>4</sub> formation (Birnbaum <i>et al.</i> , 2006) Inhibits 15-PGDH (Cho and Tai, 2002)
<b>Statin</b>	
Atorvastatin	Increase LXA <sub>4</sub> and 15-epi-LXA <sub>4</sub> formation (Birnbaum <i>et al.</i> , 2006)

Abbreviations: 15-PGDH; 15-hydroxyprostaglandin dehydrogenase; 18R-HEPE, 18R-hydroxyeicosapentaenoic acid; AA, arachidonic acid; ATL, aspirin-triggered LX; COX-2, cyclooxygenase-2; EOR, eicosanoid oxidoreductase; DHA, docosahexaenoic acid; 5-LOX, 5-lipoxygenase; LXA<sub>4</sub>, lipoxin A<sub>4</sub>; NSAID, nonsteroidal anti-inflammatory drug; Rv, resolvin.

2000, 2002, 2006b). An experimental model of contained inflammation was used for these studies, namely, murine dorsal air pouch. In this model, 4 h after TNF- $\alpha$  injection, PMN numbers drop within the exudates (Serhan *et al.*, 2000, 2002), which is the cellular definition of resolution (Cotran *et al.*, 1999). Exudates were collected in this 'spontaneous resolution' phase (Winyard and Willoughby, 2003). In this phase, lipid mediator profiles were recorded using tandem LC-UV-MS-MS to identify lipid mediators present within the exudates. When novel bioactive mediators were encountered, their structures were elucidated. This was accomplished by carrying out retrograde analysis for both biogenic (that is, using recombinant enzymes) and total organic synthesis (for example, see Petasis *et al.*, 2005). Also, this approach permitted assessment of structure-activity relationships as well as the scale-up required to confirm bioactions characterized for the novel compounds identified (Arita *et al.*, 2005a; Lu *et al.*, 2006).

## Resolvins: the 18R E-series resolvins from EPA

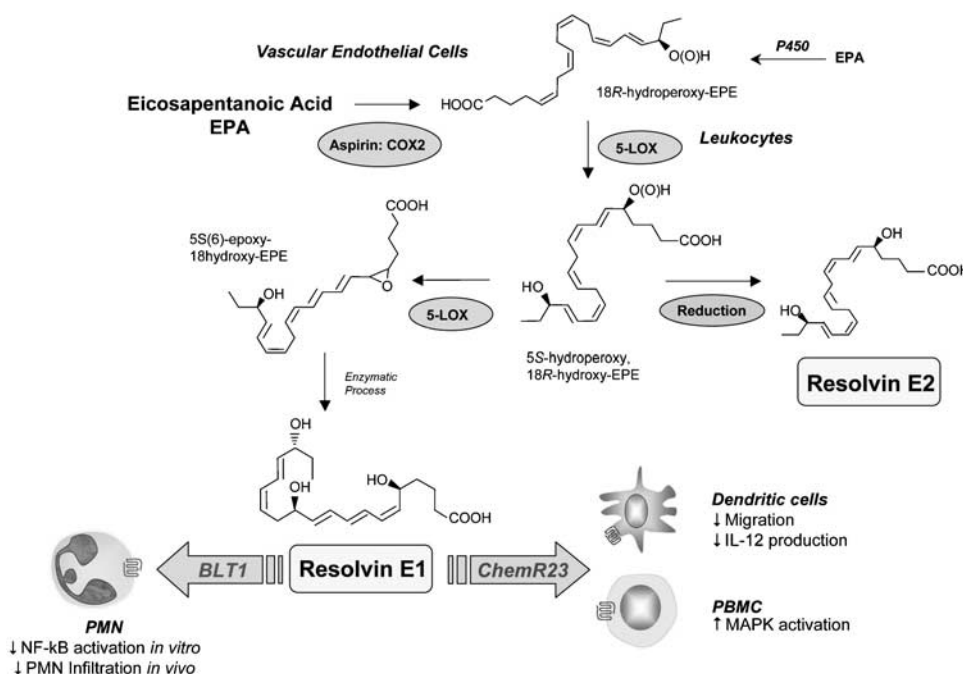
### Formation and structure elucidation

The exudates collected from murine air pouches in the resolution phase contained 18R-HEPE as well as several other related bioactive compounds (Serhan *et al.*, 2000). These novel compounds were generated from EPA. The first bioactive compound, termed resolvin E1 (RvE1), was isolated from exudates and found to reduce inflammation *in vivo* and blocks human PMN transendothelial migration *in vitro*.

Structural elucidation was carried out using both GC-MS and LC-MS-MS analyses of bioactive fractions obtained following extraction and reversed phase-high pressure liquid chromatography. The basic structure of this potent bioactive product generated from EPA proved to be 5,12,18R-trihydroxyeicosapentaenoic acid (EPE) (Serhan *et al.*, 2000). To recapitulate these *in vivo* events with human cells, isolated human vascular endothelial cells treated with aspirin convert EPA to 18R-HEPE that is released and then rapidly converted by activated human PMN to a 5(6)-epoxide-containing intermediate that is converted to bioactive RvE1 (Figure 2).

RvE1 possesses an interesting and novel distinct structure consisting of a conjugated diene plus conjugated diene chromophore present within the same molecule. Both biogenic (Serhan *et al.*, 2000) and total organic syntheses were achieved and its complete stereochemical assignment was established along with that of several related natural isomers (Arita *et al.*, 2005a). RvE1 proved to be 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid (Figure 2). RvE1 production in healthy human volunteers, marked by mass spectra-based identification in plasma, is increased with aspirin therapy (Arita *et al.*, 2005a). However, biologically significant amounts of RvE1, that is, concentrations that can evoke biological responses, are produced and present in plasma without taking aspirin.

Human recombinant 5-LOX generates resolvin E2 (RvE2) from a common precursor of E-series resolvins, namely 18-HEPE. RvE2, which is 5S,18-dihydroxyeicosapentaenoic acid, stopped zymosan-induced PMN infiltration, displaying



**Figure 2** E-series resolvins. Aspirin impacts the formation of resolvin E1 (RvE1) by acetylating COX-2 in vascular endothelial cells that stereoselectively generate 18R-hydroperoxy-EPE (18R-H(p)EPE). 18R-HEPE is further converted via sequential actions of leukocyte 5-LOX, leading to formation of RvE1. The complete stereochemistry of RvE1 was recently established. Microbial P-450s can also contribute to RvE biosynthesis via converting eicosapentaenoic acid (EPA) to 18-HEPE (Arita *et al.*, 2005b). Human recombinant 5-LOX also generates resolvin E2 (RvE2) from 18-HEPE. At least two separate GPCRs can specifically interact with RvE1: (1) ChemR23, on mononuclear cells and DCs, and (2) BLT1, on human PMN. Also, when expressed on epithelial cells ChemR23 and RvE1 stimulated CD-55-dependent clearance of PMN from the mucosal surface (Campbell *et al.*, 2007).

potent anti-inflammatory properties in murine peritonitis (Tjonahen *et al.*, 2006). In addition, RvE1 and RvE2, when given together, displayed additive action in controlling PMN infiltration. These results demonstrate that RvE2, together with RvE1, may contribute to the beneficial actions of  $\omega$ -3 fatty acids in human diseases (Table 3). Moreover, they indicate that the 5-LOX, in human leukocytes, is a pivotal enzyme that is temporally and spatially regulated *in vivo* to produce either pro- or anti-inflammatory local chemical mediators. Thus, the production of these mediators is dependent on the local environment of the leukocytes; for example, within the inflammatory exudates. Also of interest are results indicating that microbial p450 can generate 18-HEPE from EPA (Serhan *et al.*, 2000) that can be converted by human PMN to RvE1 and RvE2 (Arita *et al.*, 2005b). Hence, the microbial content in the local environment can also be a critical factor in the production of E-series resolvins *in vivo* in humans.

#### Receptors

It was of interest to determine the receptors involved in the actions of RvE1 bioactions. To this end, a panel of G-protein-coupled receptors (GPCR) was screened (Arita *et al.*, 2005a). An orphan receptor, denoted earlier as ChemR23, was found to attenuate nuclear factor (NF)- $\kappa$ B in response to RvE1. Specific binding of RvE1 to this receptor was confirmed using synthetic [<sup>3</sup>H]-labeled RvE1. The main second messenger for RvE1 agonist actions via ChemR23 appears to be activation of intracellular phosphorylation pathways rather than mobilization of intracellular calcium or cyclic AMP, as with most pro-inflammatory mediators (Arita *et al.*, 2005a, c). Recently, a second GPCR that interacts with RvE1 was identified, that is, the leukotriene B<sub>4</sub> receptor (BLT)1 (Arita *et al.*, 2007). RvE1 specifically interacts with BLT1 on human PMN, demonstrated by radioligand binding and G-protein signaling. Also, RvE1 attenuates LTB<sub>4</sub>-dependent pro-inflammatory signals, such as calcium influx and NF- $\kappa$ B activation.

RvE1-BLT1 interaction *in vivo* was demonstrated using BLT1-deficient mice. Thus, RvE1 selectively interacts with two distinct GPCRs on different cell types to control inflammation and promote resolution.

#### Actions

Resolvins of the E-series comprise several molecules. Among them, RvE1 was the first isolated and studied in depth. RvE1 displayed potent stereoselective actions *in vivo* and with isolated cells (Table 3; Figure 2). At nanomolar levels *in vitro*, RvE1 dramatically reduced human PMN transendothelial migration, dendritic cell (DC) migration and interleukin (IL)-12 production (Serhan *et al.*, 2002; Arita *et al.*, 2005a).

In several animal models of inflammatory diseases, RvE1 displays potent counterregulatory actions that protect against leukocyte-mediated tissue injury and excessive pro-inflammatory gene expression (Arita *et al.*, 2005a, c; Hasturk *et al.*, 2006). For example, administration of synthetic RvE1 blocks PMN infiltration in periodontal disease in a rabbit model (Hasturk *et al.*, 2006) and protects against the development of 2,4,6-trinitrobenzene sulfonic acid-induced colitis (Arita *et al.*, 2005c). Most recently, RvE1 was shown to reduce neovascularization in oxygen-induced retinopathy (Connor *et al.*, 2007) and enhance CD55-dependent clearance of PMN from epithelial surfaces (Campbell *et al.*, 2007). These new findings provide evidence for endogenous mechanism(s) that may account for some of the widely touted beneficial actions noted with dietary supplementation with  $\omega$ -3 PUFA (EPA and DHA), thereby providing new approaches for the treatment of gastrointestinal mucosal and oral inflammation.

#### Metabolic inactivation

One route of enzymatic inactivation of RvE1 was recently established (Arita *et al.*, 2006). In murine lung, apparently

**Table 3** *In vivo* actions of resolvin E series

Disease model	Action	Dosage (route)	Duration of postexposure	Reference
<i>Resolvin E1</i>				
Skin (dorsal air pouch)	Reduces PMN infiltration	100 ng per mouse (i.v.)	4 h	Serhan <i>et al.</i> (2000, 2002)
Peritonitis	Reduces PMN infiltration and regulates chemokine/cytokine production	300 ng per mouse (i.p.)	4–24 h	Bannenberg <i>et al.</i> (2005)
Colitis	Reduces PMN infiltration	100 ng per mouse (i.v.)	2 h	Arita <i>et al.</i> (2005a)
	Decreases PMN recruitment and pro-inflammatory gene expression Improves survival Reduces weight loss	10 ng per mouse (i.p.) 50 $\mu$ g kg <sup>-1</sup> (i.p.) on days -8, -1 and 0 before induction of colitis	2–4 h 4–12 days	Serhan <i>et al.</i> (2006a) Arita <i>et al.</i> (2005c)
Oral (periodontitis)	Reduces PMN infiltration, stops inflammation-induced tissue and bone loss	4 $\mu$ g per tooth (topical application, every other day)	6 weeks	Hasturk <i>et al.</i> (2006)
Eye (retinopathy)	Reduces vaso-obliteration and neovascularization	10 ng per day (i.p.), from postnatal day 6 to day 17	17 days	Connor <i>et al.</i> (2007)
<i>Resolvin E2</i>				
Peritonitis	Reduces PMN infiltration	10 ng per mouse (i.v., i.p.)	2 h	Tjonahen <i>et al.</i> (2006)

Abbreviation: PMN, polymorphonuclear leukocyte.



the 15-PGDH, which is abundant in lung tissues, can catalyze the conversion of RvE1 to 18-oxo-RvE1, representing the major initial metabolic route for RvE1 in lung tissues. At the same doses as RvE1, 18-oxo-RvE1 was devoid of activity in regulating/stopping PMN recruitment in zymosan-induced peritonitis. In human neutrophils, C-20 hydroxylation of RvE1 was the main route of conversion. An RvE1 analog, namely 19-(*p*-fluorophenoxy)-RvE1, was synthesized that resisted rapid metabolic inactivation and proved to retain biological activity, reducing PMN infiltration and pro-inflammatory cytokine/chemokine production *in vivo*. Thus, the designer RvE1 mimetics that resist further metabolism/inactivation could be useful tools to evaluate the agonist actions of RvE1 in complex disease models.

### Resolvins: the 17R and 17S D-series resolvins from DHA

#### Formation and structure elucidation

**17R D-series resolvins.** The resolving exudates from mice given aspirin plus DHA also contained novel 17R-hydroxy-DHA (17R-HDHA) and several related bioactive compounds (Figure 3a). The biosynthetic pathways were reconstructed *in vitro* to establish a potential origin for these novel compounds found *in vivo*. Human microvascular endothelial cells, when treated with aspirin in hypoxia, released 17R-HDHA produced from DHA. Human recombinant COX-2 converts DHA to 13-hydro(peroxy)-DHA, which is monitored as 13-hydroxydocosahexaenoic acid. With aspirin, this switches to 17R-oxygenation to give a group of AT resolvins (AT-RvD1 through RvD4) that were also found in brain (Serhan *et al.*, 2002; Hong *et al.*, 2003).

**17S D-series resolvins.** Brain, synapses and retina are highly enriched in DHA, a major  $\omega$ -3 fatty acid (Bazan, 1992; Salem *et al.*, 2001). Deficiencies in this essential fatty acid are reportedly associated with neuronal function, cancer and inflammation (Coussens and Werb, 2002; Lawrence, 2007). Using the mediator lipidomics-informatics approach (Hong *et al.*, 2007) employing tandem LC-PDA-MS-MS, it was exciting to find that neither aspirin nor exogenous DHA was required to monitor the production of these new structures *in vivo* (Hong *et al.*, 2003; Duffield *et al.*, 2006). The endogenous DHA was converted *in vivo* to a 17S alcohol-containing series of resolvins via LOX-initiated mechanisms (Hong *et al.*, 2003; Marcheselli *et al.*, 2003). The stereochemistry of both 17R- and 17S-series of resolin D1 (RvD1 and AT-RvD1) was established and total organic synthesis achieved (Sun *et al.*, 2007). The synthetic compounds matched the physical and biological properties of those enzymatically generated. RvD1 proved to be 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid, and AT-RvD1 matched 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid. Also, the total organic synthesis of resolin D2 (RvD2) was reported (Rodriguez and Spur, 2004), confirming the physical properties of the original compound identified in exudates (Serhan *et al.*, 2002).

#### Actions

RvD1 and AT-RvD1 each proved to be potent regulators of both human and murine PMN. They stopped transendothelial migration of human neutrophils (EC<sub>50</sub> approximately 30 nM) (Serhan *et al.*, 2002). In microglial cells, they block TNF- $\alpha$ -induced IL-1 $\beta$  transcripts (Hong *et al.*, 2003). In murine peritonitis *in vivo*, RvD1 and AT-RvD1 proved equipotent (at nanogram dosages), limiting PMN infiltration in a dose-dependent fashion (Table 4). Direct comparisons between RvE1, AT-RvD1 (triggered by aspirin treatment) and RvD1 (via LOX-initiated mechanisms) at equal doses of 100 ng per mouse demonstrated that RvD1 and AT-RvD1 display essentially similar actions, reducing PMN infiltration by ~50% in peritonitis and air pouch, compared to ~75–80% inhibition by RvE1 in air pouch (Serhan *et al.*, 2002; Hong *et al.*, 2003; Sun *et al.*, 2007). Thus, DHA is precursor to potent protective mediators. Both the RvDs (17S-series) and AT RvDs (17R-series) are potent regulators of PMN infiltration *in vivo*, and the *S* to *R* switch with aspirin treatment in the biosynthesis of the 17 position alcohol in the  $\omega$  side chain does not diminish their activity. Recently, RvD1 was generated in response to bilateral ischemia/reperfusion injury in mouse kidneys (Duffield *et al.*, 2006). Administration of RvDs before or after ischemia resulted in a reduction in functional and morphological kidney injury. Interstitial fibrosis after ischemia/reperfusion was also reduced in mice treated with RvDs. In addition, RvDs reduced infiltrating leukocytes and blocked Toll-like receptor-mediated activation of macrophages. These findings demonstrated previously unrecognized endogenous anti-inflammatory and antifibrotic responses of RvDs in protecting acute kidney injury, confirmed with synthetic RvD1 and PD1.

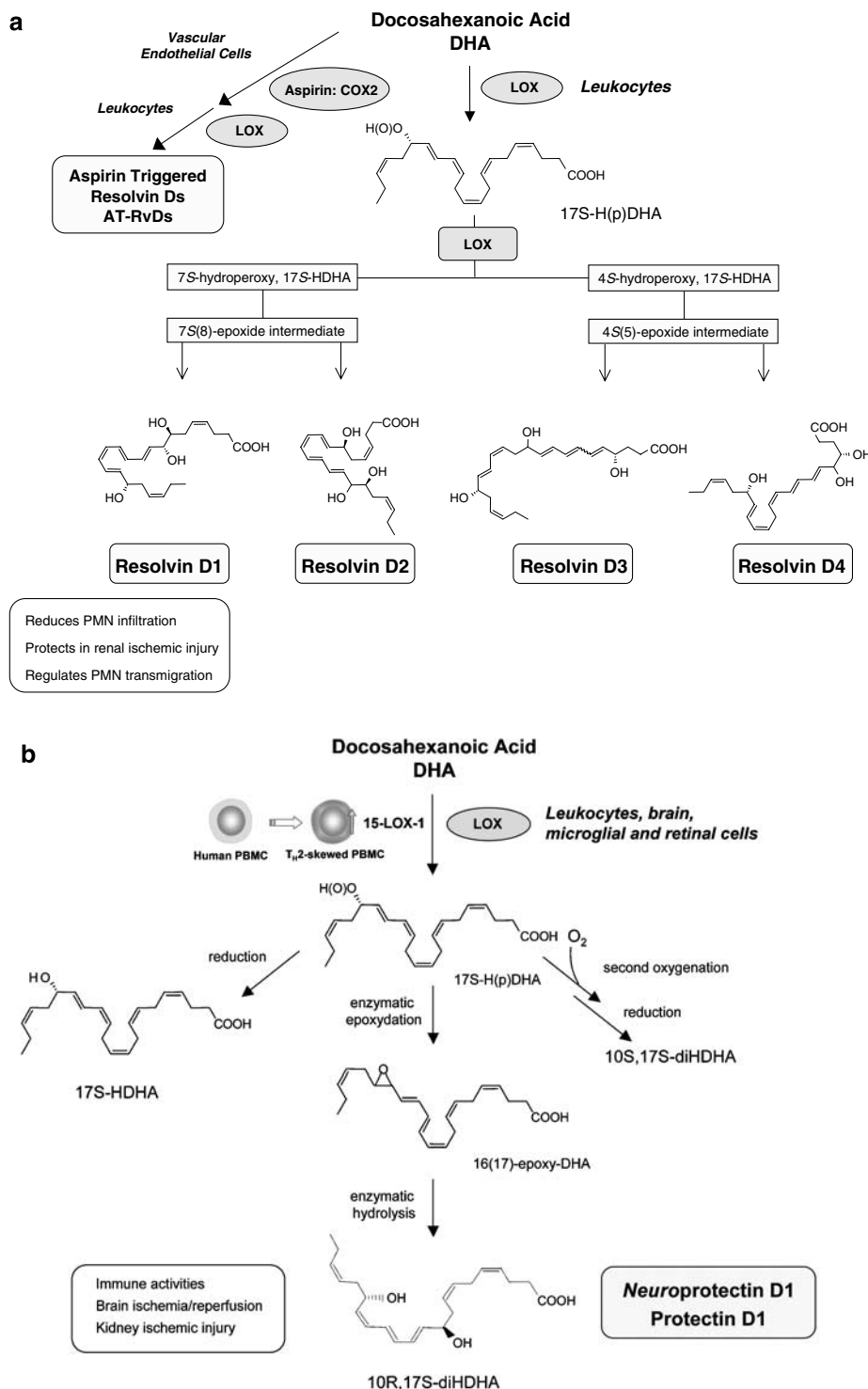
#### Metabolic inactivation

RvD1 was converted by EOR to novel 8-oxo- and 17-oxo-RvD1. 17-oxo-RvD1 gave dramatically reduced bioactivity, and the enzymatic conversion of AT-RvD1 was sharply reduced, indicating that the AT form resists rapid inactivation, suggesting that it is likely to be longer acting *in vivo* (Sun *et al.*, 2007). 8-oxo-RvD1 retained activity in reducing peritonitis.

### Protectins from DHA

#### Formation and structure elucidation

Endogenous DHA is also converted *in vivo* to a triene-containing structure via LOX-initiated mechanisms (Hong *et al.*, 2003; Marcheselli *et al.*, 2003). The LOX product 17S-H(p)DHA is converted to a 16(17)-epoxide that is enzymatically converted to the 10,17-dihydroxy-containing bioactive product (Hong *et al.*, 2003), first denoted 10,17S-docosatriene, and recently coined NPD1/protectin D1 (PD1) based on its potent actions *in vivo*. PD1 possesses a conjugated triene-containing structure as the key feature of this new family of compounds derived from DHA. The complete stereochemistry of PD1, namely chirality of the alcohol groups and geometry of the conjugated triene, was recently established. Total organic synthesis of PD1 and



**Figure 3** D-series resolvins and protectins. (a) Resolvin Ds: formed from docosahexanoic acid (DHA), the proposed biosynthetic pathways reconstructed *in vitro* involve the lipoxygenase (LOX) product 17S-H(p)DHA, which is rapidly transformed by the LOX activity in human polymorphonuclear leukocyte (PMN) into two epoxide intermediates. These two novel epoxide intermediates open to form bioactive products denoted 17S-resolvin D series (RvD1-4). Aspirin also impacts the formation of resolvin D series by catalytically switching COX-2 to a 17R-lipoxygenase-like mechanism that generates 17R-H(p)DHA, and subsequently 17R-resolvin D series (AT-RvDs). (b) Protectins: the initial enzymatic product 17S-H(p)DHA is converted to neuroprotectin D1/PD1. The complete stereochemistries of the bioactive mediators and related natural isomers are established (see text for further details).

related isomers and matching studies with biologically derived materials showed that endogenous PD1, signified as neuroprotectin D1 when produced by neural tissues,

was recently established in isolated human cells *in vitro* and murine cells *in vivo* as 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid (Serhan *et al.*, 2006a).

**Table 4** *In vivo* actions of resolvin D series

Disease model	Action	Dosage (route)	Duration of postexposure	Reference
Skin (dorsal air pouch)	Reduces PMN infiltration	100 ng per mouse (intrapouch)	4 h	Serhan <i>et al.</i> (2002) Hong <i>et al.</i> (2003)
Peritonitis	Reduces PMN infiltration	100 ng per mouse (i.v.) 0.05–50 µg kg <sup>-1</sup> (i.v.)	2 h 4 h	Hong <i>et al.</i> (2003) Sun <i>et al.</i> (2007)
Kidney ischemia-reperfusion	Protects in renal ischemic injury by limiting PMN infiltration	5 µg per mouse (i.v., s.c.)	24–48 h	Duffield <i>et al.</i> (2006)
Eye (retinopathy)	Reduces vaso-obliteration and neovascularization	10 ng per day (i.p.), from postnatal day 6 to day 17	17 days	Connor <i>et al.</i> (2007)

Abbreviation: PMN, polymorphonuclear leukocyte.

The geometry of the double bonds in PD1 and their positions during biosynthesis from the key intermediates in its biosynthesis *in situ*, namely *via* an epoxide intermediate at the 16(17) position, indicated that PD1 biosynthesis requires enzymatic steps to generate the potent bioactive molecule from DHA (Figure 3b). Other dihydroxy-docosanoids (both positional and geometric isomers) were identified natural isomers, but proved to be substantially less bioactive than native PD1 *in vivo* (Hong *et al.*, 2003; Mukherjee *et al.*, 2004; Serhan *et al.*, 2006a). The double LOX product 10S,17S-diHDHA formed by sequential enzymatic oxygenation with molecular oxygen is less active than PD1 (Serhan *et al.*, 2006a), and its conjugated triene is in the *trans*-, *cis*-, *trans*-, configuration, which is different from the configuration in PD1. Of note in humans, NPD1/PD1 is also generated by human T cells skewed toward Th2 phenotype and is biosynthesized *via* a 16(17)-epoxide intermediate in these human cells (Ariel *et al.*, 2005; Figure 3b).

### Actions

Identification of the resolvins and protectins generated from DHA now opens exploration into the essential roles of these pathways and mediators. PD1 proved to be log orders of magnitude more potent than its precursor DHA, demonstrated in several animal models of diseases (Table 5). Synthetic PD1 at 10 nM attenuates human neutrophil transmigration by ~50% *in vitro*, whereas its Δ15-*trans*-isomer is essentially inactive. PD1 is also a potent regulator of PMN *in vivo* by reducing PMN infiltration (~40% at 1 ng per mouse) in murine peritonitis. Of importance to potential treatments and uses, PD1 also reduced PMN infiltration when administered after the initiation of inflammation *in vivo* as well as acts in an additive fashion with RvE1 to stop PMN infiltration. These results indicate that PD1 is a potent, stereoselective anti-inflammatory molecule (Serhan *et al.*, 2002, 2006a; Hong *et al.*, 2003). PD1 displays potent actions in the eye, promoting corneal epithelial cell wound healing and limiting sequelae of thermal injury (Gronert *et al.*, 2005), and also protects against retinopathy (Connor *et al.*, 2007). In kidney ischemia/reperfusion injury, mouse kidneys produce RvDs and PD1. Administration of RvDs or PD1 to mice before the ischemia resulted in a reduction in functional and morphological kidney injury (Duffield *et al.*, 2006). In addition, PD1 is generated in human asthma in breath condensates and in mouse lungs *in vivo*. Administra-

tion of PD1 in an allergic airway inflammation model dampens airway hyperresponsiveness and accelerated the resolution of airway inflammation (Levy *et al.*, 2007a).

Ongoing collaborative efforts with N Bazan and colleagues at LSU (Marcheselli *et al.*, 2003; Mukherjee *et al.*, 2004; Lukiw *et al.*, 2005) have documented the formation of NPD1 in neural tissues and identified its potent immunomodulatory actions (Serhan *et al.*, 2002, 2006a; Hong *et al.*, 2003) and neuroprotective actions (Marcheselli *et al.*, 2003; Mukherjee *et al.*, 2004; Lukiw *et al.*, 2005) (Table 5). NPD1 limits stroke brain injury in part by reducing the entry of activated PMN to the stroke area (Marcheselli *et al.*, 2003) and retinal pigmented cellular damage and promotes brain cell survival, suppressing Aβ42-induced neurotoxicity (Mukherjee *et al.*, 2004) (Table 5). The protection obtained with NPD1 in the presence of oxidative stress involves upregulation of the antiapoptotic Bcl-2 and Bcl-x(L) in response to exposure to nanomolar amounts of NPD1, whereas the proapoptotic Bax and Bad are downregulated, resulting in reduced caspase-3 cleavage (Lukiw *et al.*, 2005).

In summation, when taken together these results are the first to indicate that novel potent lipid mediators are generated and present within resolving exudates that are derived from the precursors, ω-3 fatty acids EPA and DHA. This work also established that these novel compounds derived from EPA and DHA carry anti-inflammatory and pro-resolving properties and that they are log orders more potent than their precursors *in vivo*. Most importantly, their actions are stereoselective and cell-type specific.

### Conclusion

Endogenous lipid mediators (autacoids) play key roles in local controlling and programming of the acute innate inflammatory response and its resolution as an active biosynthetic process *in vivo* (Serhan, 1994; Serhan *et al.*, 2000). Systematic studies focusing on mechanisms of resolution demonstrated that PMN change their profile of lipid mediators in exudates and switch from producing initiators to stop signals that are also pro-resolving mediators in a process termed 'eicosanoid class switching' (Figure 1). This turns on production of LX biosynthetic machinery (Levy *et al.*, 2001). LX and ATL possess both anti-inflammatory and pro-resolving actions, stopping PMN infiltration and stimulating the nonphlogistic recruitment of monocytes

**Table 5** *In vivo* actions of protectin D1

Disease model	Action	Dosage (route)	Duration of postexposure	Reference
Peritonitis	Reduces PMN infiltration	100 ng per mouse (i.v.) 0.1–100 ng per mouse (i.p.)	2 h 2–4 h	Hong <i>et al.</i> (2003) Serhan <i>et al.</i> (2006a)
	Shortens resolution interval and regulates cytokines/chemokines	300 ng per mouse (i.p.)	4–24 h	Bannenberg <i>et al.</i> (2005)
Stroke	Limits T-cell recruitment in peritonitis	100 ng per mouse (i.v.)	2 h	Ariel <i>et al.</i> (2005)
	Limits stroke damage and PMN entry into the brain	0.4 µg per mouse (perfusion)	48 h	Marcheselli <i>et al.</i> (2003)
Retinal injury	Protects cellular damage from retinal injury			Mukherjee <i>et al.</i> (2004)
Alzheimer disease	Promotes neural cell survival, and reduces amyloid β-42-induced neurotoxicity			Lukiw <i>et al.</i> (2005)
Eye (wound healing)	Promotes corneal epithelial cell wound healing and repair Limits sequelae of thermal injury	1 µg per mouse three times daily (topical)	48 h	Gronert <i>et al.</i> (2005)
Eye (retinopathy)	Reduces vaso-obliteration and neovascularization	10 ng per day (i.p.), from postnatal day 6 to 17	17 days	Connor <i>et al.</i> (2007)
Kidney ischemia-reperfusion	Protects in renal ischemic injury by limiting PMN infiltration	5 µg per mouse (i.v., s.c.)	24–48 h	Duffield <i>et al.</i> (2006)
Asthma	Decreases airway eosinophil, T-lymphocyte and pro-inflammatory mediators	2–200 ng per mouse (i.v.)	4 days	Levy <i>et al.</i> (2007a)

Abbreviation: PMN, polymorphonuclear leukocyte.

*in vivo* (Maddox and Serhan, 1996; Romano *et al.*, 1996; Maddox *et al.*, 1997, 1998; Hachicha *et al.*, 1999) (for reviews, see Serhan, 1994, 1997). These systematic, temporal and differential analyses using combined cell trafficking, lipid-mediator lipidomics informatics and proteomic analyses of the resolving exudates uncovered ω-3 PUFA-derived resolvins (Rv) and protectins (PD). These small molecules have unique structures, are biosynthesized by independent pathways and share anti-inflammatory actions *in vivo*, but each member evokes specific and temporally distinct pro-resolving responses *in vivo*. The epimeric forms of these families of compounds, namely, their AT epimers (ATL, AT-Rv and AT-PD), are endogenously generated during aspirin treatment. These AT forms share the characteristic features of reducing inflammation and PMN-mediated injury from within, key culprits in many widespread human diseases, as well as stimulating resolution. The results summarized here underscore the role(s) of PUFA-derived local mediators in resolution and tissue catabasis (Tables 3–5).

The new families of EPA- and DHA-derived chemical mediators, namely the resolvins and protectins, qualify as 'resolution agonists' along with the *n*-6 derived agonists of resolution, the LX, in this new arena of immunomodulation and tissue protection. These are conserved structures in evolution, because rainbow trout biosynthesize resolvins and protectins, which are present in their neural and hematopoietic tissues (Hong *et al.*, 2005). Their functional roles in fish and lower phyla remain to be established, but are likely to involve cell trafficking, motility and protection. Additionally, they now open new avenues to design 'resolution-targeted'-based therapies where aberrant uncontrolled inflammation and/or impaired resolution are components of the disease pathophysiology. Of interest, one of the authors of this review (CNS) recently served as discussion chair for a National Institute of Dental and Craniofacial Research-hosted programmatic conference of experts to

contemplate the role of controlled resolution of inflammatory responses by LX, Rv and PD and their potential in the molecular design of nanomaterials useful in engineering tissue healing and regeneration (see the NIH commentary in Lumelsky, 2007) as novel means to harness uncontrolled inflammation in biomaterials and engineered tissues. Since LXs act on neural stem cells (Wada *et al.*, 2006), and PD1 is neuroprotective (Mukherjee *et al.*, 2004), it is likely that this new genus of endogenous pro-resolving mediators (Figure 1), their mimetics and stable analogs reviewed herein will not only prove of interest in the control of inflammation and fibrosis but will also open new opportunities for therapies and engineered materials for regenerative medicine. Thus, agonists of inflammatory resolution are likely to have a promising future in drug development for inflammatory diseases and regenerative medicine. This is best exemplified to the *BJP* readership by the recent findings that two of the most widely used drugs worldwide, aspirin and glucocorticoids, have as their *in vivo* mechanism of action the specific ability to generate endogenous mediators of resolution (Perretti *et al.*, 2002; Paul-Clark *et al.*, 2004; Gilroy, 2005; Gilroy and Perretti, 2005; Rossi and Sawatzky, 2007).

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## Conflict of interest

CNS is inventor on resolvin and lipoxin analog patents that are assigned to Brigham and Women's Hospital. These

patents as well as use patents are licensed for clinical development. CNS serves as consultant for companies developing these.

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