Resolvins and protectins in the termination program of acute inflammation

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The physiological resolution of a well-orchestrated inflammatory response is essential to maintain homeostasis. Therefore, gaining a comprehensive understanding in molecular terms of the events that direct the termination of acute inflammation is imperative. Recently, new families of local-acting mediators were discovered that are biosynthesized from the essential fatty acids eicosapentaenoic acid and docosahexaenoic acid. These new chemical mediators are endogenously generated in inflammatory exudates collected during the resolution phase, and were termed resolvins and protectins because specific members of these families control the magnitude and duration of inflammation in animals. In addition, recent results indicate novel actions of resolvins and protectins in removing chemokines ferried from the tissue by apoptotic neutrophils and T cells during resolution. Here, we review recent advances on the biosynthesis and actions of these novel anti-inflammatory and proresolving mediators.

Novel pathways to active resolution

The first indications of the indispensable roles of omega-3 fatty acids (PUFAs; see Box 1) in health date back to 1929 [2]. Nonetheless, the mechanism of action of the major omega-3 PUFAs, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in human systems is, for the most part, under scrutiny, with the general view that both are beneficial in human disease without clear in vivo evidence for a molecular basis of their numerous reported actions [3–5]. In recent years, inflammation has emerged as a new and major process underlying many prevalent diseases. These include Alzheimer’s disease, cardiovascular disease [6], and cancer [7], which now join the well-known inflammatory disorders such as arthritis and periodontal disease [8,9]. Elucidating the mode of action of omega-3 PUFAs, shown to be beneficial in many reports, is still an important challenge for evidence-based medicine. In recent years, novel enzymatic oxygenated products generated in vivo were identified in murine systems and in humans by pathways initiated from the precursors EPA and DHA. These new families of compounds are biosynthesized and contribute functionally to the resolution of inflammatory exudates [10–12] and to neuroprotection [13–15]. The term resolvins (derived from ‘resolution phase interaction products’) was first introduced to signify that the new structures were distinct, endogenous local mediators possessing stereospecific and potent anti-inflammatory in addition to immunoregulatory actions [11]. These include reducing neutrophil trafficking, cytokine and reactive oxygen species regulation, and lowering the magnitude of the inflammatory response [10,11]. The terms protectin D1 (PD1), and neuroprotectin D1 (NPD1; when generated in neural tissues) [16], were introduced given the general anti-inflammatory [12] and protective actions of this unique 10R,17S-dihydroxy-docosatriene in neural systems [14], stroke [13], animal models of Alzheimer’s disease [15], and peritonitis [16].

These new families of anti-inflammatory and proresolving mediators [10–12] contrast with the earlier

Box 1. Abbreviations used

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AA</td>
<td>arachidonic acid</td>
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<tr>
<td>CCR5</td>
<td>C-C chemokine receptor 5</td>
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<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
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<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
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<tr>
<td>LO</td>
<td>lipoxigenase</td>
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<tr>
<td>LTB₄</td>
<td>leukotriene B₄</td>
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<tr>
<td>LX₄</td>
<td>lipoxin A₄</td>
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<tr>
<td>NPD1</td>
<td>neuroprotectin D1</td>
</tr>
<tr>
<td>PD1</td>
<td>protectin D1</td>
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<tr>
<td>PMN</td>
<td>polymorphonuclear leukocyte</td>
</tr>
<tr>
<td>PUFAs</td>
<td>polyunsaturated fatty acids</td>
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<tr>
<td>Rv</td>
<td>resolvins</td>
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* Competing interests statement. The lipoxins and resolvins are biotemplates for stable analogs. Patents on these are assigned to Brigham and Women’s Hospital, and C.N. Serhan is the inventor. These analog patents are licensed for clinical development and are the subject of consultantships for C.N. Serhan.

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omega-3 PUFA-derived oxygenated products that possess similar structures to previously known arachidonic acid-originated eicosanoids, but were less potent proinflammatory mediators or devoid of bioactions (e.g. see Ref. [17]). Members of the resolvin and protectin families specifically evoke potent stereoselective bioactions evident in the nanomolar and picomolar ranges in vitro and in vivo [10–14,16] (Table 1).

### Anti-inflammatory lipid mediators in inflammation and resolution

Early studies of bioactive lipids introduced the concept that arachidonic acid is released from cellular stores and transformed by both cyclooxygenase (COX) and lipoxygenase (LO) mechanisms to several series of potent bioactive eicosanoids: prostaglandins, leukotrienes and lipoxins [18,19]. Most of the classic prostaglandin (PG) and leukotriene mediators are proinflammatory in addition to having specific and important roles in the reproductive system. It was assumed that these same mediators were formed and served in the initiation and termination of acute inflammation, in addition to the transition from acute to chronic inflammation [20]. However, in sharp contrast to the proinflammatory cascade of mediators, it has become clear that counter-regulatory substances, such as lipoxins, glucocorticoids and their product annexin 1, adenosine, PGE2 or PGD2 and its breakdown product PGJ2, and transforming growth factor (TGF)β, are generated during the resolution of acute inflammation to serve in the healthy termination of an acute response [21–24]. The first evidence that the resolution of inflammation is an active rather than a passive process, as noted in the Majno and Joris textbook [20], came with the discovery of pro-resolution biochemical signaling circuits that generate and regulate lipoxins and their nonphlogistic actions on human monocytes [25,26].

The isolation and identification of resolvins and protectins biosynthesized from omega-3 PUFAs provides a paradigm shift and an arsenal of new lipid mediators that are generated during the resolution phase, and actively promote the termination of inflammation [27,28] and the return of local tissues to homeostasis [27] (see later). The recognition that resolution is an active process and that activation of the lipoxin biosynthetic circuit and lipoxins themselves, in addition to aspirin-triggered lipoxins and their stable analogs, are potent agonists of anti-inflammatory in vivo and in many disease models (reviewed in Ref. [27]), led to concentrated efforts aimed at understanding the biochemical and molecular events activated during healthy resolution of acute inflammatory responses and ischemia reperfusion injury.

### Omega-3 PUFAs and aspirin get the jump on resolution – specialized chemical mediators in resolution

The Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardico (GISSI) results showed a reduction in sudden death of ~45% in >11,000 patients with cardiovascular disease, when taking almost a gram of omega-3 per day reduces the clinical manifestations of many diseases including inflammatory disorders and cancer [3]. In view of these observations, the mechanism(s) of the actions of this omega-3 in humans were in question. Several monohydroxy-containing products are generated from omega-3 PUFAs by the three major human LOs (5-LO, 12-LO and 15-LO). The biological importance of these, if any, was not known earlier [17,31,32]. Thus, it seemed that the long quest for elucidation of the biochemical pathways that mediate the beneficial actions of omega-3 PUFAs was about to take a new course.

Although it is clear that aspirin inhibits prostaglandin biosynthesis and, hence, a key mechanism in anti-inflammatory therapy [33], and that aspirin has well-appreciated clinical uses and ability to limit leukocyte traffic into
sites of inflammation, the key player(s) in terminating inflammation were still unknown. A self-limited resolving dorsal skin pouch model \[10,11,28,34\] was used to assess genetic contributions to resolution and develop mediator informatics and lipidomics \[35\]. These databases and informatics were geared to evaluate the role of eicosanoids and determine whether novel lipid mediators were indeed generated during the resolution phase of inflammation \[10,11\]. In this experimental system of contained inflammation, focus was directed toward the period when neutrophils are lost from the exudate and the tissue sites seem to resolve. This event was previously termed ‘spontaneous resolution’ \[34\]. In this phase of the response \textit{in vivo}, novel bioactive mediators were identified and their structures were elucidated, in particular when aspirin treatment was administered.

**Resolvins from aspirin-initiated pathways – 18R E series and 17R D series**

Resolving exudates contained bioactive compounds that reduced inflammation \textit{in vivo} and blocked human neutrophil transendothelial migration. Microscale structural elucidation gave the basic structure of the potent bioactive product generated in exudates from EPA, namely the presence and positions of the alcohol groups and double bonds in the bioactive compound, to be 5,12,18R-trihydroxy-eicosapentaenoic acid \[10\].

Pathway reconstruction using isolated human cells \textit{in vitro} determined that tandem transcellular conversions by acetylated COX2 from vascular endothelial cells treated with aspirin and LO in activated human polymorphonuclear leukocytes (PMNs) generate the bioactive 5,12,18R-trihydroxy-eicosapentaenoic acid from EPA. The biosynthesis of other members of the resolin E family is also initiated through the aspirin-COX2 pathway (Figure 1). The bioactive product 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid was termed resolvin E1 (RvE1) because of its anti-inflammatory and novel proresolving properties and unique structure. Also, a ligand-specific receptor for RvE1 (denoted Chem R23) was identified, and the complete stereochemistry of RvE1 and its geometry required for potent receptor activation in the pico- to nanomolar range was determined (see the supplementary data for Ref. \[36\]).

The resolving exudates from mice given aspirin plus DHA also contained novel 17R-hydroxy-DHA (17R-HDHA) and two novel families of bioactive compounds. Both families, resolvins and protectins, are also biosynthesized through LO mechanisms (Figure 1) without aspirin to yield the 17S series of resolvins and protectins, respectively. Of interest, lipoxins are also generated by aspirin-triggered pathways to give the 15R-hydroxy-containing lipoxins and initiated by LO to yield the 15S-hydroxy-containing lipoxins. Both lipoxin series are biosynthesized from arachidonic acid. The acetylated COX2 initiates the 15R-hydroxy-lipoxin triggered by aspirin and the 15-LO initiates native lipoxins (Figure 1). Thus, distinct families of potent anti-inflammatory and proresolving lipid mediators from different precursors can be generated by aspirin-triggering or, physiologically, through LO pathways. This gives aspirin the jump on resolution, speeding the reduction in inflammation and onset of resolution.

**Physiologically generated resolvins of the 17S D series and protectins**

Using this lipid mediator informatics approach, it was found that neither aspirin nor exogenous DHA was required to produce resolvins of the 17S D series or protectins \textit{in vivo}. Endogenous DHA is converted through LO pathways, both \textit{in vitro} and \textit{in vivo}, to a potent mediator termed PD1, which carries a 10,17S-dihydroxydocosatriene basic structure (referring to the position and presence of double bonds and alcohol groups) \[11,12,37\]. PD1 (or NPD1) proved to be log orders of magnitude more potent than its precursor DHA, an indication that it is a chemical autacoid \[11,12\], and its complete stereochemistry (i.e., the configuration of its double bonds and 10-position alcohol) was established for the biosynthesized product, which possesses potent bioactions and proved to be 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid \[16\]. It is noteworthy that the formation of potent bioactive mediators from DHA was proposed as early as 1984 \[38\]. A series of collaborative studies with Bazan and colleagues \[13–15\] documented the formation of NPD1 and identified actions that suggest it is an important mediator in neural systems (Table 1). The identification of the resolvins and protectins generated from DHA now opens avenues for exploring the essential roles of these pathways and mediators in human systems. Their basic structures and actions have been established (see later).
Early results indicated that DHA modulates T cell functions to favor a Th2 helper 2 (Th2) phenotype [39], has diminished responses to CD28 engagement [39], and increased cell death [40]. However, the mechanism that mediates the actions of DHA on T cells is still under investigation. Studies with Th2-skewed human peripheral blood mononuclear cells revealed expression of 15-LO-1 by T cells and 15-LO-dependent production of PD1 in these cultures [37]. In addition, PD1 blocked anti-CD28-induced interferon γ and tumor necrosis factor (TNF)α production from T cells, suggesting it is substantially involved in the Th1-counteracting properties of Th2 cells [41,42]. Moreover, PD1 induces T cell apoptosis [37], suggesting a disruption of the survival signal enhanced by CD28 [43]. These findings suggest PD1 is a major DHA conversion product that is generated during Th2 polarization and promotes anti-inflammation.

### Disease models and resolvins and protectins

Resolvins of the E series comprise several molecules. Among them, RvE1 was the first to be isolated and, hence, to date, studied in the most detail. Nanomolar levels of RvE1 dramatically reduce neutrophil transendothelial migration, dermal inflammation [11,36], peritonitis, dendritic cell migration and interleukin (IL)-12 production in complex disease models (Table 1). Of interest, administering RvE1 prepared by total organic synthesis blocks PMN infiltration in periodontal disease in rabbits [44] and protects against the development of colitis [45]. In several animal models of inflammatory diseases, RvE1 has proven to be a potent counter-regulatory mediator that protects tissues against leukocyte-mediated injury and excessive expression of proinflammatory genes (Table 1).

Resolvins of the D series block tumor necrosis factor (TNF)α-induced IL-1β transcripts in microglial cells and also function as potent regulators limiting PMN infiltration into inflamed brain, skin and peritoneum [11–13]. Direct comparisons between resolvins of the D series (both the 17S and the 17R epimer aspirin-triggered series) demonstrated that each is a potent regulator of PMN infiltration in vivo [12]. These findings suggest that the aspirin-triggered 17R epimers of protectins and resolvins each serve as the body’s own anti-inflammatory mediators in response to aspirin.

All compounds within the protectin family possess the conjugated triene-containing structure as the key feature of this new family derived from DHA. The 10,17S-docosatriene, termed PD1 (or NPD1 when generated in neural tissues), proved to be a potent regulator of PMN influx into exudates at the site where it is formed locally from endogenous precursors [11,12]. Each of several additional dihydroxy-docosanoids (both positional and geometric isomers) were identified and tested, and were substantially less active than PD1 [12,14,16]. PD1 (or NPD1) exhibits potent actions in multiple organ systems in vitro and in vivo (Table 1). PD1 prepared by total organic synthesis 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 PMNs in vivo by reducing PMN infiltration (~40% using 1 ng per mouse) in murine peritonitis. PD1 also reduced PMN infiltration when administered after the initiation of inflammation in vivo and acts in an additive fashion with RvE1 to stop PMN infiltration. Hence, PD1 proved to be a potent, stereoselective anti-inflammatory molecule [11,12,16]. These results, and those obtained by Bazan and colleagues in neural tissues [13–15], demonstrate that PD1 possesses potent immunoregulatory [11,12,16] and neuroprotective actions [13–15], and promotes wound healing [46]. PD1 (or NPD1) limits brain injury arising from stroke [13] and retinal pigmented cellular damage and promotes brain cell survival, suppressing Aβ42-induced neurotoxicity [14] (Table 1). Protection following oxidative stress with NPD1 involves primarily the regulation of apoptotic pathways [15]. These findings indicate that novel and potent anti-inflammatory lipid mediators such as PD1 are derived from precursor omega-3 fatty acids, and are physiologically generated and present in resolving exudates.

A recent report by Levy et al. documented lower levels of PD1 in exhaled breath condensates of patients with exacerbated asthma, compared with healthy subjects [47]. In addition, administering PD1 before Aeroallergen challenge in a murine model of asthma resulted in decreased airway eosinophil and T lymphocyte recruitment and airway mucus, in addition to diminished levels of specific proinflammatory mediators, including IL-13, cysteinyl leukotrienes and PGD₂, and a reduction in airway hyperresponsiveness to inhaled methacholine. These results are in line with the in vitro observations in Th2 cultures [37], and together underscore significant production of PD1 by human immune cells in Th2-governed milieu and potent bioactions in Th2-mediated immune responses.

### RvE1 and PD1 facilitate chemokine removal during resolution

Chemokines and their receptors [e.g. C-C chemokine receptor 5 (CCR5)] are important mediators in the pathogenesis of inflammation and autoimmune disorders [48]. To return to homeostasis and self-limit the continuous infiltration of immune cells to inflamed sites even when resolution takes over [20], an active counter-regulation or shutdown of proinflammatory mediators, including chemokines, is necessary (reviewed in Ref. [49]). Currently, chemokine proteolysis is believed to be the major mechanism responsible for neutralizing or reducing chemokine activity and, thus, local levels through proteases including elastase, cathepsin G, matrix metalloproteases, CD26 and others [50]. However, these proteolytic modulations do not account for the inactivation of CCR5 signaling following binding to its processed ligands [50–53]. Other mechanisms for abolishing CCR5 signaling, including scavenging by ‘silent’ receptors or functional decoys, were also reported [50,54,55].

Using zymosan A-initiated peritonitis for a comprehensive mapping of microbial inflammation and its resolution in mice [56,57], a swift increase was found in the level of most proinflammatory cytokines and chemokines in peritoneal exudates, reaching maximal levels after 2–4 h, followed by a rapid decline in the levels of most proinflammatory mediators in the following 8 h [56]. Observations along these lines in other models of acute
inflammation were also provided recently [58,59]. A novel mechanism that could serve to dampen chemokine activity, stop leukocyte infiltration and clear inflammatory sites was recently identified [57]. Apoptotic PMNs and T cells evolve into a professional chemokine-scavenging device as they express high levels of CCR5. These in vivo results characterize a novel property for apoptotic PMNs: namely, that they scavenge chemokines during the resolution of acute inflammation. These findings, combined with earlier evidence indicating that resolution is promoted through apoptotic cell engulfment and processing by phagocytes [60–62], complete the sequel of CCR5 ligand clearance. As illustrated in Figure 2, PMNs and T cells that are attracted to sites of inflammation by CCR5 ligands undergo apoptosis, resulting in increased CCR5 expression. This increment in CCR5 expression facilitates the scavenging of CCR5 ligands, which is followed by clearance through macrophage engulfment. The removal of proinflammatory chemokines from the resolving milieu would limit further leukocyte recruitment, prevent excessive immune response and tissue damage, and promote a return to catabasis.

Figure 2. Apoptotic leukocytes, chemokine sequestering and the proresolving lipid mediators LXA4, RvE1 and PD1 in the resolution of inflammation. Resolving pathways involving apoptotic leukocyte engulfment and chemokine sequestering, and their regulation by proresolving lipid mediators in the resolution of inflammation, are illustrated. Neutrophils that infiltrated into the inflamed tissue undergo apoptosis during resolution of the immune response. These apoptotic cells express CCR5 on their surface. Next, CCR5 ligands are sequestered by the apoptotic leukocytes that are then engulfed by resolving macrophages in a non-phlogistic manner. This process clears apoptotic cells to complete the resolution sequel. The removal of CCR5 ligands from the inflamed milieu is also mediated through degradation with secreted or cell-surface-expressed proteases. Other sequestering routes involve binding in tandem with internalization and lysosomal degradation by cells that express silent receptors, such as D6, or functional decoys, such as lipopolysaccharide- or IL-10-treated macrophages (for reviews, see Refs. [50,66]). Chemokine removal from the resolving site prevents further leukocyte recruitment by eliminating the chemoattractant gradients, thereby promoting catabasis. Proresolving lipid mediators regulate this sequence of events in resolution at three junctures: (i) LXA4, RvE1 and PD1 upregulate CCR5 expression on apoptotic neutrophils [57]; (ii) LXA4 enhances apoptotic PMN engulfment by macrophages [68]; and (iii) LXA4, RvE1 and PD1 limit PMN infiltration to inflamed sites [10,12,69]. The engulfment of apoptotic leukocytes leads to the expression of 15-LO in the non-phlogistic macrophages that release LXA4 [61] to limit inflammation further by stopping PMN influx and accelerating the macrophage removal of apoptotic PMN. Therefore, lipid mediators of resolution are potent regulators of apoptosis-associated resolving events with leukocytes.
It was also found that mediators of inflammation and its resolution counteract in regulating CCR5 expression on PMNs undergoing apoptosis. TNFα reduced CCR5 expression on PMNs after spontaneous apoptosis. By contrast, the resolution-promoting lipid mediators LXA₄, RvE1 and PD1 increase CCR5 expression on apoptotic PMNs [57]. In addition, unlike proinflammatory lipid mediators such as LTB₄ that delay PMN apoptosis [63], these proresolving mediators do not enhance PMN apoptosis. Therefore, these mediators strictly increase the expression of CCR5 on PMNs that have already initiated and engaged in apoptosis [57]. This is a novel property that can now be attributed to resolvins and protectins, in addition to lipoxins.

A recent report from Rossi et al. [64] demonstrated that the inhibition of cyclin-dependent kinases resulted in the inhibition of cyclin-dependent kinase (CDK) inhibitors might be useful as therapeutic agents in inflammatory pathologies given their resolution-enhancing capabilities.

Enhancing chemokine sequestering to limit inflammation is a strategy that was adopted by viruses and parasites to escape the immune system [66]. The production of LO-derived proresolving lipid mediators is another tactic used by pathogens to evade host defense [56]. The recent functions in resolution attributed to apoptotic leukocytes [57,61] suggest that the induction of leukocyte apoptosis by pathogenic viruses and bacteria has greater immune-debilitating potential than initially appreciated, and in fact facilitates diverse proresolving pathways. It is tempting to suggest that evolutionary-developed tactics used by microbial pathogens to limit immune responses can direct future therapeutic efforts to new areas.

Concluding remarks

Results accumulated during the past three decades indicate that the resolution of inflammation is a tightly regulated process, controlled by distinct mediators and cellular moieties [24]. This notion leads to a fresh approach in developing therapies for inflammatory disorders, in which small molecular proresolving mediators are sought after to be used as ‘inflammation terminators’. These potentially therapeutic agents should enhance or restore missing physiological resolution, and eventually replace commonly used drugs aimed at stopping the inflammatory response in an artificial manner.

The recently uncovered families of EPA- and DHA-derived chemical mediators reviewed here, namely the resolvins and protectins, now open new possibilities to design ‘resolution-targeted’ therapies for controlling unwanted side effects of excessive inflammatory responses. These omega-3-derived lipid mediators are autacoids and function as agonists at temporally and spatially distinct steps in the progression of inflammation and its resolution [156] and reviewed in Ref. [67]).

Systematic analyses focusing on mechanisms of resolution [10,11] have uncovered omega-3 PUFAs as endogenous precursors of resolvins and protectins. The aspirin-triggered epimeric forms of both families of chemical mediators, that is, the aspirin-triggered epimers of the resolin and protectin families, are generated endogenously with aspirin treatment in several murine models in vivo [11,12]. These aspirin epimers share the characteristic structural features and bioactions that dampen inflammation and PMN-mediated injury from within. These are key pathologic inflammatory and tissue injury processes that are now well-recognized in many widely occurring human diseases. Aspirin seems to be unique among anti-inflammatory drugs in that aspirin initiates resolution by generating natural epimers of resolution mediators. The potent bioactivity and specific actions within resolution of the lipoxins [23], resolvins and protectins (Table 1) and their mimetics now establish them as agonists of resolution and tissue protection within this new arena of regulated resolution, and dictate their further exploration.

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