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Deciphering the role of *n*-3 polyunsaturated fatty acid-derived lipid mediators in health and disease

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Accumulating evidence indicates that, analogous to *n*-6 PUFA, *n*-3 PUFA are enzymatically converted into diverse families of bioactive mediators that play numerous roles in physiology. These mediators, which include the resolvins, protectins and maresins, are particularly important in resolving acute inflammation and also appear to play a role in enhancing host defence. Given the protective actions of *n*-3 PUFA in human subjects and in animal models of disease, active generation of bioactive mediators may in part underlie these protective effects. Several studies have demonstrated that bioactive autacoids generated from *n*-3 PUFA have direct anti-inflammatory and pro-resolution actions, and the structures of many of these endogenous mediators have been elucidated. The diverse roles of these lipid mediators in health and disease, regulation of their biosynthesis, as well as identification of specific receptors and cellular targets, are emerging. This brief review will highlight the biosynthesis of resolvins, protectins and maresins, and discuss their receptor-mediated biological actions in promoting the resolution of inflammation. Their potential use as a new class of pro-resolution therapeutics, as well as gaps in knowledge and challenges for future research, will also be discussed. Overall, the identification of these novel families of lipid mediators has yielded insight into the protective actions of *n*-3 PUFA and may lead to the development of an entirely new class of therapeutics aimed at regulating inflammation and host defence.

Resolvins: Resolution of inflammation: DHA: EPA

PUFA of both the *n*-6 and *n*-3 classes are essential to health and must be obtained in the diet. The concept that certain types of fat are essential in the diet was put forth initially in studies of rodents consuming a fat-depleted diet, which was associated with a marked appearance of dry skin, brittle hair and skin rashes; effects that were reversed by fat replenishment[1]. A similar phenotype is apparent in human subjects with essential fatty acid deficiency, with symptoms appearing rapidly (< 1 week) in infants when their essential fatty acid levels are < 5% of total energy intake[2]. More than 80 years of research has now defined that essential fatty acids, which are components of major lipid classes including phospholipids, TAG and cholesterol esters, serve numerous roles in physiology[3,4]. These diverse functions include the regulation of cell membrane

Abbreviations: COX, cyclooxygenase; GPCR, G-protein coupled receptor; Hp, hydroperoxy; LOX, lipoxygenase; MaR1, maresin-1; PD1, protectin D1; PMN, polymorphonuclear neutrophil; RvD1, resolvin D1 (7S, 8R, 17S-trihydroxy-docosa-4Z, 9E, 11E, 13Z, 15E, 19Z-hexaenoic acid); RvD2, resolvin D2 (7S, 16R, 17S-trihydroxy-docosa-4Z, 8E, 10Z, 12E, 14E, 19Z-hexaenoic acid); RvE1, resolvin E1 (5S, 12R, 18R-trihydroxy-eicosa-6Z, 8E, 10E, 14Z, 16E pentaenoic acid); RvE2, resolvin E2 (5S, 18R-dihydroxy-eicosa-6E, 8Z, 11Z, 14Z, 16E, pentaenoic acid); SPM, specialised pro-resolving mediator.

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dynamics and cell signalling, providing a source of energy (through β-oxidation), and serving as the precursors to bioactive lipid mediators[4–6].

While mammals have the ability to elongate and desaturate both *n*-3 and *n*-6 PUFA, the biosynthetic starting materials for these pathways, namely α-linolenic acid (18:3*n*-3) and linoleic acid (18:2*n*-6), respectively, must be obtained in the diet[7–9]. Linoleic acid can be further converted into arachidonic acid (20:4*n*-6) through a series of elongation and desaturation reactions, whereas α-linolenic acid is similarly converted into EPA (20:5*n*-3) and DHA (22:6*n*-3)[9]. As such, EPA and DHA are not considered essential in the diet, but it should be noted that the conversion of α-linolenic acid into EPA and DHA is very low in human subjects and thus nutritional strategies designed to address deficiencies in *n*-3 PUFA generally utilise marine-based sources that are naturally high in EPA and DHA[2,8,9]. Importantly, studies of the biological role of *n*-3 PUFA in health and disease have been aided by the generation of transgenic mice that express an *n*-3 desaturase gene (denoted *fat-1*) not normally found in mammals that enables endogenous generation of *n*-3 PUFA from *n*-6 PUFA[10]. These mice show substantial increases in tissue levels of both EPA and DHA and are protected from inflammation and tissue injury in a myriad of animal models of disease, including colitis, cancer and pathologic angiogenesis[10–12].

While both *n*-6 and *n*-3 PUFA are essential for health, it is well-documented that the typical Western-type diet is substantially enriched in *n*-6 PUFA, shifting the balance from the optimal *n*-6:*n*-3 ratio of 1–2:1 to approximately 20:1[13]. This altered ratio of PUFA is associated with the development of numerous chronic diseases, including CVD and rheumatoid arthritis, potentially because of an imbalance between bioactive lipid mediators that are involved in regulating inflammation[8] (see later). Indeed, several clinical studies have determined that enriching the diet in *n*-3 PUFA improves outcomes in diseases such as rheumatoid arthritis[3,8]. *n*-3 PUFA are important during fetal and infant development and may be effective in preventing essential fatty acid deficiency in infants requiring parenteral nutrition[2]. The protective effects of *n*-3 PUFA are particularly convincing for CVD, such as atherosclerosis and its associated cardiac manifestations including acute myocardial infarction and heart failure[14,15]. Building upon observational studies demonstrating a low incidence of CVD in Greenland Eskimos, who have a diet rich in *n*-3 PUFA from marine sources, interventional studies have shown that dietary supplementation with purified fish oil extracts reduces the incidence of CVD[15]. In particular, the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico Prevenzione trial, which enrolled patients with a history of myocardial infarction, demonstrated reduced risk of cardiovascular death with fish oil supplementation[16]. A similar improvement in survival was also seen in heart failure patients[17]. As a testament to these and related clinical studies, the American Heart Association currently recommends eating fish rich in *n*-3 PUFA for the prevention of CVD (www.americanheart.org).

Although several epidemiological and interventional studies have established a beneficial role for *n*-3 PUFA in health and disease, the specific mechanisms whereby they exert protective actions are still emerging. Certainly, anti-arrhyrthmogenic effects via direct membrane incorporation of *n*-3 PUFA have been demonstrated and this may be related to the protective effects of *n*-3 PUFA in the context of sudden cardiac death[7]. Other mechanisms include the generation of less-potent inflammatory mediators (i.e. 3-series prostanoids and 5-series leukotrienes) that compete for arachidonic acid metabolism and pro-inflammatory effects of eicosanoids[4,8]. However, over the last decade, it has also emerged that *n*-3 PUFA, including both EPA and DHA, are converted into novel families of bioactive lipid mediators that are potent immunomodulatory agonists. This short review will highlight the biosynthesis and biological actions of these mediators, which include the resolvins, protectins and maresins, and discuss the potential for targeted therapeutics based on these specific mediators.

Bioactive lipid mediators generated from *n*-3 PUFA: biosynthetic pathways

It is well established that bioactive lipid autacoids are generated from PUFA in an enzymatic manner. Prominent examples include the generation of prostanoids and leukotrienes derived from cyclooxygenase (COX)- and lipoxygenase (LOX)-mediated conversion of arachidonic acid (20:4, *n*-6). Similarly, arachidonic acid is also a substrate for monoxygenases (i.e., cytochrome P450) that give rise to epoxyeicosatrienoic acids. Collectively, these mediators play numerous physiologic roles, including regulation of haemodynamics, inflammatory cell trafficking and blood coagulation[5,18–20]. More recently, it has been demonstrated that, like *n*-6 PUFA, *n*-3 PUFA, including EPA and DHA, can also serve as substrates for both COX and LOX enzymes and give rise to several new families of bioactive mediators[6,21].

Resolvins

Resolvins are a family of lipid mediators generated in an enzymatic manner from EPA (E-series) or DHA (D-series)[21–24]. They are so named because they were originally identified during the resolution phase of inflammation and were found to potently regulate this critical process[23] (see later). While EPA can serve as a substrate for COX-2 to give rise to 3-series prostanoids, acetylation of COX-2 by aspirin permits the generation of 18-HEPE[25,26]. Of note, 18-HEPE can also be formed through a P450-dependent route[26]. This product can be further converted by 5-LOX to 5*S*,12*R*,18*R*-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-EPA, which was later coined resolvin E1 (RvE1; Fig. 1a)[23,25]. This latter biosynthetic route is similar to that of leukotriene B4, in that the 5,6 epoxide formed

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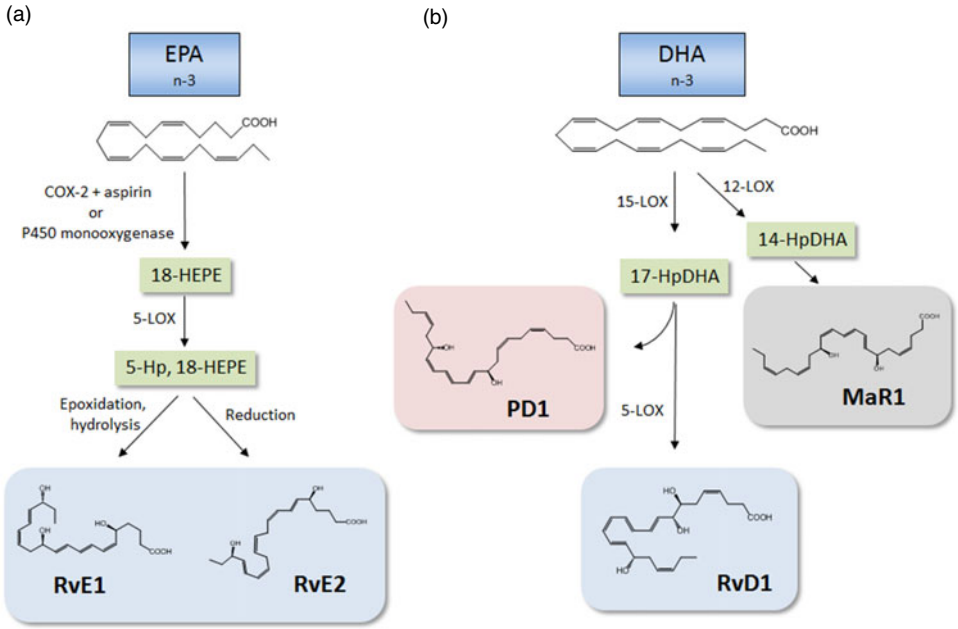

Fig. 1. (colour online) Biosynthesis of pro-resolving lipid mediators from EPA and DHA. (a) EPA serves as the substrate precursor for the E-series resolvins. In the presence of aspirin, acetylated cyclooxygenase (COX)-2 utilises EPA as a substrate and produces 18-HEPE. This intermediate, which can also be generated by a P450 route, can serve as a substrate for 5-lipoxygenase (LOX) to give rise to 5-hydroperoxy (Hp)-18-HEPE. Epoxidation and enzymatic hydrolysis generates resolvin E1 (RvE1), whereas 5-Hp, 18-HEPE can also be directly reduced to generate resolvin E2 (RvE2). (b) DHA is converted to 17-HpDHA by 15-LOX, which, through the formation of an epoxide intermediate, can form protectin D1 (PD1). Conversely, 17-HpDHA can be further converted by 5-LOX to generate resolvin D1 (RvD1). In addition to 15-LOX, DHA can also serve as a substrate for 12-LOX, giving rise to 14-HpDHA, which through enzymatic epoxidation and hydrolysis, gives rise to maresin 1 (MaR1).

by 5-LOX can be enzymatically hydrolysed by leukotriene A4 hydrolase to yield RvE1[27]. More recent studies using chiral chromatography demonstrated that both 18*S*-HEPE and 18*R*-HEPE are generated by acetylated COX-2 and are associated with the generation of 18*S*-RvE1 and 18*R*-RvE1, respectively[27]. Importantly, both mediators are bioactive and share the same specific receptor (see later). While the formation of an epoxide group and enzymatic hydrolysis mediates RvE1 biosynthesis, the 5-hydroperoxy (Hp) group can also be directly reduced to give rise to 5*S*,18*R*-dihydroxy-6E,8Z,11Z,14Z,16E-EPA, which is denoted RvE2[28,29]. This structurally unique mediator is also bioactive. More recently, a third member of the E-series resolvin family was discovered and its structure was determined. Like RvE2, resolvin E3 is a dihydroxy-containing product generated from EPA by a 12/15-LOX pathway as opposed to the 5-LOX pathway involved in RvE1 and RvE2 biosynthesis[30]. This new pathway, which is operative in eosinophils, generates two distinct stereoisomers, namely 17*R*, 18*S*-diHEPE and 17*R*, 18*R*-diHEPE, both of which are bioactive in murine models of acute inflammation[30].

Like EPA, DHA can also be converted into a family of bioactive lipid mediators generated during the resolution phase of inflammation, which are denoted D-series resolvins[6]. The common biosynthetic intermediate in D-series resolvin biosynthesis is 17-Hp DHA, which can be generated by either 15-LOX or acetylated COX-2 (Fig. 1b). However, while 15-LOX generates predominately 17*S*-HpDHA, acetylated COX-2 generates 17*R*-HpDHA and thus gives rise to 'aspirin-triggered' resolvins[31]. In either case, transcellular biosynthesis of D-series resolvins proceeds through 5-LOX, which generates a series of distinct compounds denoted resolvins D1 (RvD1)–RvD6. RvD1–D4 are trihydroxy-containing regioisomers generated via enzymatic hydrolysis of either a 7, 8 epoxide (RvD1 and RvD2) or a 4, 5 epoxide (RvD3 and RvD4)[21,32]. In contrast, RvD5 and RvD6 are dihydroxy-containing mediators that arise through a peroxidase mechanism in which the C_4 or C_7 hydroperoxide formed prior to epoxidation is converted into a hydroxyl group at position 4 in the case of RvD6 or position 7 in the case of RvD5[21]. For a detailed description of the precise stereochemistry, total organic synthesis and biosynthetic pathways giving rise to D-series resolvins, the reader is referred to a recent review by Serhan and Petasis[21]. In all cases, these diverse biosynthetic routes giving rise to D-series resolvins generate mediators with distinct stereochemistry and double-bond geometry that in turn dictate their biological actions.

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Protectins

The protectins, or neuroprotectins when generated in neural tissues, are so named because they were initially documented to have tissue protective actions in the context of neural injury associated with ischemic stroke[33–35]. The main member of this family is protectin D1 (PD1), which like D-series resolvins, is generated from DHA (Fig. 1b). The biosynthesis of PD1 proceeds via 15-LOX-mediated conversion of DHA to 17-HpDHA that, in the absence of 5-LOX (which enables biosynthesis of D-series resolvins), forms a 16,17 epoxide that is hydrolysed to 10*R*, 17*S*-diHDHA through the conjugated triene system[21]. Analogous to the leukotriene biosynthetic pathway, an isomer of PD1 can be generated from 17-HpDHA via double dioxygenation[36,37]. This isomer has an *E,Z,E* double-bond geometry, in contrast to the *E,E,Z* geometry of the conjugated triene system in PD1 and the stereochemistry of the hydroxyl group at position 10 is in the *S* configuration. Although less potent than PD1, this double-dioxygenation product reduced polymorphonuclear neutrophil (PMN) infiltration in an acute model of sterile inflammation and also appears to regulate platelet activation[36,37].

Maresins

The maresins are the newest family of pro-resolving lipid mediators generated from DHA and are so named because they are generated by macrophages (*ma*crophage mediators in *res*olving *in*flammation)[38]. Maresin-1 (MaR1) is the first member of this family and is generated from DHA by a LOX-dependent mechanism (Fig. 1b)[38]. While 15-LOX forms predominately 17-HpDHA when DHA is the substrate, the murine isoform of 15-LOX, namely 12/15-LOX, also generates a 14-hydroperoxide. Similarly, this product is also generated by 12-LOX, which is likely involved in the biosynthesis of 14-HpDHA in human subjects. This 14-HpDHA product can undergo epoxidation to form a 13,14 epoxide, which similar to the biosynthesis of PD1, can be hydrolysed to generate 7*R*, 14*S* dihydroxyDHA (MaR1) through the conjugated double-bond system (Fig. 1b)[38–40]. Of note, 14-HpDHA can also serve as a substrate for 5-LOX to generate double-dioxygenation product, 7*S*, 14*S*-diHDHA, which is markedly less potent than MaR1 in resolving acute inflammation *in vivo*[38].

Resolving inflammation and *n*-3 PUFA derived mediators

Complete resolution is the normal outcome of acute inflammation and is required to re-establish tissue homoeostasis. Key checkpoints in the resolution of inflammation include the termination of PMN infiltration into the injured tissue, the timely apoptosis of PMN, active clearance of apoptotic PMN, microbes and other dead tissue by macrophages, and the efflux of phagocytes from the injured area[41,42]. If these events are not precisely controlled, inadvertent tissue damage can occur and the later events of the wound healing response, such as angiogenesis and re-epithelialisation are impaired. It is these cell-mediated events in active resolving inflammation that are regulated by specialised pro-resolving lipid mediators (SPM), which include the resolvins, protectins and maresins.

As detailed earlier, these lipid mediators are generated in an enzymatic manner by enzymes expressed primarily in leucocytes, although other cell types, such as epithelial and endothelial cells could also be involved in transcellular SPM biosynthesis. While our current understanding of the precise mechanisms governing a switch from pro-inflammatory lipid mediator to SPM biosynthesis is incomplete, it should be noted that LOX and COX enzymes are subject to dynamic transcriptional regulation. For example, it is well documented that engagement of pattern recognition receptors initiates inflammatory signalling pathways giving rise to increased transcriptional regulators of 5-LOX and COX-2 enzymes, such as the NF-κB and activator protein-1 pathways[43,44]. Moreover, cytokines generated during a type 2 inflammatory response, such as IL-4 regulate 15-LOX expression in mononuclear leucocytes[45]. Indeed, Th2-skewed peripheral blood mononuclear cells generate SPM and alternatively activated macrophages (M2) generate more SPM in total than classically activated (M1) macrophages[46,47]. It should be noted that engulfment of apoptotic cells by macrophages is also a stimulus for SPM generation. Thus, SPM biosynthesis is regulated in a temporal manner and is dependent on the particular leucocyte subsets and enzymatic pathways operative at specific points in time. Interestingly, different types of inflammation are associated with specific SPM signatures. For example, bacterial infection biases production of RvD5, while viral infection regulates PD1 biosynthesis[48,49].

Once generated, SPM act locally to promote resolution of inflammation and they have multiple cellular targets. Most SPM identified to date block PMN infiltration into sites of inflammation. Mechanisms involved include the down-regulation of adhesion receptors (i.e. CD11b), cytoskeletal structural rearrangements, and endothelial production of anti-adhesive mediators, such as nitric oxide[21,31,50]. Modulation of this phase of the acute inflammatory response is essential to prevent excessive PMN accumulation in tissues. Next, some SPM, including RvE1, override PMN survival signalling to allow timely apoptosis to occur[51]. Like aberrant PMN infiltration, prolonged PMN survival can delay resolution of inflammation[42]. One of the most prominent defining features of actively resolving inflammation is the macrophage-dependent clearance of apoptotic cells[42]. As alluded to, this process is required to prevent post-apoptotic secondary necrosis of lingering apoptotic cells. Like the regulation of PMN chemotaxis, most SPM identified to date actively promote macrophage efferocytosis. Controlling both the magnitude of PMN infiltration, while also stimulating their removal by macrophages is a characteristic 'dual action' of SPM[31]. In addition to the clearance of apoptotic cells, SPM also promote macrophage phagocytosis of bacteria

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and promote the efflux of phagocytes from sites of inflammation[48,50,52]. This was shown in animal models of peritonitis and sepsis, in which treatment with SPM, such as PD1 and RvD2, was shown to enhance the appearance of phagocytes in the spleen and lymph nodes[50,52].

In addition to these well-defined endpoints in leucocyte trafficking, SPM also regulate other processes that allow for resolution of inflammation. For example, SPM, including PD1, stimulate the up-regulation of chemokine receptors on apoptotic cells, which promotes scavenging of soluble chemokines in inflammatory exudates[53]. Moreover, RvE1 was shown to stimulate the up-regulation of CD55 on epithelial cells to promote PMN clearance[54]. The stimulatory actions of SPM distinguish them from other *n*-3 PUFA-derived mediators, such as the 3-series prostanoids or the 5-series leukotrienes, which are primarily less potent pro-inflammatory mediators or potentially endogenous receptor antagonists[8]. While discussion of all documented biological roles of SPM are beyond the scope of this review, the key biological roles of SPM in the resolution of inflammation have identified them as a new class of mediators and have helped to study the process of resolution itself.

Pro-resolving lipid mediators: identification of specific receptors

The biological actions of most lipid mediators, including the leukotrienes and prostaglandins, are mediated primarily by G-protein coupled receptors (GPCR). Initial studies interrogating the mechanisms whereby certain SPM elicit their biological actions demonstrated that pertussis toxin abolished the effects of SPM in many cases. Indeed, stimulation of macrophage phagocytosis by RvD1 is completely blocked by pertussis toxin, as is the stimulation of nitric oxide and prostacyclin production in endothelial cells stimulated by RvD2[50,55]. This suggested that SPM may bind to specific GPCR that couple to $G\alpha_i$. The first SPM demonstrated to bind a specific GPCR was RvE1. Based on the fact that RvE1 reduced PMN recruitment during acute inflammation *in vivo* and that this process is both promoted and counter-regulated by other lipid mediators such as leukotriene B4 and lipoxin A4, a search for an RvE1 receptor began by screening receptors closely related to GPCR for these other lipid mediators. Using this unbiased approach, it was determined that RvE1 inhibited TNF-α-stimulated activation of NF-κB in cells transfected with a GPCR- denoted ChemR23[25]. Radioligand binding studies demonstrated that RvE1 specifically binds ChemR23 with high affinity (K_d~11 nM). In peripheral blood monocytes and ChemR23-transfected HEK293 cells, RvE1 stimulated phosphorylation of extracellular-signal-regulated kinase [56]. Similarly, stimulation of macrophage phagocytosis by RvE1 is blocked by an extracellular-signal-regulated kinase inhibitor, providing further evidence for receptor-mediated pro-resolving actions of RvE1. *In vivo*, RvE1 inhibited PMN infiltration in a murine model of peritonitis and the doses of RvE1 required to elicit such actions was log-orders of magnitude lower in mice with transgenic overexpression of ChemR23[57].

Further studies on the receptor-mediated biological actions of RvE1 demonstrated that direct receptor crosstalk is an important mechanism whereby RvE1 elicits its effects. In human platelets, RvE1 blocks activation, aggregation and ADP-stimulated P-selectin mobilisation[58,59]. While direct binding of ADP receptor, P2Y12 was ruled out, it was demonstrated that ChemR23-dependent activation by RvE1 inhibits P2Y12 signalling. Indeed, co-transfection of P2Y12 expressing cells with ChemR23 blocked ADP-stimulated calcium mobilisation in the presence of RvE1, whereas RvE1 failed to block P2Y12 signalling in mock-transfected cells[59]. Of interest, ChemR23 is not highly expressed on PMN and later studies demonstrated that in addition to serving as an agonist of this receptor, RvE1 also blocks leukotriene B4-induced activation of PMN in a B leukotriene receptor-1-dependent manner[60]. While a specific GPCR for RvE2 has not yet been identified, it is noteworthy that RvE2 shows the specific binding with human PMN[28].

In addition to RvE1, other SPM have also been recently shown to bind specific GPCR. In human subjects, two GPCR have been shown to mediate the pro-resolving actions of D-series resolvin, RvD1[55]. Building upon observations that regulation of actin polymerisation and adhesion receptor expression by RvD1 in human PMN was sensitive to pertussis toxin, a screening approach revealed that RvD1 binds both GPR32 (a previous orphan receptor) as well as the lipoxin A4 receptor, FPR2 (also denoted ALX). Radioligand binding studies demonstrated that RvD1 binds human leucocytes with high affinity (K_d = 0·17 nM) and binding was observed on both PMN and monocytes. In macrophages, stimulation of phagocytosis by RvD1 was blocked by shRNA-mediated knock-down of FPR2 and GPR32, establishing the involvement of receptor-mediated signalling[55]. The effects of RvD1 in regulating PMN infiltration during acute inflammation *in vivo* were enhanced in mice with transgenic overexpression of human FPR2, whereas the regulation of PMN trafficking by RvD1 is abolished in mice deficient in the closely related murine homolog of FPR2[61,62]. Collectively, these results unequivocally demonstrate that the pro-resolving actions of RvD1 are mediated by specific binding to GPCR. Lastly, while no specific GPCR for DHA product, PD1, has been identified, specific binding studies using radiolabelled PD1 have documented that PD1 specifically binds both retinal pigment epithelial cells and human PMN[63]. Importantly, PD1 binding was displaced in homoligand displacement assays, whereas its isomer, 10*S*, 17*S*-diHDHA was less effective in competing for PD1 binding, and a Δ15-trans isomer of PD1 was essentially inactive[63]. Future studies are likely to reveal important new insights into the specific receptors that mediate the biological actions of SPM and open up

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exciting new avenues for targeted pro-resolution therapeutics.

Pro-resolving lipid mediators and animal models of disease

Consistent with the potent actions of SPM on human leucocytes, these novel mediators have beneficial actions that have been demonstrated in several distinct animal models of disease. As resolvins were originally identified during the resolution phase of acute inflammation in mice, initial studies into the biological role of these mediators aimed to determine why they are generated during this specific phase and whether they are active mediators of resolution. Moving from HPLC isolates to geometrically and stereochemically pure compounds prepared by total organic synthesis, SPM including E-series resolvins, D-series resolvins, protectins and maresins, have been shown to promote resolution of acute inflammation *in vivo*[6,21,22,31]. Specifically, these SPM shorten the resolution interval, the time during which PMN decline from their maximum value by 50%[6]. This parameter, which is indicative of reduced PMN infiltration and enhanced removal of apoptotic PMN by macrophages, is a critical defining feature of pro-resolving mediators. Moving beyond acute models of sterile inflammation, multiple studies have now reported that SPM also combat bacterial infection by controlling leucocyte trafficking and actively promoting both PMN and macrophage-mediated phagocytosis and bacterial killing. This translates into improved survival in multiple distinct models of infection, including polymicrobial sepsis induced by caecal ligation and puncture, as well as direct instillation of live Gram-negative bacteria[48,50]. In the latter case, SPM were shown to lower the threshold of antibiotic therapy, suggesting that they may be effective adjunctive therapeutics in the context of infection[48]. By selectively enhancing effector functions of leucocytes and at the same time preventing overzealous inflammatory cytokine production and leucocyte recruitment, SPM have been shown to be tissue protective in several other distinct models of inflammation as well, including colitis, cancer, periodontal disease, diabetes, stroke and pathological angiogenesis. The reader is referred to more in-depth reviews regarding the role of SPM in animal models of inflammation and disease[6,21,22,31].

More recent studies on isolated SPM have shown that in addition to promoting bacterial containment and preventing excessive leucocyte accumulation in tissues, these resolution agonists may be particularly important for later phases of wound healing (i.e. tissue repair). For example, studies in diabetic rodents, which are used as a model of the delayed wound healing that occurs in human diabetics, have demonstrated that treatment with RvD1 increases the rate of wound closure and promotes granulation tissue formation[64]. In the context of diabetes, the time to wound closure is critical to prevent secondary infection. Moreover, RvD2 prevents tissue necrosis in rodent models of burn injury by preventing thrombosis and PMN sequestration and thereby enhancing microvascular access to the healing dermis[65,66]. Other SPM, such as RvD1, have been shown to stimulate keratinocyte migration *in vitro*, indicating that SPM probably have other diverse cellular targets within wounds and that they may promote other phases of wound repair beyond regulating leucocyte trafficking[67]. Finally, the newest member of the SPM genus, namely MaR1, directly stimulates tissue regeneration in brown planaria (*D. tigrina*) subject to surgical injury[40]. Given that MaR1 was also biosynthesised during the tissue regeneration process and rescued altered regeneration induced by LOX inhibition, MaR1 may also be a critical endogenous mediator of the regeneration process.

Animal models of disease have also been important to determine the endogenous role of SPM in the protective actions of *n*-3 PUFA. In particular, feeding a diet rich in *n*-3 PUFA (particularly EPA and DHA), increases endogenous production of SPM in acute sterile peritonitis, obesity, non-alcoholic fatty liver disease and pathologic retinal angiogenesis[6,11,21,68–71]. Moreover, genetic manipulation of mice to increase endogenous production of EPA and DHA via *fat-1* transgenesis (discussed earlier), also increases production of SPM in the context of inflammation associated with colitis, melanoma growth and pathologic angiogenesis[10–12]. In these studies, isolated SPM largely recapitulate the actions of increasing *n*-3 PUFA, suggesting that, not only are they generated endogenously from *n*-3 PUFA, but that they may in part underlie the protective actions of *n*-3 PUFA in these scenarios. Future studies designed to establish this cause–effect relationship using specific SPM-receptor knock-out mice for example, are likely to lend important insights into the role of *n*-3 PUFA in different disease states.

Human clinical studies: are resolvins a new class of pro-resolution therapeutics?

A myriad of studies in rodents and isolated human leucocytes have unequivocally established that SPM are generated from *n*-3 PUFA and that they have potent immunomodulatory actions that are consistent with the protective effects of *n*-3 PUFA. That generation of SPM could underlie the beneficial effects of *n*-3 PUFA supplementation have been strengthened by recent studies in human subjects. In healthy human volunteers taking a fish oil supplement for just 3 weeks (4 g/d; 35% EPA, 25% DHA), SPM including RvD1, RvD2, PD1 and SPM biosynthetic precursors, 17-HDHA and 18-HEPE, were identified[72]. Moreover, the levels of these mediators identified using a specific liquid chromatography-tandem MS based approach, were found to be within the range at which they are biologically active (e.g. about 20–40 pg/ml for RvD1 and RvD2). In addition, other studies have established that 18-HEPE and RvE1 are generated in healthy human subjects within 3–4 h of EPA and aspirin administration[25,27].

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In contrast to the generation of SPM in healthy volunteers given *n*-3 PUFA supplements, other human studies suggest that SPM generation may be deficient in the context of certain diseases associated with chronic, unresolved inflammation. For instance, PD1 and 17-HDHA were identified in exhaled breath condensates from healthy individuals, whereas only trace amounts were detected in human subjects with clinical exacerbation of asthma[73]. Similarly, SPM, including RvD1, RvD2 and PD1 were identified in human subcutaneous adipose tissue samples obtained from healthy individuals, whereas the levels of PD1 and 17-HDHA were significantly reduced in subcutaneous adipose tissue obtained from patients with peripheral vascular disease[70]. This result is similar to deficiencies observed in other pro-resolving lipid mediators, such as lipoxin A4, observed in asthma and other chronic inflammatory diseases including peripheral artery disease[74,75]. Finally, our recent studies in a rodent model of diabetic wound healing also demonstrated a reduced SPM biosynthetic capacity despite similar levels of DHA in the wounds of diabetic and non-diabetic mice[64]. Given that *n*-3 PUFA improve disease outcomes in some diseases but not others (e.g. asthma, diabetes and inflammatory bowel disease), it is possible that impaired downstream metabolism of *n*-3 PUFA could in part underlie this lack of efficacy and the development of a comprehensive understanding of the mediators of the protective actions of *n*-3 PUFA may help to identify responders *v.* non-responders[8]. Further human clinical studies will be required to elucidate these relationships fully.

Building upon evidence that the generation of SPM may in part be responsible for the protective actions of *n*-3 PUFA in human subjects, clinical studies have begun to determine whether SPM might be effective targeted therapeutics. Resolvyx (www.resolvyx.com) has been at the forefront of this effort, initiating the first clinical studies with *n*-3 PUFA-derived SPM, including RvE1. The company's product pipeline includes a synthetic RvE1 analogue, denoted RX-10045, which has been successfully used in a phase 2 clinical study for the treatment of dry eye. In 2009, Resolvyx reported positive results of the 28-d, randomised, placebo-controlled trial, which enrolled 232 patients (www.clinicaltrials.gov). The primary endpoint of the trial was the Worst Symptom Score, which is a composite indicator of dryness, ocular discomfort, stinging, burning and grittiness. Patients treated with RX-10045 showed significant improvements in this primary endpoint compared with placebo and RX-10045 was safe and well-tolerated. This particular analogue and clinical programme has now been licensed to Celtic Therapeutics for further clinical development. In addition to RX-10045, Resolvyx is also developing a programme around synthetic RvE1 (RX-10001) and PD1 (RX-20001) for other indications, such as asthma, inflammatory bowel disease and rheumatoid arthritis, and Phase 1 studies have been initiated to assess safety, tolerability, pharmacodynamics and pharmacokinetics (see www.clinicaltrials.gov).

In addition to treating chronic inflammatory pathologies, there is also considerable potential for SPM as a new genus of pain management therapeutics[76]. Recent studies have demonstrated that certain SPM, such as RvE1, RvD1 and RvD2, are potent analgesics in animal models of inflammatory pain. In particular, intrathecal or intraplantar administration of SPM have been shown to decrease pain induced by a variety of stimuli, including formalin, complete Freund's adjuvant, TNF-α and capsaicin without affecting basal pain perception[76–78]. These SPM, such as RvE1, were more potent than morphine or non-steroidal anti-inflammatory drugs *in vivo*. Mechanistically, SPM have been shown to regulate activation of transient receptor potential villinoid subtype-1 and transient receptor potential ankyryn 1 in primary sensory neurons with IC_{50} values in the low nanomolar range, suggesting that these SPM may be endogenous regulators of pain[76–78]. Although clinical studies have not yet evaluated the efficacy of SPM in regulating pain, this particular therapeutic area holds great promise as chronic pain management continues to be a challenging area of clinical medicine.

Concluding remarks

Studies over the last 10–15 years have defined that *n*-3 PUFA are enzymatically converted into a diverse array of bioactive autacoids that have potent immunomodulatory and tissue protective actions. These mediators bind specific receptors, have multiple cellular targets and their biosynthesis and actions have been demonstrated in both rodents and human subjects. The elucidation of these new pathways has provided a mechanistic understanding of the protective roles of *n*-3 PUFA in health and disease and may lead to the development of effective targeted therapeutics aimed at treating chronic inflammatory diseases.

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Conflicts of Interest

None.

Authorship

M. S. wrote and edited the manuscript.

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