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Resolution Phase Lipid Mediators of Inflammation: Agonists of Resolution

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Abstract

Lipid mediators are appreciated for their roles in leukocyte traffic required in host defense. With identification of novel resolution phase mediators, resolvins, protectins and maresins, these 3 families and their aspirin-triggered forms, given their potent stereoselective actions with human cells and animal disease models, are coined specialized pro-resolving mediators (SPM). Stereochemistries of key SPM are established and several groups reported organic synthesis. Given availability, this 2 year-review period expands their potent pro-resolving and nonredundant actions. Collectively, they support the concept that return of acute inflammation involves active biosynthesis and SPM signaling towards homeostasis.

Introducing Specialized Pro-Resolving Mediators

All cells in multi-cellular organisms require chemical gradients of signals that instruct each to move or to stop and clear tissues as needed. In acute inflammatory responses many chemical signals are produced, some from exogenous microbial origins while others are biosynthesized by the host response to tissue injury and invasion [1]. This laboratory focused on mechanisms and mediators in endogenous anti-inflammation and its resolution [2,3]. Using a systems approach with LC-MS-MS-based lipidomics, *in vivo* animal models, exudate cell trafficking and functional assessment with human cells, we identified novel bioactive products generated within the resolution phase of acute sterile inflammation that activate pro-resolving mechanisms [3,4**,5].

Focusing on self-limited resolving exudates also permitted a direct assessment of the host's responses that actively return to homeostasis. An informative bioassay that proved critical in the initial structural elucidation studies focused on human polymorphonuclear neutrophil (PMN) transmigration across endothelial cells and epithelial cells since PMN excessive infiltration is central to many pathologies [3]. Attention was focused here because PMN are among the first responders to injury and microbial invasion in all organs. Our hypothesis that endogenous mediators are produced via cell-cell interactions within the inflammatory

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exudates to control the magnitude and duration of local inflammation proved to be the case and is relevant to human translation, since anti-PMN therapy [6] to limit tissue damage and uncontrolled inflammation has increasing appeal. From these, the resolvins, protectins and maresins (Figure 1) were uncovered and these fundamental cellular processes proved predictive of SPM actions in complex disease models, because cessation of PMN entry into tissue and their removal of apoptotic PMN as well as anti-PMN regulators are central to many organs and diseases.

In these, we also learned that omega-3 essential eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are substrates for biosynthesis in self-resolving exudates of potent anti-inflammatory pro-resolving mediators [4**,5]. Identification of omega-3 as nutrients that fuel pro-resolving mechanisms opened new areas of investigation since uncontrolled inflammation is now widely appreciated to underlie many widely occurring diseases, and omega-3-derived SPM are documented in humans in health and disease (Table 1). For further detail on SPM (resolvins, protectins and maresins), readers are directed to recent reviews on biosynthesis [7], stereoselective actions and total organic synthesis [8]. Herein we review key initial observations that permitted a relatively rapid expansion and confirmation from many laboratories reported during 2010–2012.

What is pro-resolving vs. anti-inflammation?

Introducing Immunoresolvents

SPM are agonists acting on PMN and macrophages to stimulate resolution. Given this novel mechanism of pro-resolving actions, SPM demonstrate potent actions in animal disease models [9*]. Given their ability to stimulate resolution of inflammation without immune suppression [see below and ref. 10**], we recognize that SPM are immunoresolvents; by definition, *they stimulate resolution* [9*].

Confirming SPM Structures

It was essential to confirm SPM structure and actions for each family member (Figure 2). To this end, we devised a systematic approach matching endogenous SPM to those prepared by organic synthesis. This approach was deemed necessary because SPMs are produced in small quantities in vivo (i.e. picogram-nanogram range), act locally and are inactivated [reviewed in ref. 8]. These transient small quantities precluded direct NMR analysis. The original identification of D-series resolvins (Rv) reported structural elucidation of several distinct bioactive structures RvD1 through RvD6 in resolving murine exudates, their biosynthesis by human endothelial cell leukocytes and potent actions in murine as well as human acute inflammation [4**]. Recently, we established complete stereochemistry for the third member of D-series RvD3 and its aspirin-triggered form [11]. We confirmed their potent actions regulating PMN infiltration as well as enhancing macrophage phagocytosis and efferocytosis, thus establishing their specific and potent anti-inflammatory and pro-resolving actions.

In this period, we also established stereochemical assignments for AT-PD1 [12] and maresin 1 (MaR1) [9*] (Figure 2). MaR1's defining actions were confirmed with synthetic MaR1, i.e. limiting PMN infiltration in peritonitis (ng/mouse range) as well as enhancing human macrophage uptake of apoptotic PMNs. Importantly, MaR1 also accelerates regeneration in planaria. In neurons, MaR1 dose-dependently inhibits TRPV1 currents, blocks capsaicin-induced inward currents ($IC_{50} \approx 0.5$ nM) and reduces inflammatory and neuropathic pain in mice. These findings also suggest that signals are shared in resolutive cellular-trafficking key in tissue regeneration across phyla. Of note, the stereoselective actions of each SPM also proved to be highly effective in regulating human PMN and monocytes in microfluidic chambers [13], establishing the human translational potential of novel SPM. Using similar

strategies, RvE3 was recently identified as 17,18-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid, and the two stereoisomers were assigned 17R,18R- and 17R,18S-dihydroxy groups. Both 18R- and 18S-RvE3 reduce PMN infiltration [14,15], as does RvE2 [13].

Targeted LM Metabololipidomics for Resolution Lipid Mediators: Microparticles and leukocyte subpopulations

With many of the main SPM stereochemistry established, it was next possible to carry out LM-metabololipidomics profiling via LC-MS-MS-based analyses with distinct human phagocyte populations, namely PMN, apoptotic PMN, and macrophages [16]. Efferocytosis increases SPM biosynthesis, including RvD1, RvD2, and RvE2, which was further elevated in PMN-microparticle co-incubations [17]. Using deuterium-labeled precursors showed that apoptotic PMN and microparticles each contribute to SPM biosynthesis during efferocytosis. Hence, microparticles regulate specific endogenous LM during defined stages of acute inflammatory process. Also, classic M2 macrophage phenotypes produce SPM, MaR1 and lipoxin (LX) A_4 with lower leukotriene (LT) B_4 and prostaglandin than M1 cells, establishing LM signature profiles of human PMN, apoptotic PMN and macrophage subpopulations.

Infections

We recently examined underlying mechanisms for how bacterial infections impact active resolution of inflammation. In self-resolving *E. coli* infections, dominant SPM are RvD5 and PD1, levels of which are significantly greater than in exudates from higher titer *E. coli* challenged mice. Germ-free mice produce endogenous RvD1 and PD1 levels higher than conventional mice. RvD1 and RvD5 (ng/mouse) each reduce bacterial titers and increased survival. RvD5 activates the RvD1 receptor (Figure 3) enhancing *E. coli* phagocytosis. In *E. coli* infections, SPM (RvD1, RvD5, PD1) together with ciprofloxacin heighten host antimicrobial responses. SPM in skin infections enhance clearance of *Staphylococcus aureus* and lower vancomycin dose. These demonstrate that specific SPM are temporally and differentially regulated during infections and that they are anti-phlogistic, enhance containment and lower antibiotic requirements for host bacterial clearance. These endogenous resolution mechanisms are of interest in host defense because initiation of the host response is controlled by prostaglandins and leukotrienes, which when uncontrolled can lead to reduced survival during infection [10**].

SPM Receptors

Receptor identification—*RvE1:* We identified an orphan G-protein coupled receptor (GPCR) ChemR23 for RvE1 (Figure 3). ChemR23 binds ^{3}H-RvE1 and stereoselectively transduces signals to monocytes and dendritic cells [18]. Of note, RvE1 also directly interacts with BLT1, a LTB_4 receptor, inhibiting calcium mobilization, NF-kB activation and PMN infiltration [19]. Therefore RvE1 gives cell-type specific actions, serving as an agonist for ChemR23 on mononuclear and dendritic cells as well as an antagonist for BLT1 signals on PMN. ChemR23-dependent actions of RvE1 were recently confirmed in mouse fibrosis [20]. *RvD1*: RvD1's pro-resolving actions are mediated via both ALX and human GPR32 [21]. D-series Rv ligands for GPR32 identified include RvD5, RvD3 and AT-RvD3 [10**,11; see figure legend for details]. *PD1*: PD1 displays specific binding with human retinal pigment epithelial cells and PMN. Neither RvE1 nor LXA_4 competes for [^{3}H]-NPD1/PD1 specific binding with human PMN, suggesting specific receptors for PD1 [22].

Receptor overexpression and knockdown in vivo—Earlier we constructed TG mice overexpressing human ALX. RvD1 showed enhanced actions in limiting PMN infiltration in

zymosan-initiated peritonitis [23]. These actions of RvD1 were abolished in fpr2/ALX null mice [24], indicating that RvD1 dampens acute inflammation in part via activating ALX. We also prepared transgenic mice overexpressing human ChemR23/ERV, on myeloid cells. In these TG mice, RvE1 is more potent at limiting PMN infiltration in peritonitis and protecting alveolar bone loss [25].

Organs of Actions

Ocular inflammation – Clinical development—*Mouse*: In human and rat conjunctiva goblet cells, RvD1 and RvE1 reduce LTD_4-stimulated goblet cell secretion [26]. In a murine model of dry eye, RvE1 improves the outcome measures of corneal staining and goblet cell density, indicating the potential of resolvins in treatment of dry eye [27]. In HSV-induced ocular inflammation, RvE1 significantly reduces cornea lesions and angiogenesis as well as T cells and PMN. These results indicate that RvE1 represents a novel approach to control virus-induced diseases [28*]. In human corneal epithelial cells, estradiol reduced 15-lipoxygenase (LOX) type-I and LXA_4. LXA_4 addition rescues the estradiol-abrogated wound healing, demonstrating gender-specific differences in the corneal repair mediated by the 15-LOX-LXA_4 circuit [29]. *Human*. In a Phase 2 clinical trial in patients with dry eye syndrome, a RvE1 analog significantly improved signs and symptoms. This is the first demonstration of clinical efficacy for the novel class of resolvin therapeutics [30]. The Phase III clinical trial is in progress (Safety and Efficacy Study of RX-10045 on the Signs and Symptoms of Dry Eye, identifier NCT00799552; URL: http://www.clinicaltrials.gov).

Salivary – Sjögren's syndrome: With salivary epithelium, RvD1 (100 ng/ml) rescues TNF-α-induced tight junction and cytoskeletal disruption, and enhances cell migration and polarity in an ALX-dependent manner. These findings suggest RvD1 promotes repair in salivary epithelium and restores salivary gland dysfunction [31].

Oral inflammation: In localized aggressive periodontitis (LAP) patients, macrophages exhibit reduced phagocytosis. RvE1 rescues impaired phagocytic activity of LAP macrophages [32]. Humanized nanoparticles containing 17R-RvD1 or LXA_4 analog protect in a model of temporomandibular joint disease [17].

Airway inflammation: In allergic airways, RvE1 promotes resolution in part via suppressing IL-23 and IL-6 as well as increasing IFN-γ [33]. Also, RvE1 regulates NK cell migration and cytotoxicity [34]. AT-RvD1 and RvD1 each markedly shortens resolution intervals for lung eosinophilia [35]. In acute lung injury, AT-RvD1 improves epithelial and endothelial barrier integrity, decreases airway resistance and increases epinephrine levels in bronchoalveolar lavages [36]. Of interest, Fat-1 transgenic mice with increased endogenous lung n-3 [37] show higher PD1 and RvE1 after bronchoprovocation. These animals suggest a protective role for endogenous SPM in allergic airway responses, decreasing airway inflammation [38]. Human eosinophils generate PD1, which is impaired in severe asthmatics, and PD1 reduces eosinophil chemotaxis [39].

Pain and Neuroinflammation: Resolvins are potent pain regulators [40]. Intrathecal RvD1 (40ng) strongly reduces postoperative surgical pain in rats [41]. RvE1 (intrathecal) prevents nerve injury-induced mechanical allodynia [42]. Along these lines, RvD1 (100 ng/kg) significantly decreases TNBS-induced mechanical allodynia and blocked cytokine production in spinal dorsal horn [43]. RvD2 (0.01–1 ng) prevents formalin-induced pain. As part of the molecular mechanisms, RvD2, RvE1, and RvD1 each differentially regulates transient receptor potential (TRP) channels [44]. AT-RvD1 significantly reverses thermal hypersensitivity, and knockdown of epidermal TRPV3 blunts these anti-nociceptive actions [45]. In arthritis, AT-RvD1 exhibits marked anti-hyperalgesia and decreases production of

TNF-α and IL-1β in rat hind paw [46**]. RvD1 also attenuates neuroinflammation, stimulating phagocytosis of amyloid-β (Aβ) by Alzheimer disease macrophages and inhibits Aβ-induced apoptosis in a GPR32-dependent manner [47]. In addition, RvD1 strongly decreases cytokine production in amyotrophic lateral sclerosis (ALS) macrophages [48].

Skin: *Mouse*. PMN infiltration to skin was used for structure elucidation of resolvins [3] that proved critical for clinical development. In mouse burn models, RvD2 at 25pg/g given systemically post-burn prevents thrombosis of deep dermal vasculature, dermal necrosis and PMN-mediated damage [49*]. In 2,4-dinitrofluorobenzene-stimulated dermatitis, RvE1 reduces skin lesions by lowering IL-4 and IFN-γ in activated CD4(+) T cells as well as serum IgE [50]. *Human*. Topical treatment with an aspirin-triggered/LXA_4 stable analog (15(R/S)-methyl-LXA_4) significantly reduces the severity of eczema in a two-center, double-blind, placebo-controlled, randomized, parallel-group comparative study with sixty infants [51**].

Gastrointestinal tract: In murine colitis, systemic RvE1, AT-RvD1, RvD2, or 17R-hydroxy-DHA each (nanogram ranges) mitigates disease severity, prevents body weight loss, colonic damage, and PMN infiltration as well as lowers select colonic cytokines. These results suggest that SPM have potential in treating inflammatory bowel diseases [52,53].

Fibrosis: In a unilateral ureteric obstruction (UUO)-driven murine fibrosis, RvE1 (300 ng/day) reduces accumulation of myofibroblasts, deposition of collagen IV and myofibroblast proliferation. RvE1 (~1–30 nM) inhibits PDGF-BB-induced proliferation in fibroblasts that proved to be ChemR23-RvE1-receptor-dependent [20].

Metabolic syndromes: RvD1 stimulates non-phlogistic phagocytosis in adipose macrophages and reduces macrophage reactive oxygen species production [54*]. Also, in leptin receptor-deficient (db/db) mice, RvD1 (2 μg/kg) increases glucose tolerance, adiponectin production and M2 macrophages [55]. Along these lines, LXA_4 (1 nM) also attenuates adipose inflammation and improves insulin sensitivity in a model of age-associated adipose inflammation [56]. In diabetic wounds, local RvD1 accelerates closure and reduces accumulation of apoptotic cells in wounds [57*]. Together, SPM may give new therapeutic approaches to reduce adipose inflammation and insulin resistance, key components of type-2 diabetes.

Micro RNAs in resolution: SPM-GPCR-microRNA circuits

RvD1 accelerates resolution and controls specific miRNA expression including miR-146b, 208a, and 219. This panel of miRs is temporally regulated during self-limited inflammation and controlled by RvD1 *in vivo* as well as in RvD1-GPCR-dependent human macrophage responses [58**]. Macrophages overexpressing miR-219 down-regulate 5-LOX and reduce LTB_4. Hence, 5-LOX is a target gene of miR-219 [58]. In addition, RvD1 at low doses (10 ng) increases miR-219 in ALX-TG mice, whereas this dose is not effective in non-TG [23]. Of note, delayed resolution initiated by high-dose zymosan challenges decreases miR-219-5p expression along with higher LTB_4 and lower SPM [59]. Thus, both ALX and miR-219 are components of RvD1-initiated resolution circuits.

Conclusions

Pus bonum et laudabile -- from ancient Latin “good and laudable pus” [1,2]. Hence within pus are indeed endogenous molecules that are beneficial that we now call resolvins, protectins and maresins (the SPM), which terminate inflammation and stimulate resolution. In this review period 2010–2012, the complete stereochemistries of several additional SPM

were obtained and several groups reported on their total organic synthesis. With availability of reliable commercial resolvins, in two years a body of literature emerged that confirms and expands their potent pro-resolving and anti-inflammatory actions. The SPM have also entered into human trials and are reported to be effective in skin inflammation and ocular disease. These new findings together provide further support for the novel concept that return of acute inflammatory responses to homeostasis involves active biosynthesis of pro-resolving autacoids that are receptor agonists activating resolution programs returning tissues to homeostasis and regenerating function.

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References

1. Majno, G. Inflammation and infection: historic highlights. In: Majno, G.; Cotran, RS.; Kaufman, N., editors. Current Topics in Inflammation and Infection. Williams & Wilkins; 1982. p. 1-17.
2. Serhan CN. A search for endogenous mechanisms of anti-inflammation uncovers novel chemical mediators: missing links to resolution. Histochem. Cell Biol. 2004; 122:305–321. [PubMed: 15322859]
3. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J. Exp. Med. 2000; 192:1197–1204. [PubMed: 11034610]
4. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac R-L. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter pro-inflammation signals. J. Exp. Med. 2002; 196:1025–1037. [PubMed: 12391014] . **This article reports the original identification of DHA-derived resolvins that are generated in inflammatory exudates, vascular, leukocytes and neural cells, which are potent regulators stimulating resolution of acute inflammation.
5. Hong S, Gronert K, Devchand P, Moussignac R-L, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autacoids in anti-inflammation. J. Biol. Chem. 2003; 278:14677–14687. [PubMed: 12590139]
6. Takano T, Clish CB, Gronert K, Petasis N, Serhan CN. Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A_4 and novel lipoxin B_4 stable analogues. J. Clin. Invest. 1998; 101:819–826. [PubMed: 9466977]
7. Bannenberg G, Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: an update. Biochim. Biophys. Acta. 2010; 1801:1260–1273. [PubMed: 20708099]
8. Serhan CN, Petasis NA. Resolvins and protectins in inflammation-resolution. Chem. Rev. 2011; 111:5922–5943. [PubMed: 21766791]
9. Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA. Macrophage pro-resolving mediator maresin 1 stimulates tissue regeneration and controls pain. FASEB J. 2012; 26:1755–1765. [PubMed: 22253477] . *This article demonstrates that MaR1 is an immunoresolvent, regulating inflammation resolution, tissue regeneration, and pain resolution.
10. Chiang N, Fredman G, Bäckhed F, Oh SF, Vickery TW, Schmidt BA, Serhan CN. Infection regulates pro-resolving mediators that lower antibiotic requirements. Nature. 2012; 484:524–528. [PubMed: 22538616] . **This report demonstrates that specific SPMs are temporally and differentially regulated during infections and that they are anti-phlogistic, enhance containment and lower antibiotic requirements for bacterial clearance.
11. Dalli J, Winkler JW, Colas RA, Arnardottir H, Cheng CYC, Chiang N, Petasis NA, Serhan CN. Resolvin D3 and aspirin-triggered resolvin D3 are potent immunoresolvents. Chem. Biol. 2013; 20:188–201. [PubMed: 23438748]

12. Serhan CN, Fredman G, Yang R, Karamnov S, Belayev LS, Bazan NG, Zhu M, Winkler JW, Petasis NA. Novel proresolving aspirin-triggered DHA pathway. Chem. Biol. 2011; 18:976–987. [PubMed: 21867913]

13. Jones CN, Dalli J, Dimisko L, Wong E, Serhan CN, Irimia D. Microfluidic chambers for monitoring leukocyte trafficking and humanized nano-proresolving medicines interactions. Proc Natl Acad Sci U S A. 2012; 109:20560–20565. [PubMed: 23185003]

14. Isobe Y, Arita M, Matsueda S, Iwamoto R, Fujihara T, Nakanishi H, Taguchi R, Masuda K, Sasaki K, Urabe D, et al. Identification and structure determination of novel anti-inflammatory mediator resolvin E3, 17,18-dihydroxyeicosapentaenoic acid. J Biol Chem. 2012; 287:10525–10534. [PubMed: 22275352]

15. Isobe Y, Arita M, Iwamoto R, Urabe D, Todoroki H, Masuda K, Inoue M, Arai H. Stereochemical assignment and anti-inflammatory properties of the omega-3 lipid mediator resolvin E3. J Biochem. 2013

16. Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. Blood. 2012; 120:e60–e72. [PubMed: 22904297]

17. Norling LV, Spite M, Yang R, Flower RJ, Perretti M, Serhan CN. Cutting edge: Humanized nano-proresolving medicines mimic inflammation-resolution and enhance wound healing. J. Immunol. 2011; 186:5543–5547. [PubMed: 21460209]

18. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN. Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J. Exp. Med. 2005; 201:713–722. [PubMed: 15753205]

19. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B_4 receptor BLT1 and ChemR23 to regulate inflammation. J. Immunol. 2007; 178:3912–3917. [PubMed: 17339491]

20. Qu X, Zhang X, Yao J, Song J, Nikolic-Paterson DJ, Li J. Resolvins E1 and D1 inhibit interstitial fibrosis in the obstructed kidney via inhibition of local fibroblast proliferation. J Pathol. 2012

21. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee C-H, Yang R, Petasis NA, Serhan CN. Resolvin D1 binds human phagocytes with evidence for pro-resolving receptors. Proc. Natl. Acad. Sci. U.S.A. 2010; 107:1660–1665. [PubMed: 20080636]

22. Marcheselli VL, Mukherjee PK, Arita M, Hong S, Antony R, Sheets K, Petasis N, Serhan CN, Bazan NG. Neuroprotectin D1/protectin D1 stereoselective and specific binding with human retinal pigment epithelial cells and neutrophils. Prostaglandins Leukot. Essent. Fatty Acids. 2010; 82:27–34. [PubMed: 19931440]

23. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 receptor stereoselectivity and regulation of inflammation and pro-resolving microRNAs. Am. J. Pathol. 2012; 180:2018–2027. [PubMed: 22449948]

24. Norling LV, Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 limits polymorphonuclear leukocytes recruitment to inflammatory loci: receptor dependent actions. Arterioscler. Thromb. Vasc. Biol. 2012; 32:1970–1978. [PubMed: 22499990]

25. Gao L, Faibish D, Fredman G, Herrera BS, Chiang N, Serhan CN, Van Dyke TE, Gyurko R. Resolvin E1 and Chemokine-like Receptor 1 Mediate Bone Preservation. J Immunol. 2013; 190:689–694. [PubMed: 23241890]

26. Dartt DA, Hodges RR, Li D, Shatos MA, Lashkari K, Serhan CN. Conjunctival goblet cell secretion stimulated by leukotrienes is reduced by resolvins D1 and E1 to promote resolution of inflammation. J Immunol. 2011; 186:4455–4466. [PubMed: 21357260]

27. de Paiva CS, Schwartz CE, Gjorstrup P, Pflugfelder SC. Resolvin E1 (RX-10001) Reduces Corneal Epithelial Barrier Disruption and Protects Against Goblet Cell Loss in a Murine Model of Dry Eye. Cornea. 2012; 31:1299–1303. [PubMed: 22257864]

28. Rajasagi NK, Reddy PBJ, Suryawanshi A, Mulik S, Gjorstrup P, Rouse BT. Controlling herpes simplex virus-induced ocular inflammatory lesions with the lipid-derived mediator resolvin E1. J. Immunol. 2011; 186:1735–1746. [PubMed: 21187448] . *This is the first demonstration that a RvE1 analog control lesion severity in a virally induced immunopathological disease.

29. Wang SB, Hu KM, Seamon KJ, Mani V, Chen Y, Gronert K. Estrogen negatively regulates epithelial wound healing and protective lipid mediator circuits in the cornea. FASEB J. 2012; 26:1506–1516. [PubMed: 22186873]
30. Science Blog. Resolvyx announces positive data -- Phase 2 trial of resolvin RX-10045 for dry eye syndrome. 2009. http://www.scienceblog.com
31. Odusanwo O, Chinthamani S, McCall A, Duffey ME, Baker OJ. Resolvin D1 prevents TNF-alpha-mediated disruption of salivary epithelial formation. Am J Physiol Cell Physiol. 2012; 302:C1331–C1345. [PubMed: 22237406]
32. Fredman G, Oh SF, Ayilavarapu S, Hasturk H, Serhan CN, Van Dyke TE. Impaired phagocytosis in localized aggressive periodontitis: rescue by resolvin E1. PLoS One. 2011; 6:e24422. [PubMed: 21935407]
33. Haworth O, Cernadas M, Yang R, Serhan CN, Levy BD. Resolvin E1 regulates interleukin-23, interferon-gamma and lipoxin A_4 to promote resolution of allergic airway inflammation. Nat. Immunol. 2008; 9:873–879. [PubMed: 18568027]
34. Haworth O, Cernadas M, Levy BD. NK cells are effectors for resolvin E1 in the timely resolution of allergic airway inflammation. J Immunol. 2011; 186:6129–6135. [PubMed: 21515793]
35. Rogerio AP, Haworth O, Croze R, Oh SF, Uddin M, Carlo T, Pfeffer MA, Priluck R, Serhan CN, Levy BD. Resolvin D1 and aspirin-triggered resolvin D1 promote resolution of allergic airways responses. J Immunol. 2012; 189:1983–1991. [PubMed: 22802419]
36. Eickmeier O, Seki H, Haworth O, Hilberath JN, Gao F, Uddin M, Croze RH, Carlo T, Pfeffer MA, Levy BD. Aspirin-triggered resolvin D1 reduces mucosal inflammation and promotes resolution in a murine model of acute lung injury. Mucosal Immunol. 2012
37. Hudert CA, Weylandt KH, Wang J, Lu Y, Hong S, Dignass A, Serhan CN, Kang JX. Transgenic mice rich in endogenous n-3 fatty acids are protected from colitis. Proc. Natl. Acad. Sci. U.S.A. 2006; 103:11276–11281. [PubMed: 16847262]
38. Bilal S, Haworth O, Wu L, Weylandt KH, Levy BD, Kang JX. Fat-1 transgenic mice with elevated omega-3 fatty acids are protected from allergic airway responses. Biochim Biophys Acta. 2011; 1812:1164–1169. [PubMed: 21616147]
39. Miyata J, Fukunaga K, Iwamoto R, Isobe Y, Niimi K, Takamiya R, Takihara T, Tomomatsu K, Suzuki Y, Oguma T, et al. Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma. J Allergy Clin Immunol. 2012
40. Xu Z-Z, Zhang L, Liu T, Park J-Y, Berta T, Yang R, Serhan CN, Ji R-R. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. Nat. Med. 2010; 16:592–597. [PubMed: 20383154]
41. Huang L, Wang C-F, Serhan CN, Strichartz G. Enduring prevention and transient reduction of post-operative pain by intrathecal resolvin D1. Pain. 2011; 152:557–565. [PubMed: 21255928]
42. Xu ZZ, Berta T, Ji RR. Resolvin E1 Inhibits Neuropathic Pain and Spinal Cord Microglial Activation Following Peripheral Nerve Injury. J Neuroimmune Pharmacol. 2012
43. Quan-Xin F, Fan F, Xiang-Ying F, Shu-Jun L, Shi-Qi W, Zhao-Xu L, Xu-Jie Z, Qing-Chuan Z, Wei W. Resolvin D1 reverses chronic pancreatitis-induced mechanical allodynia, phosphorylation of NMDA receptors, and cytokines expression in the thoracic spinal dorsal horn. BMC Gastroenterol. 2012; 12:148. [PubMed: 23092159]
44. Park CK, Lü N, Xu ZZ, Liu T, Serhan CN, Ji RR. Resolving TRPV1 and TNF-α-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1. J. Neurosci. 2011; 31:15072–15085. [PubMed: 22016541]
45. Bang S, Yoo S, Yang TJ, Cho H, Hwang SW. 17(R)-resolvin D1 specifically inhibits transient receptor potential ion channel vanilloid 3 leading to peripheral antinociception. Br. J. Pharmacol. 2012; 165:683–692. [PubMed: 21718307]
46. Lima-Garcia J, Dutra R, da Silva K, Motta E, Campos M, Calixto J. The precursor of resolvin D series and aspirin-triggered resolvin D1 display anti-hyperalgesic properties in adjuvant-induced arthritis in rats. Br. J. Pharmacol. 2011; 278:683–293.. **This article demonstrates the anti-hyperalgesic properties of AT-RvD1 in arthritis pain. Thus SPM might have implication in treating pain associated with chronic inflammation.

47. Mizwicki MT, Liu G, Fiala M, Magpantay L, Sayre J, Siani A, Mahanian M, Weitzman R, Hayden E, Rosenthal MJ, et al. 1alpha,25-Dihydroxyvitamin D3 and Resolvin D1 Retune the Balance between Amyloid-beta Phagocytosis and Inflammation in Alzheimer's Disease Patients. J Alzheimers Dis. 2012
48. Liu G, Fiala M, Mizwicki MT, Sayre J, Magpantay L, Siani A, Mahanian M, Chattopadhyay M, La Cava A, Wiedau-Pazos M. Neuronal phagocytosis by inflammatory macrophages in ALS spinal cord: inhibition of inflammation by resolvin D1. Am J Neurodegener Dis. 2012; 1:60–74. [PubMed: 22787561]
49. Bohr S, Patel SJ, Sarin D, Irimia D, Yarmush ML, Berthiaume F. Resolvin D2 prevents secondary thrombosis and necrosis in a mouse burn wound model. Wound Repair Regen. 2013; 21:35–43. [PubMed: 23110665] . *This is the first demonstration that RvD2 mitigates the deleterious impact of the inflammatory response in burn wounds, preventing thrombosis of the deep dermal vascular network and subsequent dermal necrosis.
50. Kim TH, Kim GD, Jin YH, Park YS, Park CS. Omega-3 fatty acid-derived mediator, Resolvin E1, ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis in NC/Nga mice. Int Immunopharmacol. 2012; 14:384–391. [PubMed: 22951188]
51. Wu SH, Chen XQ, Liu B, Wu HJ, Dong L. Efficacy and Safety of 15(R/S)-Methyl-Lipoxin A(4) in Topical Treatment of Infantile Eczema. Br J Dermatol. 2013; 168:172–178. [PubMed: 22834636] . **This article is the first report using a LXA_4 and ATL analog in topical treatment of eczema in humans.
52. Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, Blumberg RS, Serhan CN. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc. Natl. Acad. Sci. U.S.A. 2005; 102:7671–7676. [PubMed: 15890784]
53. Bento AF, Claudino RF, Dutra RC, Marcon R, Calixto JB. Omega-3 fatty acid-derived mediators 17(R)-hydroxy docosahexaenoic acid, aspirin-triggered resolvin D1 and resolvin D2 prevent experimental colitis in mice. J. Immunol. 2011; 187:1957–1969. [PubMed: 21724996]
54. Titos E, Rius B, González-Périz A, López-Vicario C, Morán-Salvador E, Martínez-Clemente M, Arroyo V, Clária J. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by elicitying macrophage polarization toward a pro-resolving phenotype. J. Immunol. 2011; 187:5408–5418. [PubMed: 22013115] . *This report illustrates novel mechanism of RvD1 initiating macrophage polarization toward an M2-like phenotype, thereby conferring anti-inflammatory and proresolving actions in inflamed adipose tissue.
55. Hellmann J, Tang Y, Kosuri M, Bhatnagar A, Spite M. Resolvin D1 decreases adipose tissue macrophage accumulation and imiproves insulin sensitivity in obese-diabetic mice. FASEB J. 2011; 25:2399–2407. [PubMed: 21478260]
56. Borgeson E, McGillicuddy FC, Harford KA, Corrigan N, Higgins DF, Maderna P, Roche HM, Godson C. Lipoxin A4 attenuates adipose inflammation. FASEB J. 2012; 26:4287–4294. [PubMed: 22700871]
57. Tang Y, Zhang MJ, Hellmann J, Kosuri M, Bhatnagar A, Spite M. Proresolution Therapy for the Treatment of Delayed Healing of Diabetic Wounds. Diabetes. 2013; 62:618–627. [PubMed: 23043160] . *This article shows that diabetes impairs wound healing. RvD1 accelerates wound closure and decreased accumulation of apoptotic cells and macrophages in the wounds, representing a novel approach to treating chronic, nonhealing wounds in patients with diabetes.
58. Recchiuti A, Krishnamoorthy S, Fredman G, Chiang N, Serhan CN. MicroRNAs in resolution of acute inflammation: identification of novel resolvin D1-miRNA circuits. FASEB J. 2011; 25:544–560. [PubMed: 20956612] . **This is the first report demonstrating that RvD1 regulates specific micro RNAs and their target genes involved in resolution and establish a novel resolution circuit with RvD1 receptor-dependent regulation of specific miRNAs.
59. Fredman G, Li Y, Dalli J, Chiang N, Serhan CN. Self-limited versus delayed resolution of acute inflammation: temporal regulation of pro-resolving mediators and microRNA. Sci Rep. 2012; 2:639. [PubMed: 22957142]
60. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature. 2007; 447:869–874. [PubMed: 17568749]

61. Samuelsson B. Role of basic science in the development of new medicines: examples from the eicosanoid field. J. Biol. Chem. 2012; 287:10070–10080. [PubMed: 22318727]
62. Spite M, Norling LV, Summers L, Yang R, Cooper D, Petasis NA, Flower RJ, Perretti M, Serhan CN. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. Nature. 2009; 461:1287–1291. [PubMed: 19865173]
63. Rodriguez AR, Spur BW. Total synthesis of the macrophage derived anti-inflammatory lipid mediator Maresin 1. Tetrahedron Lett. 2012; 53:4169–4172.
64. Rodriguez AR, Spur BW. First total synthesis of the anti-inflammatory lipid mediator Resolvin D6. Tetrahedron Lett. 2012; 53:86–89.
65. Rodriguez AR, Spur BW. Total synthesis of the anti-inflammatory lipid mediator Resolvin E2. Tetrahedron Lett. 2012; 53:1912–1915.
66. Ogawa N, Kobayashi Y. Total synthesis of the antiinflammatory and proresolving protectin D1. Tetrahedron Lett. 2011; 52:3001–3004.
67. Ogawa S, Urabe D, Yokokura Y, Arai H, Arita M, Inoue M. Total synthesis and bioactivity of resolvin E2. Org Lett. 2009; 11:3602–3605. [PubMed: 19637859]
68. Ogawa N, Kobayashi Y. Total synthesis of resolvin E1. Tetrahedron Lett. 2009; 50:6079–6082.
69. Ohira T, Arita M, Omori K, Recchiuti A, Van Dyke TE, Serhan CN. Resolvin E1 receptor activation signals phosphorylation and phagocytosis. J. Biol. Chem. 2010; 285:3451–3461. [PubMed: 19906641]
70. Oh SF, Pillai PS, Recchiuti A, Yang R, Serhan CN. Pro-resolving actions and stereoselective biosynthesis of 18*S* E-series resolvins in human leukocytes and murine inflammation. J. Clin. Invest. 2011; 121:569–581. [PubMed: 21206090]
71. Oh SF, Dona M, Fredman G, Krishnamoorthy S, Irimia D, Serhan CN. Resolvin E2 formation and impact in inflammation resolution. J. Immunol. 2012; 188:4527–4534. [PubMed: 22450811]
72. Vong L, Ferraz JG, Dufton N, Panaccione R, Beck PL, Sherman PM, Perretti M, Wallace JL. Up-regulation of Annexin-A1 and lipoxin A(4) in individuals with ulcerative colitis may promote mucosal homeostasis. PLoS One. 2012; 7:e39244. [PubMed: 22723974]
73. Gutierrez AD, Sathyanarayana P, Konduru S, Ye Y, Birnbaum Y, Bajaj M. The effect of pioglitazone treatment on 15-epi-lipoxin A4 levels in patients with type 2 diabetes. Atherosclerosis. 2012; 223:204–208. [PubMed: 22687642]
74. Giera M, Ioan-Facsinay A, Toes R, Gao F, Dalli J, Deelder AM, Serhan CN, Mayboroda OA. Lipid and lipid mediator profiling of human synovial fluid in rheumatoid arthritis patients by means of LC-MS/MS. Biochim Biophys Acta. 2012; 1821:1415–1424. [PubMed: 22841830]
75. Yamaguchi H, Higashi N, Mita H, Ono E, Komase Y, Nakagawa T, Miyazawa T, Akiyama K, Taniguchi M. Urinary concentrations of 15-epimer of lipoxin A(4) are lower in patients with aspirin-intolerant compared with aspirin-tolerant asthma. Clin Exp Allergy. 2011; 41:1711–1718. [PubMed: 22093074]
76. Ho KJ, Spite M, Owens CD, Lancero H, Kroemer AH, Pande R, Creager MA, Serhan CN, Conte MS. Aspirin-triggered lipoxin and resolvin E1 modulate vascular smooth muscle phenotype and correlate with peripheral atherosclerosis. Am. J. Pathol. 2010; 177:2116–2123. [PubMed: 20709806]
77. Clària J, Dalli J, Yacoubian S, Gao F, Serhan CN. Resolvin D1 and resolvin D2 govern local inflammatory tone in obese fat. J. Immunol. 2012; 189:2597–2605. [PubMed: 22844113]
78. Mas E, Croft KD, Zahra P, Barden A, Mori TA. Resolvins D1, D2, and Other Mediators of Self-Limited Resolution of Inflammation in Human Blood following n-3 Fatty Acid Supplementation. Clin Chem. 2012; 58:1476–1484. [PubMed: 22912397]
79. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, et al. The human serum metabolome. PLoS One. 2011; 6:e16957. [PubMed: 21359215]
80. Yanes O, Clark J, Wong DM, Patti GG, Sánchez-Ruiz A, Benton HP, Trauger SA, Desponts C, Ding S, Siuzdak G. Metabolic oxidation regulates embryonic stem cell differentiation. Nat. Chem. Biol. 2010; 6:411–417. [PubMed: 20436487]

Highlights

- The n-3 specialized pro-resolving mediators (SPM) evoke stereoselective actions
- SPM control excessive PMN and stimulate macrophage clearance, vital for resolution
- SPM promote resolution of inflammation, regeneration and are anti-hyperalgesia.
- The complete stereochemistries of key SPM are now established
- During past 2 years a body of literature confirms and expands potent actions of SPM

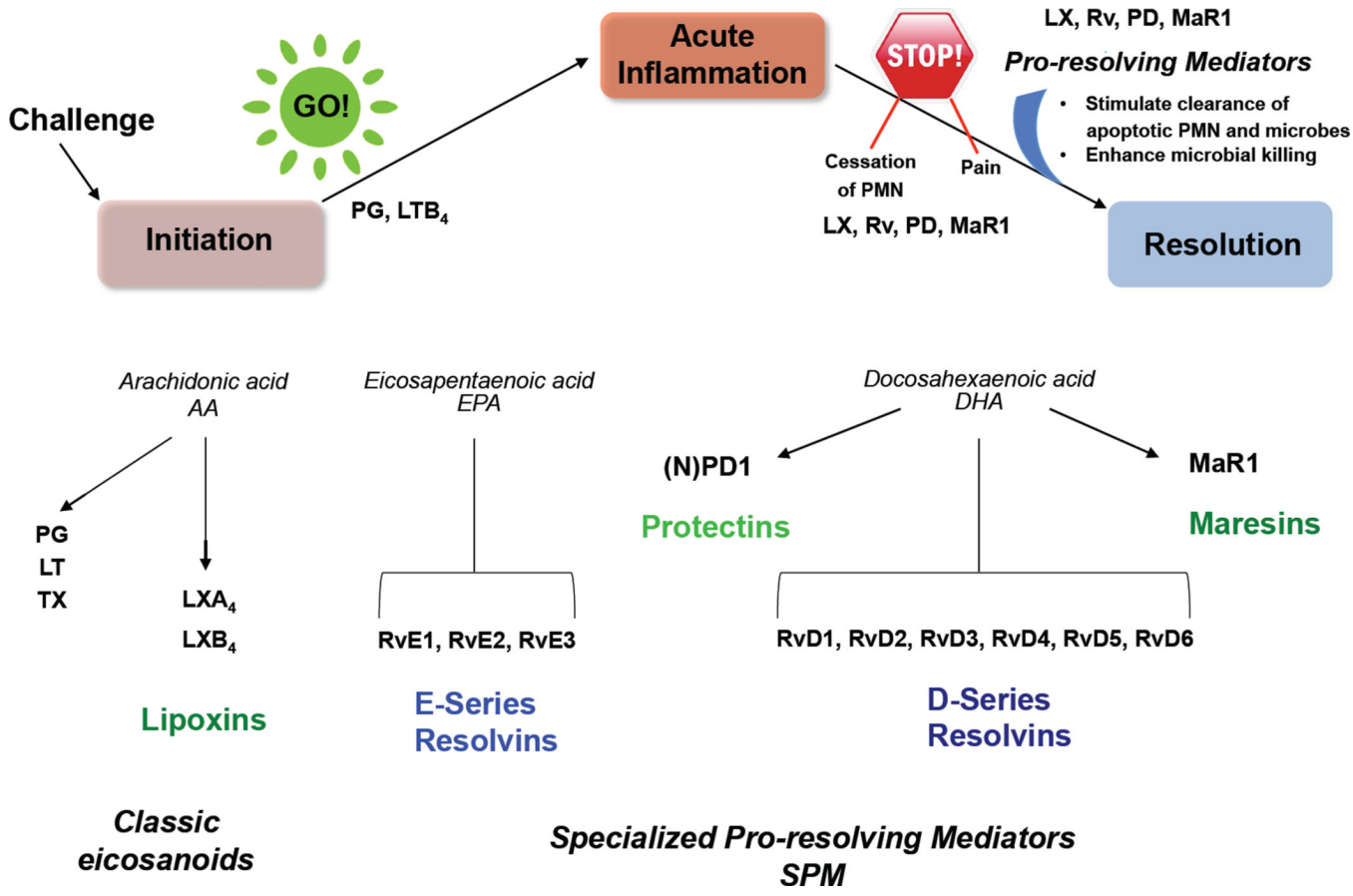

Figure 1. Specialized pro-resolving mediators (SPM): chemical structures

Among chemical signals at the site of an acute inflammatory response, those that originate from host essential fatty acids are of particular interest because of their nutritional regulation of the response via essential fatty acids EPA and DHA and the potential to design small molecule mimetics of these molecules. Those produced from arachidonic acid, including prostaglandins and leukotriene B_4, are involved in the initiating steps that permit leukocytes to leave post-capillary venules, i.e. diapedesis. Mediators produced from EPA and DHA, i.e. SPM, possess potent pro-resolving actions that include cessation of PMN tissue infiltration, counter-regulation of chemokines and cytokines, reduction in pain [40] and stimulation of macrophage-mediated actions, i.e. efferocytosis, phagocytosis of microbes [10**,60].

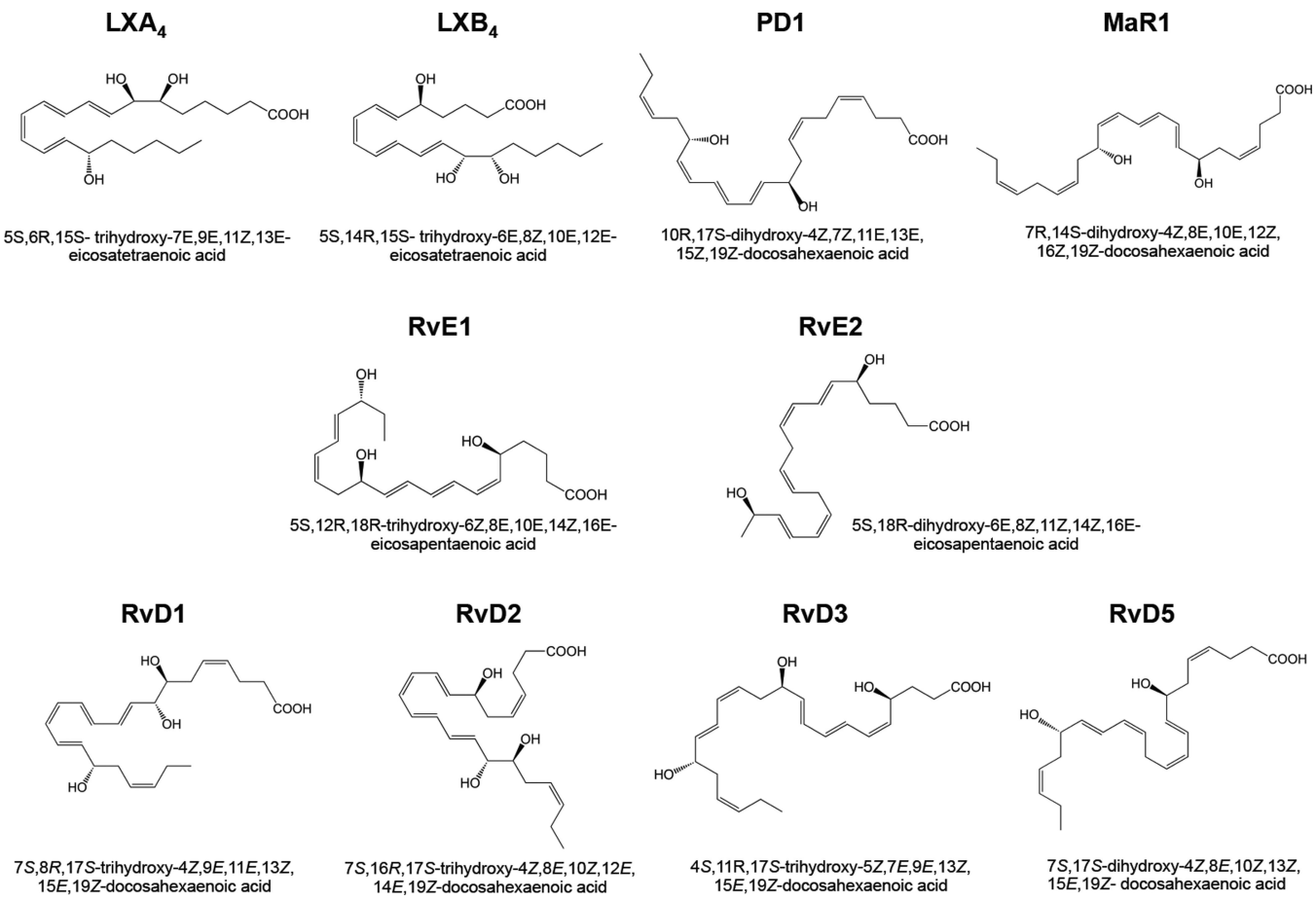

Figure 2. Structures of SPM

Classic eicosanoids each carry established stereoselectivity in their actions [61]. Establishing the complete stereochemical assignment for each of the separate resolvin, protectin and maresin (SPM) structures shown herein was key to affirming novel actions on leukocytes. In this period 2010–2012, the structures of RvD1, its aspirin-triggered 17*R*-epimer, RvD2 [62], aspirin-triggered (AT)-protectin D1 [12] and MaR1 [9*] were assigned and several were made commercially available. Recently, using LC-MS/MS-based metabololipidomics we matched RvD3 with 4*S*,11*R*,17*S*-trihydroxydocosa-5*Z*,7*E*,9*E*,13*Z*,15*E*,19*Z*-hexaenoic acid, and AT-RvD3 matched 4*S*,11*R*,17*R*-trihydroxydocosa-5*Z*,7*E*,9*E*,1Z*E*,15*E*,19*Z*-hexaenoic acid. In this period, we also established the stereochemical assignments for AT-PD1 [12] and that of maresin 1 (MaR1; 7*R*,14*S*-dihydroxydocosa-4Z,8*E*,10*E*,12*Z,16Z,19Z*-hexaenoic acid) [9*]. The total organic syntheses of MaR1 and additional SPM were also achieved by Rodriguez and Spur using Sonogashira coupling [63]; these investigators also reported resolvin D6 [64] and synthesis of resolvin E2 [65]. Kobayashi et al. reported stereoselective total synthesis of protectin D1 [66], resolvin E2 [67] and resolvin E1 [68]. See reference [8] for further details.

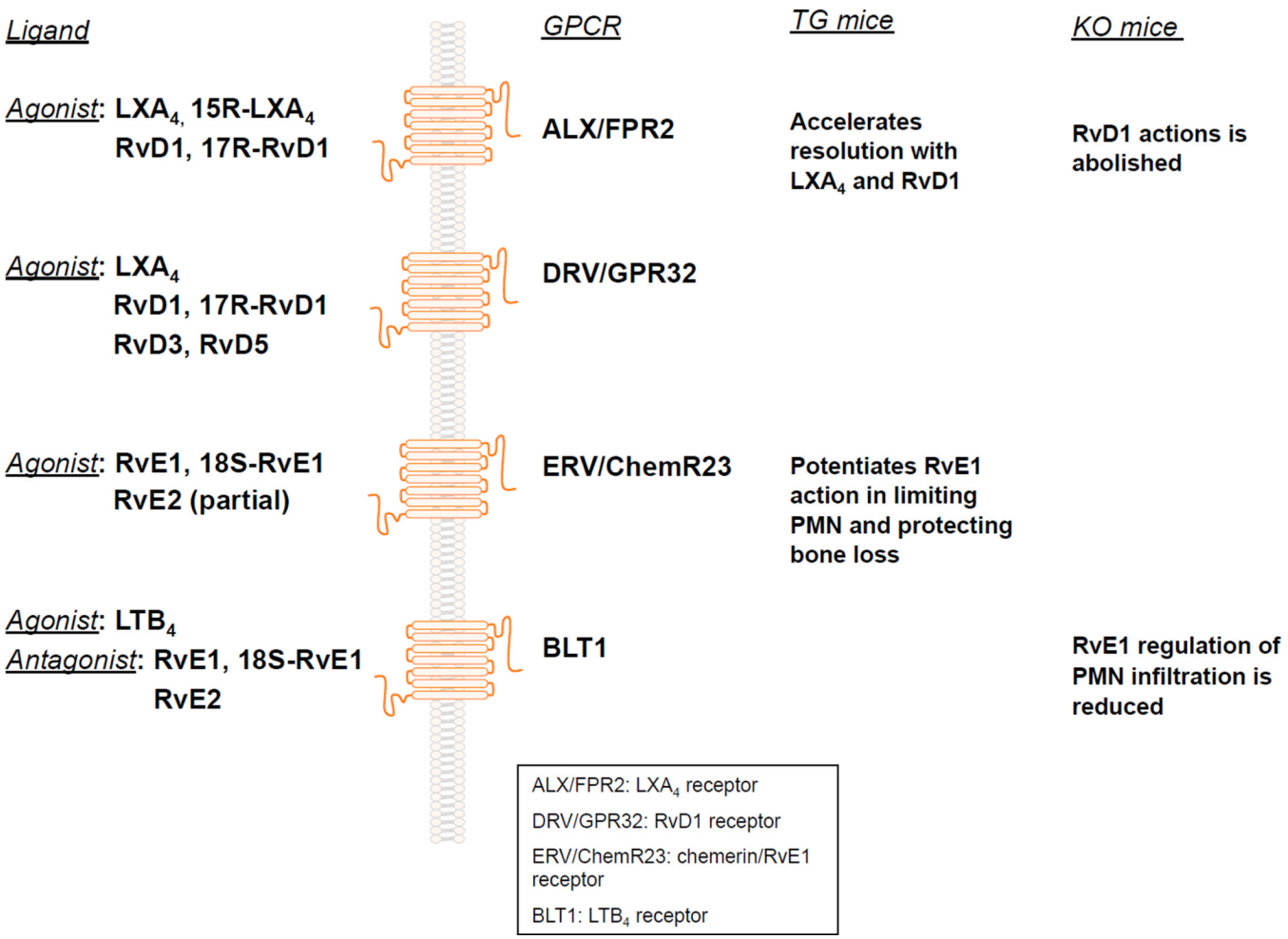

Figure 3. SPM and their receptors

ALX and GPR32, 2 GPCRs for RvD1 on human phagocytes. ALX is a lipoxin A_4 receptor and GPR32. RvD1 displays specific binding, reduces actin polymerization and CD11b on PMN, as well as stimulates macrophage phagocytosis in an ALX and GPR32-dependent manner [21]. The aspirin-triggered epimer 17R-RvD1 and stable analog 17-R/S-methyl-RvD1 each dose-dependently activate ALX/FPR2 and GPR32 in GPCR-overexpressing β-arrestin systems and electric cell-substrate impedance sensing [23]. RvD5 also activates human GPR32-β-arrestin systems and stimulates macrophage phagocytosis of *E.coli* enhanced by GPR32 [10**]. RvD3 and AT-RvD3 each activate this GPCR, contributing to their pro-resolving actions in stimulating macrophage phagocytosis [11].

ChemR23, a specific receptor for RvE1, is related to lipoxin and leukotriene receptors in deduced amino acid sequences. ChemR23 specifically binds tritiated RvE1 and signals to activate monocytes, reduce dendritic cell migration and IL-12 production [18]. RvE1-ChemR23 interactions also stimulate macrophage phagocytosis via phosphorylation signaling pathways including ribosomal protein S6, a downstream target of PI3K/Akt signaling and the Raf/ERK pathways [69]. 18S-RvE1 also binds to ChemR23 with increased affinity and potency compared with the R-epimer, but is rapidly inactivated [70]. RvE2 is a partial agonist for ChemR23 [71].

BLT1, a leukotriene B_4 receptor, also directly interacts with RvE1, which inhibits calcium mobilization, NF-kB activation and PMN infiltration in vivo [19]. Both 18S-RvE1 and

RvE2 also bind to BLT1 [70,71] demonstrating ligand specificity and related structural features of resolvins.

TG mice. We constructed mice overexpressing human ALX. The pro-resolving actions of RvD1 were further enhanced in ALX-TG mice. Transgenic mice were also prepared overexpressing human ChemR23, the RvE1 receptor, on myeloid cells. In these TG mice, RvE1 is 10-fold more potent in limiting PMN infiltration in zymosan-initiated peritonitis, and ligature-induced alveolar bone loss was diminished in ChemR23tg mice. Hence, RvE1 modulates osteoclast differentiation and bone remodeling by direct actions on bone, in addition to anti-inflammation and pro-resolution [23].

KO mice. In fpr2/ALX-deficient mice (mouse orthologue of human ALX), anti-inflammatory actions of RvD1 are lost [24]. RvD1 regulates acute inflammation in part via human ALX and GPR-32 receptors. In BLT1 knockout mice, anti-inflammatory actions of RvE1 were sharply reduced when given at low doses (100 ng i.v.) in peritonitis. In contrast, higher doses of RvE1 (1.0 μg i.v.) reduce PMN infiltration in a BLT1-independent manner. Taken together, RvE1 binds to BLT1 as a partial agonist and serves as a local damper of BLT1 signals on leukocytes along with ChemR23-mediated counter-regulatory actions to mediate the resolution of inflammation [19].

Table 1

Specialized pro-resolving mediators (LX, Rv, PD and MaR1) in humans

SPM	Disease/tissues	Formation
Lipoxins & Aspirin-triggered lipoxins (ATL)	Colitis	Elevated mucosal LXA_4 in ulcerative colitis individuals in remission [72]
	Type 2 Diabetes	Increased plasma ATL with intake of pioglitazone [73]
	Rheumatoid Arthritis	LXA_4 present in synovial fluid from rheumatoid arthritis patients [74]
	Localized Aggressive Periodontitis (LAP)	Less LXA_4 in LAP whole blood compared to healthy donors [32]
	Asthma	Higher urinary ATL levels in aspirin-tolerant asthma than in aspirin-intolerant asthma [75]
	Peripheral artery disease	Lower plasma levels of ATL in patients with symptomatic peripheral artery disease than in healthy volunteers [76]
	Adipose tissues	LXA_4 identified in human adipocytes from obese patients [77]
Resolvins	Synovial fluid	RvD5 present in synovial fluid from rheumatoid arthritis patients [74]
	Blood (healthy volunteers)	Plasma RvD1, RvD2 and 17-HDHA identified with oral omega-3 supplementation [78]
	Adipose tissues	RvD1 and RvD2 identified in human adipocytes from obese patients [77]
	Human plasma	RvE1 and RvD1 identified in human plasma (~1nM) [79]
Protectin	Asthma	Decreased PD1 in eosinophils from patients with severe asthma compared to healthy individuals [39]
	Embryonic stem cells	PD1 produced in embryonic stem cells [80]
Maresins	Synovial fluid	MaR1 is identified in synovial fluid from rheumatoid arthritis patients [74]