

REVIEW ARTICLE

Identification and structure elucidation of the pro-resolving mediators provides novel leads for resolution pharmacology

Correspondence Dr Jesmond Dalli, William Harvey Research Institute, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK. E-mail: j.dalli@qmul.ac.uk

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Jesmond Dalli¹  and Charles N Serhan² 

¹Lipid Mediator Unit, William Harvey Research Institute, Barts and the London School of Medicine, Queen Mary University of London, London, UK, and ²Center for Experimental Therapeutics and Reperfusion Injury, Department of Anaesthesia, Perioperative and Pain Medicine, Building for Transformative Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Inflammatory diseases are a major socio-economic burden, with the incidence of such conditions on the rise, especially in western societies. For decades, the primary treatment paradigm for many of these conditions was to develop drugs that inhibit or antagonize the production and biological actions of molecules that were thought to be the culprits in propagating disease; these include cytokines and eicosanoids. This approach is effective in controlling disease propagation; however, long-term exposure to these anti-inflammatories is also associated with many side effects, some of which are severe, including immune-suppression. The discovery that termination of self-limited acute inflammation is an active process orchestrated by endogenous mediators, including the essential fatty acid-derived resolvins, protectins and maresins, has provided novel opportunities for the design of therapeutics that control inflammation with a lower burden of side effects. This is because at variance to anti-inflammatories, pro-resolving mediators do not completely inhibit inflammatory responses; instead, these mediators reprogramme the immune response to accelerate the termination of inflammation, facilitating the regain of function. The scope of this review is to highlight the biological actions of these autacoids and their potential utility as lead compounds in developing resolution pharmacology-based therapeutics.

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Abbreviations

13-HDPA, 13-(R)-hydroxy-7Z,10Z,13R,14E,16Z,19Z-docosapentaenoic acid; 13-HpDPA, 13-(R)-hydroxyperoxy-7Z,10Z,13R,14E,16Z,19Z-docosapentaenoic acid; 13S, 14S-epoxy-MaR, 13S,14S-epoxy-4Z,7Z,9E,11E,16Z,19Z-docosahexaenoic acid; 17R-RvD3, 4S,11R,17R-trihydroxy-5Z,7E,9E,13Z,15E,19Z-docosahexaenoic acid; ALX, lipoxin A₄ receptor; BLT1 receptor, LTB₄ receptor; DHA, docosahexaenoic acid; DSS, dextran sodium sulphate; EPA, eicosapentaenoic acid; LOX, lipoxygenase; LXA₄, 5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid; MaR, maresin; MaR1, 7R,14S-dihydroxy-4Z,8E,10E,12Z,16Z,19Z-docosahexaenoic acid; MaR1_{n-3} DPA, 7R,14S-dihydroxy-8E,10E,12Z,16Z,19Z-docosapentaenoic acid; MaR2, 13R,14S-dihydroxy-4Z,7Z,9,11,16Z,19Z-docosahexaenoic acid; MaR_{n-3} DPA, n-3docosapentaenoic acid-derived maresins; MCTR, maresin conjugates in tissue regeneration; MCTR1, 13R-glutathionyl,14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid; MCTR2, 13R-cysteinylglycyl,14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid; MCTR3, 13R-cysteinyl,14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid; miRNA, microRNA; n-3 DPA, n-3 docosapentaenoic acid; NALP3, NACHT, LRR and PYD domains-containing protein 3; NSAIDs, non-steroidal anti-inflammatory drugs; PCTR, protectin conjugates in tissue regeneration; PCTR1, 16R-glutathionyl,17S-hydroxy-4Z,7Z,10Z,12E,14E,19Z-docosahexaenoic acid;

PD, protectin; PD1, 10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid; PD1_{n-3} DPA, 10R,17S-dihydroxy-7Z,11E,13E,15Z,19Z-docosapentaenoic acid; PD_{n-3} DPA, n-3 docosapentaenoic acid-derived protectin; RCTR, resolvin conjugates in tissue regeneration; Rv, resolvin; RvD, docosahexaenoic acid-derived resolvins; RvD1, 7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid; RvD2, 7S,16R,17S-trihydroxy-4Z,8E,10Z,12E,14E,19Z-docosahexaenoic acid; RvD3, 4S,11R,17S-trihydroxy-5Z,7E,9E,13Z,15E,19Z-docosahexaenoic acid; RvD4, 4S,5R,17S-trihydroxy-6E,8E,10Z,13Z,15E,19Z-docosahexaenoic acid; RvD5, 7S,17S-dihydroxy-4Z,8E,10Z,13Z,15E,19Z-docosahexaenoic acid; RvD_{n-3} DPA, n-3 docosapentaenoic acid-derived resolvins; RvE, eicosapentaenoic acid derived resolvins; RvE1, 5S,12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid; RvE2, 5S,18R-trihydroxy-6E,8Z,11Z,14Z,16E-eicosapentaenoic acid; RvE3, 17,18-dihydroxy-5Z,8Z,11Z,13E,15E-eicosapentaenoic acid; RvT, thirteen series resolvins; SPM, specialized pro-resolvin mediators; TRPV1, transient receptor potential cation channel subfamily V member 1

During evolution, multicellular organisms arose and cells within an organism became specialized to perform specific tasks leading to the formation of organs and tissues. These specialized organs required protection from invading microbes as well as mechanisms to repair and regenerate damaged tissues and organs, thus paving the way to the evolution of the immune system (Malagoli, 2016). In higher organisms, the immune system is divided into two arms, the innate arm provides the front line defence system and the adaptive arm provides protection from insults and/or pathogens to which the body was previously exposed. In some instances, immune responses may become dysregulated leading to disease. It is now appreciated that a significant portion of diseases that afflict western societies are associated with unbridled inflammation leading to damage of vital organs and tissues resulting in malaise and ultimately death (Kumar *et al.*, 2014; Majno, 1991).

Classically, the inflammatory response has been divided into two phases, the initiation and resolution (or termination) phases (Kumar *et al.*, 2014). Many studies conducted primarily in the last century focused on determining the mechanisms and molecules produced during the initiation phase, demonstrating that this was a tightly orchestrated response with the production of several classes of molecules that are involved in the recruitment of different leukocyte subsets and include the cytokines, chemokines and the classic eicosanoids (Kumar *et al.*, 2014). Interested readers on the biology of these mediators are directed to articles in this special edition as well as to Samuelsson (2012) and Dinarello and Joosten (2016). The identification of these molecules also paved the way to the development of innovative therapeutics for the treatment of many inflammatory diseases that revolutionized medicine and medical practice. The drugs were designed to inhibit or antagonize the biological actions and the production of local mediators that were identified to be important in disease onset and progression. Longitudinal studies on the use of many of these therapeutics demonstrate that while these molecules are effective at inhibiting inflammation, they also come with many side effects. For example, non-steroidal anti-inflammatory drugs (NSAIDs) lead to an increased incidence of gastrointestinal bleeds (Goldstein and Cryer, 2015), and anti-TNF increase the incidence of infections (Minozzi *et al.*, 2016). Thus, these observations underscore an urgent need for the development of alternative approaches, especially in the treatment of chronic diseases, which would not interfere with the immune system and would carry a lower burden of side effects.

For many years, termination of inflammation was thought to occur *via* the simple dilution of inflammatory signals from the site of injury or infection, leading to the egress of white blood cells and re-establishment of tissue function (Robbins and Cotran, 1979). Studies focusing on mechanisms that control the termination of inflammation demonstrated that arachidonic acid is not only a substrate in the biosynthesis of inflammation-initiating molecules, such as PGs and leukotrienes, but is converted to protective and anti-inflammatory molecules that include the lipoxins (Serhan *et al.*, 1984; Levy *et al.*, 2001) and the cyclopentenone PGs (Gilroy *et al.*, 1999). Identification of these molecules indicated that resolution of inflammation is an active process coordinated by autacoids produced at the site that control immune cell responses. Introducing quantitation indices to mark resolution permitted the delineation of the *in vivo* biological actions of pro-resolving mediators, including the lipoxins, resolvins and protectins, during acute inflammation (Bannenberg *et al.*, 2005; Schwab *et al.*, 2007). Detailed studies investigating mechanisms controlling the termination of inflammation also uncovered a link between the inflammation-initiating eicosanoids **PGE₂** and **PGD₂** and lipoxin biosynthesis. These PGs are important in up-regulating the expression of 15-lipoxygenase type 1 (15-LOX-1), the initiating enzyme in the lipoxin biosynthetic pathway (Levy *et al.*, 2001). Of note, inhibition of PG biosynthesis using NSAIDs is linked to a reduction in the biosynthesis of specialized pro-resolving mediators and a delay in the termination of inflammation in experimental systems (Fukunaga *et al.*, 2005). Thus, these studies demonstrated that the acute inflammatory response is a coordinated process with the initiation and termination phases being intricately linked.

These initial observations also raised the question whether novel molecules are produced during acute inflammation to promote its termination and activate reparative and regenerative responses thereby paving the way to the re-establishment of tissue and organ function. Using a systems approach, we uncovered three novel mediator superfamilies that actively reprogramme the host immune response to halt inflammation and re-establish organ function (Serhan *et al.*, 2000; 2002; 2009; Dalli *et al.*, 2013; 2014; 2015a). Given their potent biological actions, these mediators were termed as specialized pro-resolving mediators (SPM). This superfamily is composed of mediators that are produced *via* the stereoselective conversion of essential fatty acids and include the docosahexaenoic acid derived resolvins (RvD), protectins and maresins and the eicosapentaenoic acid

derived resolvins (RvE) (Serhan *et al.*, 2017). The production of these mediators is regulated in a time, organ and stimulus-dependent manner (see Dalli, 2017), and their relative concentrations to classic eicosanoids are also dependent on these factors. For example, in cerebrospinal fluids from patients with multiple sclerosis PGE₂ and **RvD1** concentrations were present at similar concentrations (~1 pg·mL⁻¹) (Pruss *et al.*, 2013). In experimental models of eye infections, **LXA₄** concentrations were between 2- and 10-fold higher than those of PGE₂. The production of eicosanoids and SPM is also regulated in a sex-dependent manner where LXA₄ concentrations in females were elevated when compared to males during experimental eye infections (Livne-Bar *et al.*, 2017), whereas RvD and RvE are elevated during experimental inflammation in humans (Rathod *et al.*, 2017). The scope of the present review is to discuss the evidence underpinning the protective actions of SPM and how insights into their biological actions may provide leads on the utility of harnessing SPM as templates for the development of resolution pharmacology-based therapeutics that would carry a lower burden of side effects.

The identification and structure elucidation of novel immunoresolvents

The EPA bioactive metabolome

In order to establish whether novel molecules are produced during the resolution phase that actively promote the termination of inflammation, it is essential to employ a systems approach. For this purpose, we developed metrics to measure the kinetics of cellular trafficking as well as tissue regeneration responses (Bannenberg *et al.*, 2005; Schwab *et al.*, 2007). Using this approach, we found that during acute, self-limited inflammation, **eicosapentaenoic acid (EPA)** was converted in inflammatory exudates to novel mediators that potently and stereospecifically promoted the termination of inflammation and were thus coined as E-series resolvins (RvE) (Serhan *et al.*, 2000).

Investigations into the mechanisms activated by these molecules demonstrated that these mediators directly counter-regulate the production of pro-inflammatory mediators, including TNF- α and inflammatory eicosanoids. In addition, the biological actions of **RvE1** at controlling leukocyte responses were found to be more potent than those of aspirin and dexamethasone. RvE1 was also found to carry potent antinociceptive actions reducing inflammatory pain by regulating both central and peripheral responses with activities of 10 ng per mouse when administered intrathecally and 285–570 pmol when administered peripherally *in vivo*. These actions were also displayed *in vitro* at concentrations as low as 3 nM (Xu *et al.*, 2010; Jo *et al.*, 2016; Fonseca *et al.*, 2017). Of note, the antinociceptive properties of RvE1 are more potent than those exerted by the COX-2 inhibitor **NS398** and **morphine** (Xu *et al.*, 2010).

In studies assessing the role of omega-3 supplementation in patients with arthritis, Barden and colleagues (2016) found an association between the synovial fluid concentrations of RvE2 and joint pain, where higher RvE2 concentrations were

associated with lower pain scores in these patients. RvE2 is also antidepressant; i.c.v. infusions (10 ng per mouse) reduced LPS-induced depression in mice *via* the activation of mTORC1 signalling in the medial prefrontal cortex and hippocampal dentate gyrus (Deyama *et al.*, 2018). Isobe and colleagues also found that the RvE1 and RvE2 precursor 18-hydroxy-eicosapentaenoic acid is converted by eosinophils to a novel bioactive mediator denoted as RvE3. This mediator, at 10–100 ng per mouse, displays potent anti-inflammatory properties characteristic of the SPM family, including the ability to regulate neutrophil recruitment (Isobe *et al.*, 2012; Isobe *et al.*, 2013).

The DHA bioactive metabolome – protectins

EPA is not the only omega-3 essential fatty acid that is converted during acute self-limited inflammation to novel bioactive mediators. Docosahexaenoic acid is also a substrate for the formation of two SPM families produced *via* a key 17-hydroperoxy-docosahexaenoic acid intermediate and coined as D-series resolvins and protectins (Serhan *et al.*, 2017). In peripheral tissues, these mediators are produced by leukocytes and their biosynthesis is temporally regulated (Dalli *et al.*, 2013a; Winkler *et al.*, 2016). In neural tissues, the protectin biosynthetic pathway regulates both stromal and immune cell responses. In retinal pigmented cells, protectin D1 (PD1; 50 nM; referred to as NPD1 in neuronal systems) potently counteracts H₂O₂/TNF- α oxidative-stress-triggered DNA damage. PD1 also up-regulates the expression of several anti-apoptotic proteins including **Bcl-2** and **Bcl-xL** and decreases the expression of the pro-apoptotic factors **Bax** and **Bad** as well as the executioner caspase, **caspase 3** (Mukherjee *et al.*, 2004). In cytokine-stressed human neural cells, PD1 formation was associated with an attenuation of **amyloid- β** secretion (Lukiw *et al.*, 2005). This SPM was also reduced in the hippocampal *cornu ammonis* region 1 from patients with Alzheimer's disease but not in the thalamus or occipital lobes from the same brains. Furthermore, the expression of key enzymes in the biosynthesis of PD1, cytosolic **PLA₂** and 15-LOX, was altered in the hippocampus of patients with Alzheimer's disease (Lukiw *et al.*, 2005). NPD1 at 300 ng per eye also reduces the severity and incidence of stromal keratitis and corneal neovascularization following herpes simplex virus infections (Rajasagi *et al.*, 2013).

Recent studies have implicated a subset of eosinophils in the biosynthesis of PD1 during the course of self-limited inflammation. Eosinophil recruitment to inflamed loci during the resolution phase of acute inflammation correlates with an increase in PD1 production (~100 pg per exudate; Yamada *et al.*, 2011). Depletion of eosinophils results in a delay in resolution responses, including an impairment of lymphatic drainage with a reduction in the appearance of phagocytes carrying engulfed zymosan in the draining lymph node and sustained numbers of polymorphonuclear leukocytes in inflamed tissues. The resolution deficit caused by eosinophil depletion was rescued by eosinophil restoration or the administration of PD1, 5 μ g per mouse (Yamada *et al.*, 2011). Of note, PD1 production is reduced from 2.23 ± 1.55 ng·mL⁻¹ in healthy volunteers to trace concentrations in exhaled breath from asthmatics (Levy *et al.*, 2007). This reduction in PD1 biosynthesis is also observed in isolated eosinophils from patients with severe asthma, suggesting a role for

defective production of this SPM in disease onset and/or propagation (Miyata *et al.*, 2013).

The DHA bioactive metabolome – resolvins

The D-series resolvins are now thought to regulate host immune responses in a number of disease settings. In *Pseudomonas aeruginosa*-mediated lung infection **RvD1**, at 100 ng per mouse, significantly reduces *P. aeruginosa* titres, leukocyte infiltration and lung tissue damage (Codagnone *et al.*, 2018). In murine lung macrophages sorted during *P. aeruginosa* chronic infection, RvD1 regulates the expression of Toll-like receptors (TLRs), downstream genes, as well as microRNA (miR)-21 and 155, resulting in a reduction in inflammatory signalling. *In vitro*, RvD1 up-regulates *P. aeruginosa* phagocytosis by both neutrophils and macrophages (Codagnone *et al.*, 2018).

RvD2 was recently identified in skeletal muscle biopsies from humans with peripheral artery disease at concentrations of $\sim 150 \text{ pg}\cdot\text{g}^{-1}$ of tissue. When this mediator was administered to mice (100 ng per mouse) during ischaemia/reperfusion, it enhanced perfusion recovery and promoted arteriogenesis (Zhang *et al.*, 2016). In contrast to other strategies used for revascularization that exacerbate inflammation, RvD2 does not enhance vascular permeability, it reduces neutrophil accumulation in the damaged tissues and plasma inflammatory cytokine levels, including TNF- α and granulocyte macrophage colony-stimulating factor. RvD2 also increases myocyte regeneration, enhances endothelial cell migration in a Rac-dependent manner and restores defective revascularization in diabetic mice (Zhang *et al.*, 2016). In alipoprotein E (ApoE) deficient mice, RvD2 concentrations also correlated with the signs of plaque stability, and an injection of RvD2 (100 ng per mouse) to ApoE^{-/-} mice prevented atheroprotection, reduced macrophage recruitment, increased fibrous cap thickness and smooth muscle cell numbers (Viola *et al.*, 2016).

Additionally, RvD3 displays potent tissue protective actions. RvD3 was identified in uninjured lungs, suggesting that it plays a functional role in regulating tissue resolution tone (Colby *et al.*, 2016). The concentrations of this mediator are rapidly up-regulated following hydrochloric acid-initiated injury from ~ 22 to $\sim 75 \text{ pg}$ per lung. Administration of the metabolically stable endogenous isomer of RvD3, 17R-RvD3 (10 ng per mouse), affords significant tissue protection during hydrochloric acid-initiated injury, reducing alveolar wall thickening, lung oedema and leukocyte infiltration (Colby *et al.*, 2016). This mediator also increases lung epithelial cell proliferation, bronchoalveolar lavage fluid levels and keratinocyte growth factor promoting cutaneous re-epithelialization (Colby *et al.*, 2016).

In murine tissues infected with *Staphylococcus aureus*, recent studies found that RvD4 persists late into the resolution phase at concentrations of 0.75 pg per exudate (Winkler *et al.*, 2016). Administration of this mediator (200 ng per mouse) during *S. aureus* infection reduces neutrophilic infiltration, increases bacterial clearance and restores resolution responses. RvD4 also enhanced the uptake of apoptotic neutrophils by human dermal fibroblasts at concentrations as low as 0.1 nM (Winkler *et al.*, 2016). RvD5 displays potent anti-bacterial actions, up-regulating neutrophil and macrophage phagocytosis of bacteria in a dose- and receptor-

dependent manner (Chiang *et al.*, 2012). Of note, RvD biosynthesis in tissues is regulated in both a stimulus- and tissue-dependent manner, thereby demonstrating that these mediators exert distinct biological actions during self-limited inflammation (Figure 1) (Chiang *et al.*, 2012; Dalli *et al.*, 2013a; Winkler *et al.*, 2016).

The DHA bioactive metabolome – maresins

Macrophages are central players in regulating the host immune response. We recently found that these cells also produce a novel family of mediators termed maresins (macrophage mediators in resolving inflammation) (Serhan *et al.*, 2009). The biosynthesis of this family of mediators is initiated by lipoxygenation and subsequent epoxidation of DHA to yield 13S,14S-epoxy-MaR (13S,14S-eMaR). This product regulates the production of the leukocyte chemoattractant **LTB₄** via the direct inactivation of the LTA₄ hydrolase (Dalli *et al.*, 2013b). 13S,14S-eMaR (10 nM) also increases the expression of markers associated with an alternatively activated phenotype in human macrophages, including CD163 and CD206, and down-regulating the expression of markers linked with a classically activated phenotype including CD54 (Dalli *et al.*, 2013b). In addition to directly regulating aspects of the immune response, 13S,14S-eMaR is also converted to MaR1 and MaR2 via enzyme-mediated hydrolysis (Serhan *et al.*, 2009; Dalli *et al.*, 2013b; Deng *et al.*, 2014).

MaR1, the first member of this family identified, is antinociceptive regulating the activation of **TRPV1** by capsaicin at concentrations as low as $0.5 \text{ ng}\cdot\text{mL}^{-1}$. Administration of MaR1 at 10 ng per mouse also inhibits chemotherapy-elicited neuropathic pain (Serhan *et al.*, 2012). MaR1 exerts protective actions on hepatocytes, reducing lipotoxicity-induced apoptosis by activating the unfolded protein response pro-survival mechanisms and limiting the up-regulation of pro-apoptotic pathways (Rius *et al.*, 2017). This macrophage-derived mediator regulates the expression of miRNA targeting both protein folding and apoptosis and enhances the phagocytic capacity of Kupffer cells (Rius *et al.*, 2017).

Recent studies also demonstrate that MaR1 is produced by circulating neutrophil-platelet aggregates. During these heterotypic cell aggregates, DHA is converted to 13S,14S-eMaR by the platelet 12-LOX. This intermediate is then donated to neutrophils that, via enzyme-mediated hydrolysis, produce MaR1 (Abdulnour *et al.*, 2014). The production of MaR1 by these heterotypic cell aggregates in the vasculature is a protective mechanism engaged to limit tissue damage following acid-induced lung injury and results in a reduction of activated neutrophil recruitment into the damaged tissues (Abdulnour *et al.*, 2014). At the site of infectious inflammation, MaR1 is subjected to further metabolism by exudate leukocytes. Macrophages convert this mediator to 14-oxo-MaR1, which displays blunted biological actions in comparison to the parent mediator. Neutrophils convert MaR1 to 22-OH-MaR1 that retains the potent biological actions of the parent SPM, including its ability to regulate the activation of the LTB₄ receptor (**BLT₁ receptor**) by its cognate ligand (Colas *et al.*, 2016). In the CNS, MaR1 also displays reparative actions, where MaR1 (1 μg per mouse, i.v.) administration to animals subjected to spinal cord injury improved locomotor recovery and reduced secondary injury progression (Francos-Quijorna *et al.*, 2017).

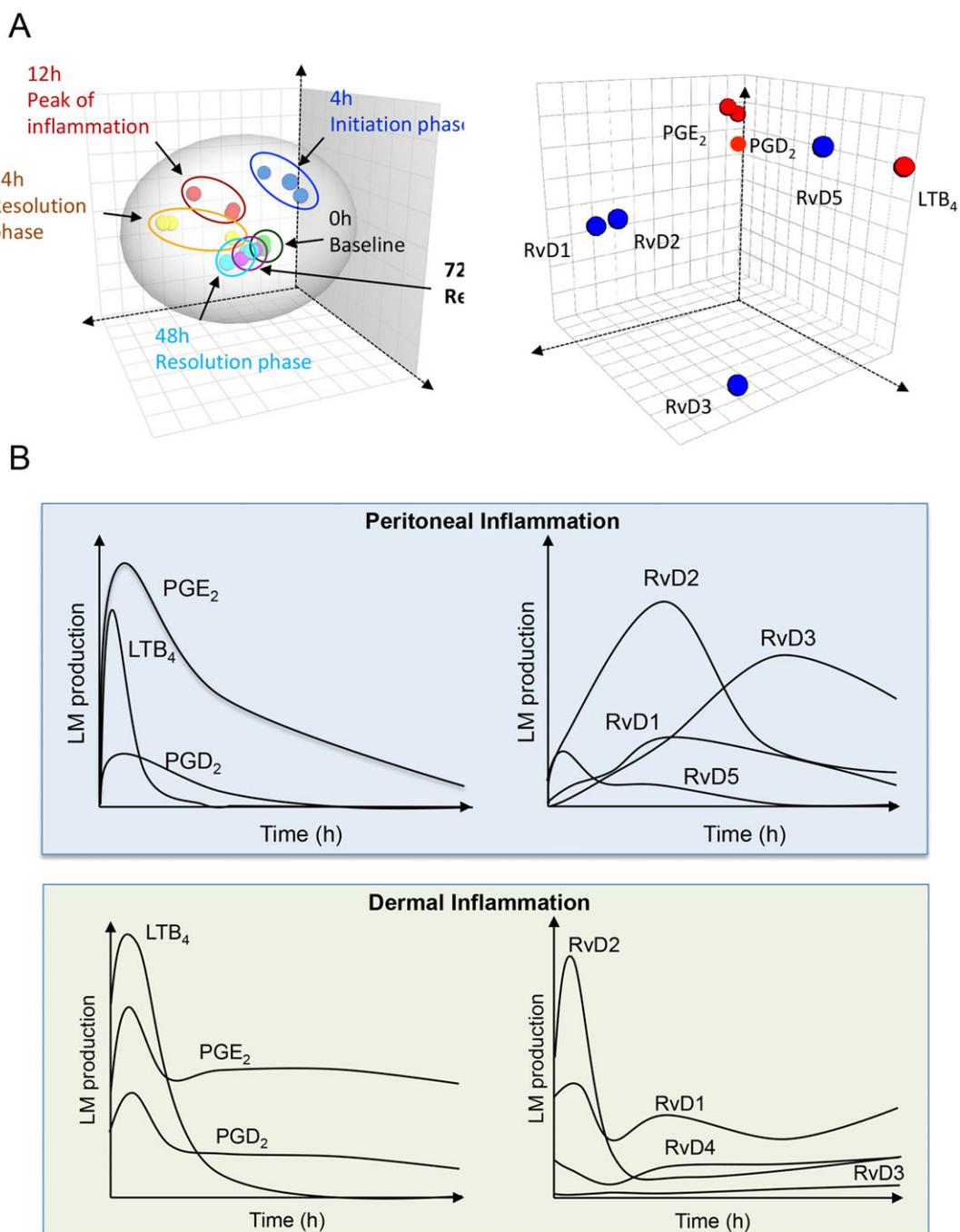


Figure 1

Temporal, tissue and stimulus specific SPM regulation during sterile and infectious inflammation. (A) Mice were challenged with 1 µg of zymosan; inflammatory exudates were collected at the indicated intervals and lipid mediators quantified as in Dalli *et al.* (2013a). Results were analysed using multivariate analysis. (A, left panel) Depicts the temporal shift in lipid mediator clusters during the course of initiation and resolution. (A, right panel) Denotes the contribution of each mediator to the temporal clustering. (B, top panel) Mice were challenged and lipid mediators were identified and quantified. Panels are illustrations of results presented in Dalli *et al.* (2013a). (B; bottom panel) Mice were challenged with *Staphylococcus aureus* and lipid mediators identified and quantified. Panels are illustrations of results presented in Winkler *et al.* (2016).

The DHA bioactive metabolome – sulfido conjugated SPM

In recent studies, we found that 13S,14S-eMaR is also an intermediate in the biosynthesis of a novel family of peptide-lipid conjugated molecules termed maresin conjugates in tissue

regeneration (MCTR) (Dalli *et al.*, 2014). This family of mediators is evolutionary conserved being identified in planaria, mice and humans. Furthermore, in each of these species, MCTR displays potent tissue protective and tissue regenerative actions. During murine infections, MCTRs are produced late within the

resolution phase, coinciding with the turning on of reparative and regenerative responses (Dalli *et al.*, 2014). Administration of MCTRs (100 nM) to planaria following surgical injury leads to the up-regulation of genes involved in tissue regeneration. In mice following ischaemia–reperfusion mediated injury, MCTR1 and MCTR2 (50 ng per mouse each) up-regulate the expression of proteins involved in lung repair and regeneration, including Ki67 and **R-spondin 3** (Dalli *et al.*, 2014). Identification of the MCTRs also paved the way to the identification of two novel peptide lipid conjugated mediator families termed as protectin conjugates in tissue regeneration (PCTR) and resolvin conjugates in tissue regeneration (RCTR) (Dalli *et al.*, 2015b). These mediators display tissue regenerative and leukocyte directed actions controlling a host's response to the Gram-negative bacterium *Escherichia coli* (Dalli *et al.*, 2015b). PCTR1 production in the murine peritoneum is under neural control (Dalli *et al.*, 2017). This mediator is central in maintaining tissue resolution tone *via* regulating tissue resident macrophage phenotype and function. Disruption of the vagus nerve or inhibiting 15-LOX, the initiating enzyme in the PCTR biosynthetic pathway, perturbs PCTR production leading to impaired host responses to *E. coli* infections, including delayed resolution of self-limited inflammation and a reduction in the ability of recruited leukocytes to phagocytose and kill bacteria (Dalli *et al.*, 2017).

The n-3 docosapentaenoic acid bioactive metabolome

We recently found that n-3 docosapentaenoic acid (DPA) was not simply a precursor in the biosynthesis of DHA from EPA.

This essential fatty acid is converted by leukocytes to several mediator families that are congeners to those produced when DHA is the substrate (Figure 2) (Dalli *et al.*, 2013). In murine plasma and inflammatory exudates following ischaemia–reperfusion injury, we found that n-3 DPA is converted to the D-series resolvins, RvD1_{n-3 DPA}, RvD2_{n-3 DPA} and RvD5_{n-3 DPA} as well as PD1_{n-3 DPA} with concentrations at the site of inflammation for these SPM ranging between 20 and 100 pg per exudate (Dalli *et al.*, 2013). These new mediators each display potent leukocyte-directed properties, reducing neutrophil recruitment during acute peritonitis and leukocyte-mediated lung damage during ischaemia–reperfusion (Dalli *et al.*, 2013; Aursnes *et al.*, 2014). Human leukocytes, in addition to producing RvD_{n-3 DPA} and PD_{n-3 DPA}, also convert n-3 DPA to MaR_{n-3 DPA}, which displays leukocyte and endothelial directed actions reducing the expression of CD54 in TNF- α activated endothelial cells (Dalli *et al.*, 2013; Tungen *et al.*, 2014). The biosynthesis of RvD5_{n-3 DPA} and PD1_{n-3 DPA} is disrupted in colonic biopsies from patients with inflammatory bowel disease as well as in mice given dextran sodium sulphate (DSS) (Gobbetti *et al.*, 2017). Administration of either of these mediators provided significant protection against DSS-initiated colon inflammation. Furthermore, increasing the endogenous production of these mediators in mice during DSS-initiated colitis also led to a significant reduction in tissue damage (Gobbetti *et al.*, 2017). In this context, recent studies in healthy volunteers found that supplementation with n-3 DPA increased plasma RvD5_{n-3 DPA} concentrations (Markworth *et al.*, 2016). Using a lipid mediator profiling approach, we found that in humans systemic concentrations of RvD_{n-3 DPA} diurnally regulated

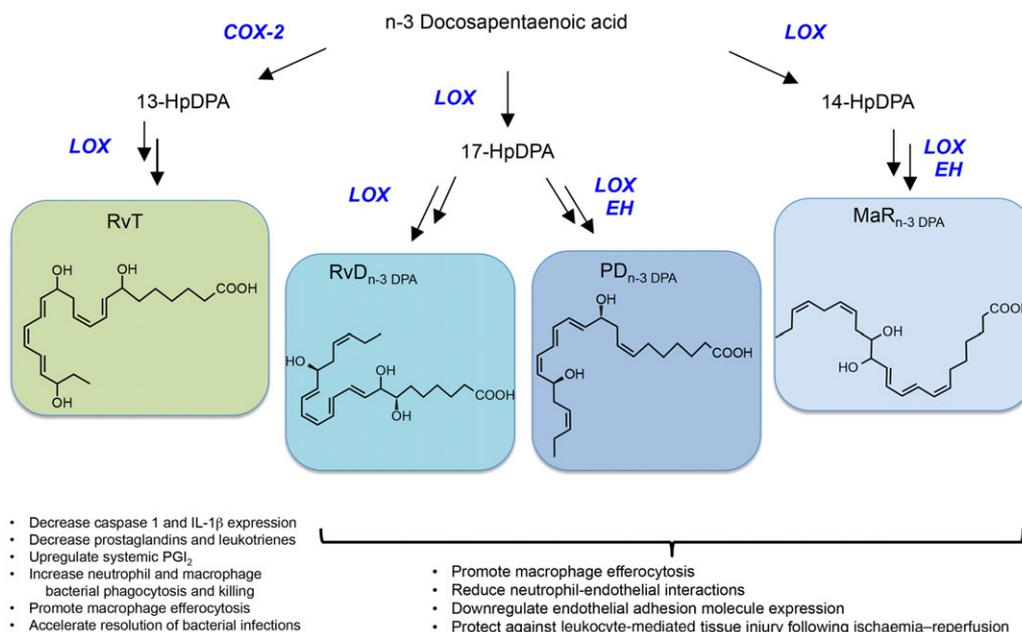


Figure 2

Novel immunoresolvents biosynthesized from n-3 docosapentaenoic. In the vasculature, n-3 docosapentaenoic acid is the substrate for conversion, by endothelial COX-2, to 13-HpDPA that is then donated to neutrophils and converted to RvT that display potent protective actions in infectious inflammation (Dalli *et al.*, 2015a). The n-3 docosapentaenoic is also converted by leukocytes to 17-HpDHA that is a precursor to RvD_{n-3 DPA} and PD_{n-3 DPA}. Conversion of n-3 DPA *via* 14-lipoxygenation yields 14-HpDHA that is converted to MaR_{n-3 DPA}. Each of these mediator families displays potent leukocyte-directed and host-protective actions (Dalli *et al.*, 2013; Gobbetti *et al.*, 2017).

and their concentrations correlate with markers of both platelet and leukocyte activation including CD11b and CD62P (Colas *et al.*, 2018).

In the vasculature during ongoing inflammation, n-3 DPA is also converted to the 13-series resolvins (RvT) (Dalli *et al.*, 2015a). The biosynthesis of these mediators is initiated by endothelial **COX-2** that converts the essential fatty acid to 13-(R)-hydroxyperoxy-7Z,10Z,13R,14E,16Z,19Z-docosapentenoic acid (13-HpDPA). This or its reduced form, namely, 13-HDPA, are then donated to neutrophils for transcellular biosynthesis to produce RvT (Dalli *et al.*, 2015a; Primdahl *et al.*, 2016). These mediators are produced during the early stages of self-limited bacterial infections with plasma concentrations reaching concentrations of ~30–50 pg·mL⁻¹. These mediators regulate the ability of leukocytes to uptake and kill bacteria. RvT also down-regulate the activation of the NACHT, LRR and PYD domain-containing protein 3 (**NALP3**) inflammasome in murine and human macrophages, reducing the expression of **caspase 1** and the production of **IL-1 β** as well as the release of LDH, a hallmark of pyroptosis (Dalli *et al.*, 2015a). In addition, RvTs are also important in mediating the biological actions of atorvastatin and pravastatin (Dalli *et al.*, 2015a; Walker *et al.*, 2017). Administration of these statins regulates COX-2 activity by up-regulating the **NOS**-mediated nitrosylation of the enzyme, increasing its catalytic turnover and 13-HDPA formation (Dalli *et al.*, 2015a). Inhibition of either NOS or COX-2 using specific pharmacological inhibitors led to a reversal of the protective actions of atorvastatin and pravastatin in both infectious inflammation and rheumatoid arthritis (Dalli *et al.*, 2015a; Walker *et al.*, 2017).

How do the SPM exert their biological actions?

Agonists of GPCRs

The biological actions of SPM are stereoselective and are mediated *via* the activation of cognate receptors and signalling pathways. The first of these receptors to be identified was the LXA₄ receptor, which up until then was thought to be a low affinity receptor for endogenous formylated peptides (Fiore *et al.*, 1994). The LXA₄ receptor (**ALX**) is part of the GPCR family (Figure 3). Its activation by cognate endogenous ligands, including LXA₄ and the pro-resolving protein **annexin A1**, displays a characteristic bell shape, where at either end of the dose range, the ability of the ligand to activate the receptor is significantly reduced (Hayhoe *et al.*, 2006; Rovira *et al.*, 2010). Of note, ALX receptor activation by its pro-resolving ligands leads to distinct downstream signalling pathways that are cell specific. For example, LXA₄ increases intracellular calcium levels in conjunctival goblet cells *via* the ALX receptor (Hodges *et al.*, 2016); whereas activation of this receptor by LXA₄ does not elicit a calcium response in human neutrophils (Fiore and Serhan, 1995). Studies conducted by Cooray and colleagues demonstrate that the ALX receptor on cell membranes is expressed as a homo- or heterodimer with the formyl peptide receptor **FPR1** or **FPR3** (the two other family members) and operates in a ligand-biased fashion. Furthermore, signalling pathways activated by these different dimerization states are distinct (Cooray *et al.*, 2013). This process

of dimerization is at least in part influenced by the ligands themselves whereby annexin A1 promotes homodimerization and the activation of p38/MAPK-activated protein kinase/heat shock protein 27 signalling and IL-10 up-regulation (Cooray *et al.*, 2013). On the other hand, the pro-inflammatory acute phase protein serum amyloid protein A did not lead to receptor homodimerization. These findings shed light on how one receptor can mediate the biological actions of functionally distinct molecules (Cooray *et al.*, 2013). In addition to mediating the biological actions of LXA₄ and its aspirin triggered epimer (Ortiz-Munoz *et al.*, 2014; Wang *et al.*, 2014; Romano *et al.*, 2015), the ALX receptor also mediates the actions of the D-series resolvins RvD1, RvD3 and their aspirin triggered epimers (Krishnamoorthy *et al.*, 2012; Arnardottir *et al.*, 2016; de Oliveira *et al.*, 2017; Mottola *et al.*, 2017; Serhan *et al.*, 2017).

Activation of the orphan receptor **GPR32** by RvD1 leads to the regulation of a number of miRNA involved in the orchestration of acute inflammation, including miR-146b, miR-208a and miR-219 (Krishnamoorthy *et al.*, 2012; Serhan *et al.*, 2017). This receptor also mediates the biological actions of RvD5 in the context of bacterial infections, whereby its activation by RvD5 leads to enhanced bacterial phagocytosis in human macrophages and a down-regulation of several pro-inflammatory genes, including NF- κ B and TNF- α (Chiang *et al.*, 2012). In lung cancer cells, GPR32 also mediates the biological actions of RvD1 in preventing **TGF β 1**-induced epithelial-mesenchymal-transition (Lee *et al.*, 2013). Additionally, this receptor is involved in regulating macrophage phenotype and function, whereby it mediates RvD1-initiated regulation of pro-inflammatory cytokines IL-1 β and IL-8. GPR32 is also involved in mediating the inhibitory actions of RvD1 on macrophage chemotaxis towards **chemerin**, fMLF and **monocyte chemoattractant protein-1 (also known as CCL2)** (Schmid *et al.*, 2016).

The biological actions of the E-series resolvins, RvE1 and RvE2, are mediated by **chemerin receptor 2/resolvin E1 (chemerin₁) receptor** (ERV1; Arita *et al.*, 2005; Oh *et al.*, 2012). RvE1 signalling *via* the chemerin₁ receptor (ERV1) leads to the activation of PI3K and ERK resulting in the phosphorylation of both Akt and the ribosomal protein S6 (Arita *et al.*, 2005). The expression of this receptor in human diabetic patients is up-regulated. Of note, despite increased expression of the RvE receptor in diabetic neutrophils, the biological actions of RvE1 in these patients are blunted suggesting that impaired/defective signalling of this receptor in diabetes may be a component in the ethiopathogenesis of the disease (Freire *et al.*, 2017). The expression of chemerin₁ receptors (ERV1) in human monocyte-derived macrophages was recently shown to be regulated by **LPS** and **IFN- γ** , where incubation of monocyte-derived macrophages with these inflammatory signals activates the promoter of these receptors (Herova *et al.*, 2015).

GPR18 (resolvin D2 receptor; DRV2) is a receptor for RvD2. The binding of the agonist to its receptor leads to an increase in intracellular cAMP as well as CREB, ERK1/2 and STAT3 phosphorylation. Loss of this receptor in transgenic animals is associated with delayed resolution response to both sterile and infectious inflammatory insults (Chiang *et al.*, 2015; Zhang *et al.*, 2016) as well as increased mortality during polymicrobial sepsis (Chiang *et al.*, 2017). Silencing

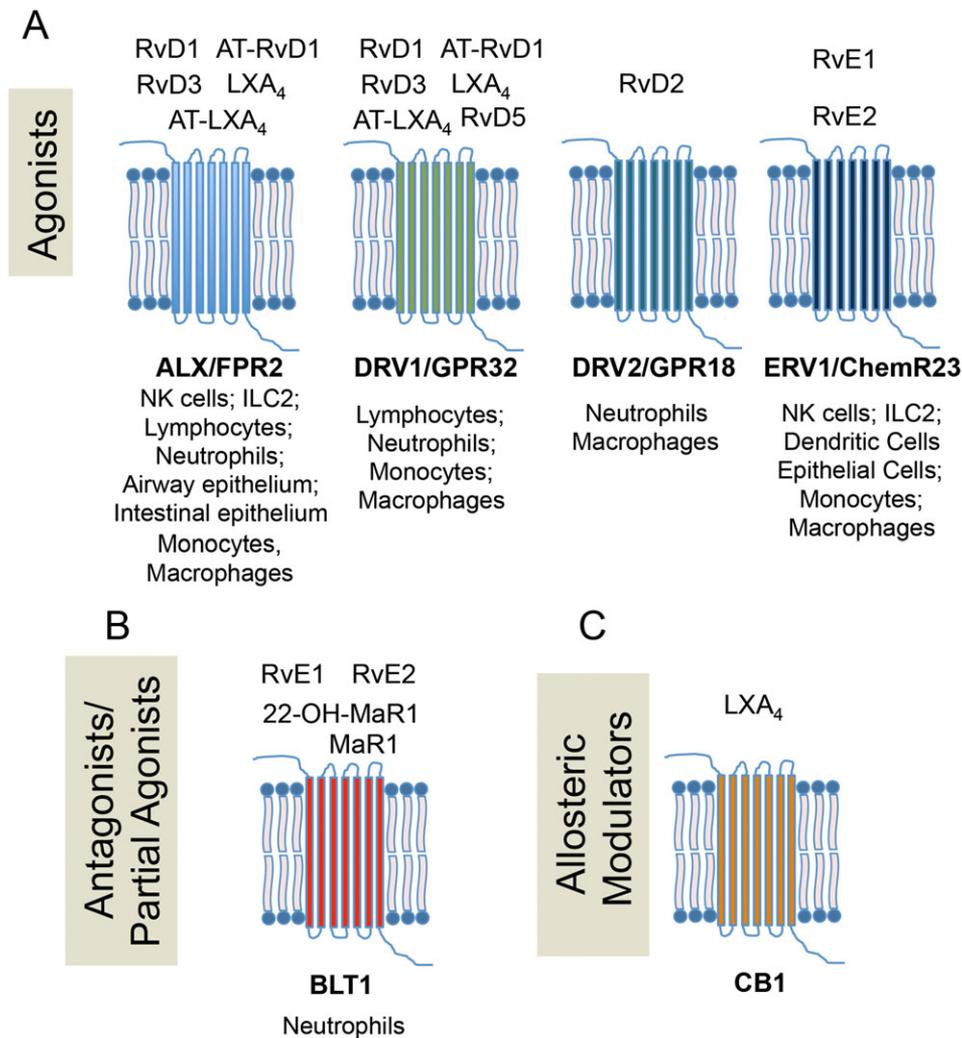


Figure 3

Distinct interactions between SPM and GPCRs in controlling host responses. (A) Depicts the receptors activated by select pro-resolving mediators and the target cells in which these receptors mediate the actions of their cognate SPM. (B,C) Depicts the receptors at which each SPM acts as a (B) antagonists/partial agonist or (C) allosteric modulator.

of GPR18 (DRV2) on human macrophages also leads to a loss in their ability to clear bacteria and apoptotic cells, two key resolution responses, thereby underscoring the role of this RvD2-GPR18 axis in restabilising tissue function following an inflammatory insult (Chiang *et al.*, 2015; 2017).

SPM as allosteric modulators and antagonists

In addition to activating cognate receptors, SPM also regulate the activity of receptors for other endogenous mediators. LXA₄ was recently found to be an endogenous allosteric modulator of the cannabinoid CB₁ receptor (Pamplona *et al.*, 2012). This SPM enhanced the affinity of anandamide at the CB₁ receptor potentiating its biological actions without competing for the orthosteric binding site of the CB₁ receptor and altering endocannabinoid metabolism (Figure 3). In addition, LXA₄ displayed protective actions against β -amyloid

(1–40)-induced spatial memory impairment, actions that were CB₁ receptor-dependent (Pamplona *et al.*, 2012).

Some SPM also act as antagonists to receptors of pro-inflammatory eicosanoids. RvE1, MaR1 and its further metabolite 22-OH-MaR1 are all competitive antagonists of BLT1 receptors (Figure 3) inhibiting the signalling and biological actions of the potent leukocyte chemoattractant LTB₄ (Arita *et al.*, 2005; El Kebir *et al.*, 2012; Oh *et al.*, 2012; Colas *et al.*, 2016). In human neutrophils, BLT1 antagonism enhances neutrophil apoptosis (El Kebir *et al.*, 2012). Of note, during self-limiting infectious inflammation, MaR1 concentrations reach a maximum early on in the course of the inflammatory process, concomitant with a rise in LTB₄ (Colas *et al.*, 2016). This suggests that, MaR1 production may play an important role in antagonizing the actions of LTB₄, thereby limiting neutrophil recruitment. These results also indicate that disruptions in the biosynthesis of this mediator may lead to

Table 1

Biological actions of SPM analogues

Analogue	Biological action	Dose	Reference
Lipoxin analogues			
15(R/S)-methyl-LXA ₄	Inhibits vascular permeability change and PMN infiltration	3–130 nM 2.5–10 µg·kg ⁻¹	(Takano <i>et al.</i> , 1998) (Mitchell <i>et al.</i> , 2002)
16-phenoxy-LXA ₄	Stimulates phagocytosis of apoptotic PMN by macrophages <i>in vivo</i> Inhibits neutrophil infiltration in response to LTB ₄	240 nM	(Takano <i>et al.</i> , 1997)
15-epi,16-phenoxy-LXA ₄	Inhibits neutrophil infiltration in response to LTB ₄	240 nM	(Takano <i>et al.</i> , 1997)
16-parafluoro-phenoxy-LXA ₄	Inhibits PMN infiltration	26 nM	(Takano <i>et al.</i> , 1998)
5(S)-methyl-LXB ₄	Inhibits PMN infiltration	26 nM	(Takano <i>et al.</i> , 1998)
15-epi, 16-para-fluorophenoxy-LXA ₄ -methyl ester	Tissue protection and reduced neutrophil infiltration during kidney ischaemia–reperfusion. Reduces tissue IL-1β, IL-6, and GRO1 mRNA levels Prevents airway hyper-responsiveness to methacholine Reduces eosinophils and lymphocytes infiltration Protects against vascular injury	15 µg per mouse 10 µg per mouse 100–1000 µg·cm ⁻² 0.1 nM–1 µM 10 µg·mL ⁻¹ (p.o) 1–100 nM	(Leonard <i>et al.</i> , 2002) (Levy <i>et al.</i> , 2002) (Schottelius <i>et al.</i> , 2002) (Gewirtz <i>et al.</i> , 2002) (Cezar-de-Mello <i>et al.</i> , 2008)
	Reduces skin inflammation Inhibits human neutrophil chemotaxis		
	Reduces colon inflammation		
	Inhibits VEGF-induced EC migration and proliferation		
o-[9,12]-benzo-ω6-epi-lipoxin A ₄	Reducec leukocyte infiltration into the tempo mandibular joint following CFA administration Reduces neutrophil recruitment in response to zymosan	10 ng per mouse 100 ng per mouse 1 µg per site	(Norling <i>et al.</i> , 2011) (Van Dyke <i>et al.</i> , 2015)
	Promotes regeneration of hard and soft tissues irreversibly lost to periodontitis in the Hanford miniature pig		
D-series resolvins analogues			
7R/S methyl RvD1 methyl ester	Reduces DC expression of MHC II, CD40 and IL-12 following LPS stimulation Reduces allosensitization during corneal transplant and enhances graft survival and angiogenesis	100 µg per mouse	(Hua <i>et al.</i> , 2014)
Benzo-diacetylenic-17R-RvD1-methyl ester	Shortens the resolution interval, Ri, during <i>E. coli</i> peritonitis Reduces ischaemia-reperfusion-initiated second organ injury	100 ng per mouse 1 µg per mouse	(Orr <i>et al.</i> , 2015)

continues

Table 1

(Continued)

Analogue	Biological action	Dose	Reference
E-series resolvin analogues			
RX-10045	Substrate/inhibitor for efflux transporters multidrug resistance-associated protein, breast cancer-resistant protein and organic cation transporter-1	50–300 μ M	(Cholkar <i>et al.</i> , 2015)
		0.01% solution	(Torricelli <i>et al.</i> , 2014)
	Reduces corneal opacity after haze-generating after opacity-generating high correction photorefractive keratectomy	0.05–0.1% nanomicellar solution	(Wire, 2009)
	Reduction from baseline in controlled adverse environment-induced staining of the central cornea Improvement in human dry eye disease symptoms, including dryness, stinging, burning, grittiness and ocular discomfort		
α -cyclopropane resolvin E2	Reduces the number of exudate leukocytes in response to <i>Propionibacterium acnes</i> infection	300 fg–3 ng per mouse	(Fukuda <i>et al.</i> , 2016)
β -cyclopropane resolvin E2	Reduces the number of exudate leukocytes in response to <i>P. acnes</i> infection	300 fg–3 ng per mouse	(Fukuda <i>et al.</i> , 2016)

delayed resolution of responses. Together, these findings highlight the utility of SPM as templates for the development of novel receptor modulators.

Harnessing SPM biology towards resolution pharmacology

SPM display potent biological actions without the side effects displayed by traditional anti-inflammatories. The reason for this lack of unwanted side effects may be attributable to the mechanisms activated by SPM, which are distinct from those regulated by anti-inflammatories. Indeed, SPM do not completely abolish the production and actions of molecules and cells considered to be inflammatory. Instead, these mediators reprogramme both the immune response and stromal cells limiting the production of inflammatory molecules and up-regulate the expression of protective mediators (Mukherjee *et al.*, 2004; Pamplona *et al.*, 2012; Lee *et al.*, 2013; Miyata *et al.*, 2013; Dalli *et al.*, 2014; Herova *et al.*, 2015; Colby *et al.*, 2016; Codagnone *et al.*, 2018; de Oliveira *et al.*, 2017). This reprogramming of the immune system is also linked to the apparent lack of immunosuppressive action. Thus, prompted by these observations, several studies have explored the potential of harnessing these actions to control excessive inflammation characteristic of many

diseases that afflict modern societies (Takano *et al.*, 1997; Leonard *et al.*, 2002; Mitchell *et al.*, 2002; Guilford *et al.*, 2004).

From these efforts, several generations of analogues and mimetics are now developed (see Table 1) with the aim of obtaining molecules with enhanced pharmacokinetics and pharmacodynamics that are also amenable to scaled-up synthesis (Takano *et al.*, 1997; Leonard *et al.*, 2002; Mitchell *et al.*, 2002; Guilford *et al.*, 2004). The first of these developed was the lipoxin analogues that retain the biological actions of their parent molecules but with enhanced stability. One of these analogues, a benzo-LXA₄, displays potent host protective actions that include the ability to regulate neutrophil responses and promote tissue repair and regeneration in periodontal disease (Van Dyke *et al.*, 2015). These observations have now been extended to humans where the protective actions of this analogue are being investigated in the context of human periodontal disease (ClinicalTrials.gov Identifier: NCT02342691). A later generation analogue of RvE1 displays potent protective actions in dry eye syndrome in humans. In a phase 2 trial, this RvE1 analogue significantly reduced disease incidence, controlling dry eye-related inflammation, findings that are under further investigation in a phase 3 trial (Wire, 2009.).

Additionally, recent studies have demonstrated the protective actions of analogues of RvD1 (Orr *et al.*, 2015) and RvE2 (Fukuda *et al.*, 2016). The RvD1 analogues retain

the ability to activate GPR32 (DRV1) as well as the tissue protective actions of the parent SPM (Orr *et al.*, 2015). Enhanced biological actions are also displayed the cyclopropane congeners of RvE2 (Fukuda *et al.*, 2016), thereby supporting a role for these analogues as novel therapeutics.

Conclusion

The appreciation that resolution of inflammation is an active process and SPM are central in controlling both cellular trafficking and responses has opened up new and exciting horizons for the development of new therapeutics. Given that resolution pharmacology-based medicines will harness endogenous reparative responses, it is anticipated that they will be burdened with lower side effects since they will not interfere with natural host immunity, potentially also increasing patient compliance. Furthermore, these findings shed new light on the roles of omega-3 essential fatty acids in the control of acute inflammation. The protective actions observed in many clinical studies using these fatty acids are not simply due to the inhibition of inflammatory eicosanoid formation by competing for the biosynthetic enzymes. Indeed, these substrates are precursors to structurally unique molecules, the SPM, that carry potent tissue protective actions. Thus, these mediators may represent novel markers to determine the pharmacodynamics and pharmacokinetics of omega-3 supplements and their ability to influence the host response in both healthy people as well as patients with inflammatory diseases. While the evidence in humans for the utility of this approach is still being investigated, results from animal and initial human experiments are encouraging (Dalli *et al.*, 2013; Arnardottir *et al.*, 2016; Barden *et al.*, 2016; Markworth *et al.*, 2016; Gobbetti *et al.*, 2017). Therefore, resolution-based therapeutics provide an exciting new paradigm for personalized medicine where supplementation can be utilized to maintain/boost endogenous SPM levels to preserve tissue resolution tone and health. Whereas in disease settings, SPM-based drugs may be useful in regulating host responses to both local and systemic inflammation. In this context, nanomedicines enriched in SPM or their analogues/mimetics (Norling *et al.*, 2011) may provide a tractable system for targeted tissue delivery that can control both inflammation and promote tissue repair and regeneration (Van Dyke *et al.*, 2015). Hence, resolution pharmacology could provide the basis for reprogramming host immunity in order to expedite microbial clearance, limit collateral tissue damage and stimulate tissue regeneration.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018) and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b,c,d).

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Conflict of interest

The authors declare no financial conflicts of interest.

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