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# Structural Elucidation and Physiologic Functions of Specialized Pro-Resolving Mediators and Their Receptors

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## Abstract

The acute inflammatory response is host protective to contain foreign invaders. Many of today's pharmacopeia that block pro-inflammatory chemical mediators can cause serious unwanted side effects such as immune suppression. Uncontrolled inflammation is now considered a pathophysiologic basis associated with, many widely occurring diseases such as cardiovascular disease, neurodegenerative diseases, diabetes, obesity and asthma, as well as the classic inflammatory diseases, e.g. arthritis, periodontal diseases. The inflammatory response is designated to be a self-limited process that produces a superfamily of chemical mediators that stimulate resolution of inflammatory responses. Specialized proresolving mediators (SPM) uncovered in recent years are endogenous mediators that include omega-3-derived families resolvins, protectins and maresins, as well as arachidonic acid-derived (n-6) lipoxins that stimulate and promote resolution of inflammation, clearance of microbes, reduce pain and promote tissue regeneration via novel mechanisms. Here, we review recent evidence from human and preclinical animal studies, together with the structural and functional elucidation of SPM indicating the SPM as physiologic mediators and pharmacologic agonists that stimulate resolution of inflammation and infection. These results suggest that it is time to develop immunoresolvents as agonists for testing resolution pharmacology in nutrition and health as well as in human diseases and during surgery.

### Keywords

omega-3 PUFA; leukocytes; resolvins; inflammation; protectins; maresins

## Introduction

Phagocytes in the innate immune system play a central role to protect the host from invading organisms and foreign objects. The repertoire and cell trafficking of the acute inflammatory

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response is protective, and the cardinal signs of inflammation – heat, redness, swelling, and eventual loss of function – were recognized by physicians of ancient civilizations (Majno, 1975; Cotran, 1999). It is now clear that excessive or uncontrolled inflammation is associated with many widely occurring diseases. The therapeutic approach to treating excessive inflammation and the resulting collateral tissue damage has not significantly changed since ancient practitioners of folk medicine used willow bark (Yedgar et al., 2007); namely, therapeutic approaches to inflammation have focused on suppressing, blocking or inhibiting proinflammatory mediators of inflammation (Serhan, 2017). While many of these are effective, relieving gross signs and symptoms, but can give rise to immune suppression and infections (Dinarello and Joosten, 2016; Delano and Ward, 2016). Therefore, new therapeutic interventions need to be developed to address infection, inflammatory diseases, aging and the tissue damage-evoked inflammation associated with surgery (Nathan, 2012).

Temporal events in many physiologic inflammation or self-limited acute inflammatory responses are recognized to resolve at the histologic level (Figure 1) with the loss of inflammatory infiltrates from these tissue with the return of function or catabasis (Robbins and Cotran, 1979; Serhan et al., 2004). The cellular steps and tissue histology of the stage were set as for example viewed clinically in the resolution of lung inflammation (Henson, 1991; Savill et al., 1989) yet the role and function of resolution phase mediators remained to be uncovered. Focus on the fundamental mechanisms in the resolution response using a modern systems approach, in the authors' laboratories, led to the isolation and complete structural elucidation of several novel families of pro-resolving mediators of inflammation that together constitute a superfamily of structurally distinct bioactive mediators. These are biosynthesized from essential polyunsaturated fatty acids precursors to function as potent local resolution agonists. CNS coined the first of these new mediators "resolvins", indicating their potent bioactions and biosynthetic origins as in the *resolution phase interaction products* that function in catabasis to return the host tissues from leukocyte defensive position with microbial invaders to normal functions (Serhan et al., 2000; Serhan et al., 2002; Hong et al., 2003; and reviewed in refs. Serhan, 2011; Serhan, 2014). The identification of pro-resolving pathways and mediators that expedite resolution of inflammation was rapidly embraced by colleagues studying immunopharmacology and opened the potential for considering new therapeutic directions (Gilroy et al., 2004; Serhan et al., 2007). The cells, mediators, and mechanisms in the resolution of inflammation are the subjects of several recent in-depth reviews that interested readers are directed to (Jones et al., 2016; Perretti, 2015; Robb et al., 2016; Tabas and Glass, 2013; Fullerton and Gilroy, 2016; Buckley et al., 2014; Spite et al., 2013; Serhan et al., 2015b; Serhan et al., 2015a).

This update and overview addresses the emergence of the concepts and potential for endogenous mediators of resolution and tissue regeneration within the resolution terrain that give rise to a new field, namely resolution pharmacology. There hasn't been a conceptual change in pharmacology of patient care for inflammatory diseases or infections for centuries. New approaches are needed to minimize or eliminate unwanted side effects and immune suppression that can accompany prolonged use of traditional anti-inflammatory therapies e.g. steroids, non-steroidal anti-inflammatory drugs (cyclooxygenase inhibitors) as well as the newer biologics that act by blocking cytokines such as anti-TNF therapies. The currently available pharmacopeia for treatment consists mainly of inhibitors and receptor

antagonists to manage excessive inflammation and/or infectious inflammation. The new terrain of natural, active resolution, namely the pro-resolving pathways and endogenous mediators uncovered give us new concepts with physiologic and pharmacologic means that are in alignment with the self-limited inflammatory response. Importantly, its natural timely resolution with tissue regeneration and return of function together provides fertile ground for treating excessive inflammation as well as controlling the communication between the innate and adaptive immune responses.

## The Inflammatory Cascade – Alpha Signals Omega

The acute inflammatory response works in our defense daily, protecting us from microbial invades and tissue injury. It was important to understand and determine the molecular decision paths that can lead from acute inflammation to either chronicity or the ideal out come of complete resolution (Figure 1). In textbooks the resolution of acute inflammation was thought to be a passive process, meaning that inflammatory mediators from the initiation of the acute response (e.g. chemoattractants, complement components, C5a, C3b, prostaglandins, chemokine and cytokines) would just simply dilute and dissipate (Dinarello, 2010; Nathan, 2012; Robbins and Cotran, 1979) to stop the infiltration of leukocytes into the tissues. By studying self-limited acute inflammatory responses with inflammatory exudates formed in vivo from mice that we adopted from the well-known rat air pouch (Winyard and Willoughby, 2003), we learned that, during self-limited responses in animal models using a systems approach, this process gives rise to active resolution where resolvins and other specialized proresolving mediators (SPMs), a superfamily of pro-resolving mediators that include resolvins, protectins, maresins and lipoxins (Serhan, 2014), take part to bring about a state of resolution and tissue function (Serhan et al., 2000; Serhan et al., 2002; Hong et al., 2003; Bannenberg et al., 2005).

Considering chronic inflammation and infections, we first focused our research efforts on periodontal disease (Van Dyke, 2008) because it's a chronic inflammation that is certainly a public health need (Serhan et al., 2003; Van Dyke and Serhan, 2006). Patients with periodontal disease are a very good example of leukocyte-mediated tissue destruction primarily by neutrophils that can amplify inflammation via debris produced around the ligaments of the tooth, so we thought that this would be an ideal clinical scenario to test the principles of stimulating resolution to control the unwanted side effects of this form of infectious inflammation. The main questions of interest in the author laboratories are: 1) What are the endogenous controllers of excessive inflammation and infection? 2) How are these linked and 3) what are the signaling molecules involved? The specialized pro-resolving mediators – each of these are a separate family of bioactive mediators, the resolvins, protectins and maresins, with separate biosynthesis pathways and receptors (Figure 2 and 3) (Serhan, 2014). Recent findings introduced three new pathways identified that stimulate in tissue regeneration (Dalli et al., 2015c). Their original structural elucidations were confirmed using the complete stereochemical assignments and total organic synthesis, thus, providing the basis for resolution pharmacology.

The results from our studies, illustrated in Figure 1, emphasize that the resolution of inflammation as an active process with novel mediators and checkpoint endogenous

controlled mechanisms. Prof. Bengt Samuelsson and colleagues of the Karolinska Institute, Stockholm, Sweden, contributed seminal discoveries of the biosynthetic pathways for the prostaglandins and leukotrienes (Samuelsson, 1983), which are important mediators that evoke the classic cardinal signs of inflammation (Robbins and Cotran, 1979). Prostaglandins and leukotriene $B_4$ play critical roles at the beginning of the acute inflammatory response, and in a controlled laboratory setting (with a fixed time zero of the initiation of the acute inflammatory response) we've introduced this temporal sequence of key cellular and molecular events for the signs of resolution (Dalli et al., 2015a; Serhan, 2014), which are regulated by the pro-resolving mediators. There is a temporal lipid mediator class switch that leads to the production of the lipoxins (Levy et al., 2001) that also involve phospholipases (Norris et al., 2014) and the resolvins that gives signals to macrophages and the non-phlogistic recruitment of monocytes (Maddox and Serhan, 1996; Jones et al., 2012), as they eat and take up the apoptotic neutrophils via phagocytosis (Godson et al., 2000). When added back pharmacologically, resolvins and other SPM such as the protectins regulate inflammatory mediators by counter-regulation (TNFα, PAF, prostaglandins, leukotrienes chemokines, cytokine) to head off inflammation in animal models (Serhan, 2014) as well as organ fibrosis (Börgeson et al., 2012; Martins et al., 2009; Qiu et al., 2014; Qu et al., 2012). The maresins and resolvin E1 were first recognized (Serhan et al., 2009; Dalli et al., 2013b) to stimulate resolution as well as tissue regeneration and subsequent wound healing (Serhan et al., 2012; Spite et al., 2013). Importantly, inhibitors of prostanoid biosynthesis like the classic NSAID disrupt timely resolution (Buckley et al., 2014; Levy and Serhan, 2014; Schwab et al., 2007; Chan and Moore, 2010). Keep this in mind in terms of thinking about immune suppression of classic anti-inflammatory therapies, NSAIDs and steroids that are widely used (Koopman and Moreland, 2005) as in, for example, the treatment of arthritis or other widely occurring inflammatory conditions and diseases, such as neurodegenerative diseases, obesity, metabolic syndrome. Prostaglandin biosynthesis is critical to mounting resolution because $PGE_2$ and $PGD_2$ stimulate the induction of 15 type I lipoxygenase required to produce lipoxins and some specific SPM, resolvins and protectins. The novel concept from these results is that the resolution process begins at time zero experimentally, and is an active process (Bannenberg et al., 2005; Serhan and Savill, 2005): namely, alpha signals omega; the beginning signals the end or termination of the acute inflammatory response (Serhan and Savill, 2005).

The main physiologic responses that we used to structurally elucidate the pro-resolving mediators include evoking cessation of neutrophilic infiltration, reducing diapedesis to limit further neutrophilic infiltration to a site of inflammation at the postcapillary venule, and stimulating macrophage phagocytosis. When added back pharmacologically this is perceived in experimental animals as anti-inflammation or anti-inflammatory. This is quite different from the pro-resolving action, which is actually comprised of both of these, where the pro-resolving mediators increase the clearance and killing, efferocytosis and phagocytosis of the apoptotic PMN, so it took us quite some time to discover that pro-resolution was not identical to anti-inflammation. To be able to do this systematically and gain the evidence, we introduced resolution indices to be quantitative and to pinpoint the actions of SPM in experimental animal models (Table 1) (Bannenberg et al., 2005). This extended on to the uptake and killing of microbes (Table 2) (Chiang et al., 2012; Haas-Stapleton et al., 2007).

We systematically assigned stereochemistries and biosynthesis of the E-series resolvins (Figure 2A). There are three: resolvin E1 (Serhan et al., 2000; Arita et al., 2005; Oh et al., 2011), resolvin E2 (Oh et al., 2012), and the third family member recently discovered and denoted resolvin E3 (Isobe et al., 2012). Docosahexaenoic acid is enriched in human organs such as the brain, eye, breast milk and testes (Lands, 2005; Bracco and Deckelbaum, 1992), and DHA is also used by inflammatory exudates, or pus cells. DHA is transformed into six separate and structurally distinct D-series resolvins; we've carried out the complete stereochemical assignments of each (Serhan and Petasis, 2011) (Figure 2B). During this process, a hydroperoxide-containing intermediate is converted into an epoxide that is precursor to the protectin family (Serhan et al., 2015b) and neuroprotectin D1 (Figure 2C); we collaborated with Professor Nicolas Bazan and his colleagues to introduce neuroprotectin (NPD1) (Mukherjee et al., 2004). Macrophages arrive in resolving exudates later within the inflammatory response and convert DHA to the maresins (Figure 2C) (Serhan et al., 2009). The resolvins, the lipoxins, the protectins and maresins as SPM at this time have been studied by many independent laboratories (more than >2000 PubMed citations) documenting their potent actions in reducing inflammation and a wide range of preclinical disease models using validated commercial available resolvins and other synthetic SPM. In nanogram to microgram amounts, they are stereoselective, they have novel mechanism of action, they are active in the airway, cardiovascular, ocular, in renal function, within the brain, periodontal and, for example, gastrointestinal disease (Campbell et al., 2011), as well as in the liver (Rius et al., 2014). Table 2 lists only a few of these potent and more recently discovered actions in animal models of infections. These results illustrate the agonist actions of SPM and their potential to control and treat excessive inflammation in human infections.

## How do SPMs work? SPM receptors

In the postcapillary venule, results indicate that SPMs key function is to limit the further recruitment of PMN, yet stimulate the nonphlogistic recruitment of mononuclear cells (Figure 1). When macrophages encounter SPMs they increase phagocytosis resulting in the removal of apoptotic PMN as well as microbes and clear PMN from the sites. The SPMs are proven not to be immunosuppressive in multiple in vivo experimental animal models (those cited in Table 2 for example). Each biosynthesized SPM counterregulates the early initiators of acute inflammation, the prostaglandins; they regulate COX-2 expression, leukotrienes and PAF formation, counterregulate the pro-inflammatory cytokines and increase IL-10 (Serhan et al., 2007). Importantly, they regulate NF-κB gene products and ultimately lead to the regulation of edema (Arita et al., 2005). SPM activate five separate GPCR receptors (Figure 3A) (Serhan and Chiang, 2013; Chiang et al., 2015). Prof. Mauro Perretti studied the lipoxin $A_4$ receptor that can form heterodimers to evoke differential intracellular signaling (Cooray et al., 2013; Filep, 2013), which is also shared by resolvin D1 (Krishnamoorthy et al., 2010), confirmed in receptor knockout mice (Norling et al., 2012). SPM receptors are expressed on different cell types giving rise to tissue selectivity. This has been qualified with knockouts and transgenic mice (Devchand et al., 2003), as most recently the resolvin D2 receptor (Chiang et al., 2015). These SPM receptors counterregulate NFκB and they increase heme-1-oxygenase and endogenous carbon monoxide in many target organs to give tissue protection (Chiang et al., 2013; Biteman et al., 2007; Jin et al., 2007).

### ALX and DRV1/GPR32

RvD1's pro-resolving actions are mediated via its interaction with both ALX and human GPR32 (Krishnamoorthy et al., 2010) (Figure 3A). Also, aspirin-triggered (AT) epimer 17R-RvD1 and stable analog 17-R/S-methyl-RvD1 each dose dependently activates ALX/FPR2 and GPR32 (Krishnamoorthy et al., 2012). D-series Rv ligands for GPR32 identified recently also include RvD5, RvD3 and AT-RvD3 (Chiang et al., 2012; Dalli et al., 2013a). RvD1 displays specific binding and reduces actin polymerization and CD11b on PMN, as well as stimulate macrophage phagocytosis in an ALX and GPR32-dependent manner (Krishnamoorthy et al., 2010). In addition to RvD1, its aspirin-triggered epimer 17R-RvD1 and stable analog 17-R/S-methyl-RvD1 each dose dependently activates ALX/FPR2 and GPR32 in GPCR-overexpressing β-arrestin systems and electric cell-substrate impedance sensing (Krishnamoorthy et al., 2012). Recently we demonstrated that RvD5 also activates human GPR32 in the GPR32-β-arrestin systems, and stimulates macrophage phagocytosis of *E. coli* in a GPR32-dependent manner (Chiang et al., 2012). In addition, RvD3 and AT-RvD3 each activates GPR32, contributing to their pro-resolving actions in stimulating macrophage uptake of microbial particles (Dalli et al., 2013a).

### ERV/ChemR23

We identified an orphan GPCR, ChemR23, as a receptor for RvE1 (Figure 3A). ChemR23 binds $^{3}$H-RvE1 and stereoselectively transduces signals to monocytes, and dendritic cells to reduce IL-12 production (Arita et al., 2005). ChemR23 is closely related to lipoxin and leukotriene receptors in amino acid sequences. RvE1-ChemR23 interactions also stimulates macrophages phagocytosis via phosphorylation-signaling pathways including Ribosomal protein S6, a downstream target of the PI3K/Akt signaling pathway and the Raf/ERK pathway (Ohira et al., 2010). 18*S*-RvE1 also activates this receptor ChemR23 with increased affinity and potency compared with the R-epimer, but was more rapidly inactivated than RvE1 (Oh et al., 2011). RvE2 is a partial agonist for ChemR23 (Oh et al., 2012).

### RvE1-BLT1 interactions

a leukotriene $B_4$ receptor, also directly interacts with RvE1, which inhibits calcium mobilization, NF-κB activation in vitro and PMN infiltration in vivo (Arita et al., 2007). 18S-RvE1 and RvE2 also bind to BLT1 (Oh et al., 2011; Oh et al., 2012). In BLT1 knockout mice, in vivo anti-inflammatory actions of RvE1 were sharply reduced when given at low doses (100 ng i.v.) in peritonitis. In contrast, RvE1 at higher doses (1.0 μg i.v.) significantly reduced PMN infiltration in a BLT1-independent manner. These results indicate that RvE1 binds to BLT1 as a partial agonist, serving as a local damper of BLT1 signals on leukocytes along with other receptors (e.g., ChemR23-mediated counterregulatory actions) to mediate the resolution of inflammation (Arita et al., 2007).

### DRV2/GPR18

Recently we identified a new resolvin D2 receptor, namely GPR18/DRV2 that is expressed in human leukocytes including polymorphonuclear neutrophils (PMN), monocytes and macrophages (Chiang et al., 2015) (Figure 3B). Specific binding of RvD2 to recombinant DRV2 was confirmed using a synthetic $^{3}$H-labeled-RvD2 and gave a Kd~10nM consistent

with RvD2 bioactive concentration range. In human macrophages, RvD2-stimulated phagocytosis of *E. coli* and apoptotic PMN (efferocytosis) were enhanced with DRV2 overexpression and significantly reduced by shRNA knockdown (Chiang et al., 2015).

### SPM receptor transgenic mice

We constructed mice overexpressing human ALX. hALX transgene was placed under the control of CD11b promoter, directing receptor expression in myeloid cells (Devchand et al., 2003). In non-TG littermates, RvD1 as low as 10 ng given together with zymosan, reduced leukocyte numbers by ~38% at 24h. This action was further enhanced in ALX-TG mice giving 53% reduction of leukocytes. Also with RvD1 treatment, PMN numbers in TG-mice was 50% lower than non-TG controls (Krishnamoorthy et al., 2012). Lipid mediator metabololipidomics carried out with 24-hour exudates revealed that RvD1 in vivo gave a significant reduction in a number of pro-inflammatory mediators including prostaglandins and $LTB_4$ in wt mice.

We also prepared transgenic mice 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, compared with non-TG littermates. In addition, ligature-induced alveolar bone loss was diminished in chemR23tg mice. Local RvE1 treatment of uniform craniotomy in the parietal bone significantly accelerated regeneration of the bone defect. These results indicate that RvE1 modulates osteoclast differentiation and bone remodeling by direct actions on bone, in addition to its anti-inflammatory and proresolving actions (Gao et al., 2013).

### SPM receptor deficient mice

In mice deficient of *alx/fpr2* (mouse orthologue of human ALX), the anti-inflammatory actions of RvD1 were abolished. Administration of RvD1 (1 ng/mouse) significantly reduces PMN infiltration in WT mice, but not in fpr2 null mice. Also in peritoneal exudates, RvD1 activates lipoxin biosynthesis stimulating the production of the anti-inflammatory mediator $LXB_4$ and stimulated the biosynthesis of the cyclooxygenase-derived $PGE_2$ while downregulating production of the proinflammatory $LTB_4$. This regulation of lipid mediator by RvD1 is lost in the fpr2 null mice (Norling et al., 2012). These results indicate that RvD1 dampens acute inflammation in part via ALX receptor. In addition, in the ischemia reperfusion (IR) injury of the mesenteric artery, administration of $LXA_4$ attenuated IR-mediated inflammation in WT but not alx/Fpr2(-/-) mice (Brancaleone et al., 2013). Further, AT-$LXA_4$ blocked atherosclerosis progression in the aortic root and thoracic aorta of $ApoE^{-/-}$ mice. This protection by AT-$LXA_4$ was abolished in $ApoE^{-/-}$ *alx/fpr2* $^{-/-}$ mice (Petri et al., 2017).

**DRV2/GPR18-deficient mice—**In both *E. coli* and *S. aureus* infections, RvD2 limited PMN infiltration, enhanced phagocyte clearance of bacteria and accelerated resolution (Chiang et al., 2015). These actions were lost in DRV2 deficient mice. During PMN-mediated second organ injury, RvD2's protective actions were also significantly diminished in DRV2 deficient mice. In addition, in polymicrobial sepsis initiated by cecal ligation and puncture (CLP), RvD2 (~2.7 nmol/mouse) significantly increased survival (>50%) of wild-

type (WT), reduced hypothermia and bacterial titers compared to vehicle-treated CLP mice that succumbed at 48h (Chiang et al., 2017). Protection by RvD2 was abolished in DRV2-KO mice. Mass spectrometry-based lipid mediator metabololipidomics demonstrated that DRV2-KO infectious exudates gave higher pro-inflammatory leukotriene (LT) $B_4$ and pro-coagulating thromboxane (TX) $B_2$, as well as lower SPM, including RvD1 and RvD3, compared to WT. RvD2-DRV2-initiated intracellular signals were investigated using mass cytometry (CyTOF) which demonstrated that RvD2 enhanced phosphorylation of CREB, ERK1/2 and STAT3 that were absent in DRV2-KO macrophages. Monitored by real-time imaging, RvD2-DRV2 interaction significantly enhanced phagocytosis of live *E. coli*, an action dependent on PKA and STAT3 in macrophages. Together, these results provide evidence for a novel RvD2-GPR18 resolution axis that activates intracellular signaling pathways, stimulates human and mouse phagocyte functions to control bacterial infections and promote organ protection (Figure 3B). In addition, RvD2 enhanced endothelial cell migration in a Rac-dependent manner, via DRV2/GPR18, and *Gpr18*-deficient mice had an endogenous defect in perfusion recovery following hind limb ischemia (Zhang et al., 2016). RvD2 and its receptor reduce neuroinflammation that impacts systemic obesity (Pascoal et al., 2017).

## Functional SPM Metabolomics enables personalized nutrition and precision medicine

Recently we operationalized lipid mediator metabolomics (Colas et al., 2014). We routinely identify six diagnostic ions in each mediator, a key point for accurate and rigorous identification. Routinely, we perform computational and cluster analysis of these pathway mediators. These are critical not only in organ and tissue profiles such as with blood and plasma (Colas et al., 2014; Dalli et al., 2017b), which is an example that can also be useful in personalized and precision medicine in the near future. From this approach, we and others have now shown by mass spectrometry that many human tissues produce pro-resolving mediators (Table 3 and references within). SPM are produced in human whole blood where they function to increase both phagocytosis and bacterial killing (Colas et al., 2014). Of interest are the recent results with human breast milk (Arnardottir et al., 2016a) that have high levels of SPM that are functional and stimulate resolution. This finding suggests that there are organs and tissues that produce SPM in addition to the inflammatory exudate and innate immune system. Recently synovial fluids were profiled (Norling et al., 2016) as well as by Barden et al. (Barden et al., 2016b) that showed increases in SPM upon supplementation with RvE2 amounts correlating with reduced pain scores.

Some of the highest levels of the proresolving mediators are found in the human placenta (Keelan et al., 2015), which is quite intriguing, and in the human and murine lymph nodes as well (Colas et al., 2014). SPM and eicosanoids are present in intensive care unit sepsis patients that reflect the disease time course and dynamics (Dalli et al., 2017b). Resolvin D1, 17-epi-RvD1 and resolvin D2 as well as PD1 were each identified in 51 samples from human placenta. Supplementation with omega-3 PUFA increased placental SPM precursors 17-HDHA and 18-HEPE, while increases in placental resolvins and PD1 were not significantly increased by oral supplementation (Keelan et al., 2015). This is different in rat

placenta, where increased consumption of n-3 PUFA increased pathway precursors (Figure 2) and resolvin D1 and 17-epiresolvin D1. Protectins, e.g. PD1 and PDx (10*S*,17*S*-diHDHA), also increase in late gestation rat placenta along with increased expression of ALOX15 m-RNA (Jones et al., 2013). Hence, these placental studies demonstrate that the local organ control of resolvins and protectins is apparently tightly regulated in placenta from humans and rats, illustrating species differences in the relationship between oral consumption of omega-3 PUFA and local biosynthesis of SPM in mammalian organs. In obese individuals supplemented with n-3, resolvin D1 and resolvin D2 were essentially doubled in peripheral blood in months to functional levels (Polus et al., 2016).

To establish the relationships and governing principles between oral supplementation of omega-3 and local SPM production and organ levels, detailed randomized and controlled studies are required. These are in progress, and have already given compelling evidence for the n-3 PUFA supplementation relationship to tissue resolvins and other SPM (Mas et al., 2015; Barden et al., 2016a). The further optimization of SPM profiling approaches via LC-MS-MS along with available deuterium-labeled internal and synthetic SPM standards for calculating recoveries and amounts of specific SPM family members (Colas et al., 2014; Dalli et al., 2015a; Le Faouder et al., 2013) will continue to permit rigorous interrogation of these relationships with human tissues (Elajami et al., 2016; Dalli et al., 2017b) to determine age, gender and organ-dependent levels for healthy individuals versus specific pathologic conditions. Of interest, human emotional tears contain SPM and their presents revealed a gender preference (English et al., 2017).

## SPM in human clinical trials

Our approach to human translation was to prepare a series of SPM analogs that resist local inactivation (Serhan and Petasis, 2011; Sun et al., 2007; Kasuga et al., 2008), hence using the body's own stop signals of inflammation as agonists of resolution as templates (Leslie, 2015) to make new proresolving therapeutics. We pursued this approach, for example with resolvin D1, of receptor mimetics in animal models and elsewhere to advance the development of agonists for the pro-resolving actions (Kasuga et al., 2008; Orr et al., 2015; Tang et al., 2014). This approach was used with resolvin E1 and other SPM since they are inactivated within tissues near the site of formation (Serhan and Petasis, 2011). These trial results were reported from a Phase I and Phase II multicenter double-blinded placebo trial with > 232 patients treated with an RvE1 analog. The results from this study from Resolvyx Pharmaceuticals, for which CNS was the original scientific founder, were the first demonstration of clinical efficacy with pro-resolving agents in humans (Business Wire, 2009; de Paiva et al., 2012). The Phase II trial controlling inflammation in the eye with topical drops (Business Wire, 2009) was based on the physiologic roles (Gronert, 2010; Li et al., 2010; Erdinest et al., 2014) of SPM such as resolvins and lipoxins in the eye (Li et al., 2013; Hodges et al., 2016; Dartt et al., 2011; Cortina and Bazan, 2011) and apparent gender differences, where females display delayed wound healing of cornea tissue and reduced 15-LOX-derived SPM such as lipoxin $A_4$ (Gao et al., 2015; Wang et al., 2012). Since 15-*R/S*-methyl lipoxin $A_4$, an analog of the aspirin-triggered form of lipoxin $A_4$, one of the first stop signals and pro-resolving mediators, is effective in infantile eczema in a double-blind, placebo-controlled study randomized at two different centers (Wu et al., 2013), and $LXA_4$,

resolvin D1, RvD2 and maresin 1 act on T-cell responses (Chiurchiu et al., 2016; Gao et al., 2015), it is likely that SPM have physiologic roles in human skin and barrier functions that remain to be studied, and their functions extend from the resolution phase into the adaptive immune response (Ramon et al., 2012). It is hoped that results from these studies can open up new directions for treatment of a wide range of human diseases by controlling the resolution and endogenous catabasis mechanisms of the inflammatory response via resolution and regeneration agonists.

## Resolution mediators and tissue regeneration

SPM control the severity of infections in animal pre-clinical models enhancing both phagocytosis and killing as well as reducing collateral tissue damage and pro-inflammatory mediators (Table 2) (Russell and Schwarze, 2014). Are there signals from resolving infectious exudate that act in tissue regeneration? To address this, we investigated the mouse model of peritonitis, where very rapid neutrophilic infiltration occurs followed by the resolution phase of self-limited infections (Dalli et al., 2014), using FACS analysis and LM-metabololipidomics to interrogate the infectious pus that's produced. With extract from *E. coli* infections, we employed another model organism, the planaria, a primordial organism (Serhan et al., 2012; Dalli et al., 2013b; Dalli et al., 2014), for surgical injury experiments. RvE1 and MaR1 each stimulate tissue regeneration in this system following surgical intervention there we introduce a quantitative index for tissue regeneration rates (Serhan et al., 2012). In these, we can surgically remove their heads or tails that grow back in a very short period of time on the order of days. In these experiments, we systematically took extracts of the resolving exudates from *E. coli* infection to assess whether mediators/signal molecules are produced in the resolution phase of infections that can accelerate tissue regeneration.

In these model systems primordial chemical signals were produced that activate this evolutionally conserved tissues regeneration isolated from infectious inflammatory exudates. The first two structures that were identified through structural elucidation carried carbon 14 position alcohols, so we knew that this placed them into the family of maresins, which have a conjugated double bond system (Figure 2C). The first was a glutathione adduct that is a 13-glutathionyl-14-hydroxy, which is 13R-glutathionyl, 14S-hydroxy-4Z,7Z,9E,11E,13R, 14S,16Z,19Z-docosahexaenoic acid, so coined the term maresin conjugates in tissue regeneration 1 (MCTR1). The structure of second or MCTR2 is 13R-cysteinylglycinyl, 14S-hydroxy-4Z,7Z,9E,11E,13R,14S,16Z,19Z-docosahexaenoic acid. These are both bioactive, structural elucidation and biosynthesis studies permitted the pathway assembly (Dalli et al., 2014). We found these novel bioactive structures in human milk, in the mouse exudate, in human macrophages that stimulate the regeneration of tissue in planaria substantially shortening the time interval required for complete regeneration. This was exciting, as were the results from add-back experiments. Mice with *E. coli*-induced peritonitis take about 20 hours to resolve the *E. coli* infection. When given at just 50 nanograms of MCTR per mouse, the resolution interval is reduced from 20 hours to 10 hours ~50% shortening in time employing the resolution indices (Table 1) (Bannenberg et al., 2005). Importantly, where this might be considered to lead to some reduction of neutrophilic responses or immunosuppression, instead there is a statistically significant increase in *E. coli*

phagocytosis and killing of the bacteria by the neutrophils that are at the site of infection (Dalli et al., 2014). Continued investigation along these lines permitted elucidation of three separate pathways, coined MCTRs, PCTRs and RCTRs. The stereochemistry of MCTR1, MCTR2 and MCTR3 and PCTR1 (Dalli et al., 2016a; Ramon et al., 2016) are established (Figure 2), and they are produced in mouse lymphoid tissues and in infectious inflammatory exudates as well as in specific human tissues (Dalli et al., 2017a).

## Failed Resolution: Contributions to Human Disease?

From the initial studies on the actions of resolvins and SPM (Hong et al., 2003; Serhan et al., 2000; Serhan et al., 2002) and early results on lipoxins in humans (Brezinski et al., 1992), it was clear that reduced amounts of SPM could contribute to disease pathologies. Failed mechanisms in resolution could arise from multiple factors. Reduced dietary intake of n-3 essential fatty acids (EPA, DHA), genetic polymorphisms in the enzymes involved in SPM biosynthesis or SPM receptors, dysfunctional SPM receptors or diminished expression, and abnormal intracellular post-SPM receptor signaling are a few components that can contribute to failed resolution, as well as drugs that are resolution toxic in animal disease models (Serhan et al., 2007). In humans, new evidence is now available indicating that pathologic conditions associated with reduced SPM can contribute to chronicity and magnitude of persistent inflammation. Impaired resolution can contribute to acute cardiovascular diseases such as atherosclerosis (Ho et al., 2010; Libby et al., 2014). Resolvin D1 and the ratio of SPM to leukotriene $B_4$ are reduced in vulnerable plaque in human carotid atherosclerotic plaques that may lead to plaque instability (Fredman et al., 2016). In females, reduced ocular lymph node $LXA_4$ correlates with increase in the number of T effector cells ($T_H1$ and $T_H17$), decrease in regulatory T cells ($T_{reg}$) and increase in dry eye pathogenesis (Gao et al., 2015). Sex differences in resolution mechanism in inflammation are also observed in humans, where healthy females on skin challenge produce higher levels of D-series resolvins than males that is associated with accelerated resolution (Rathod et al., 2016). These results suggest that specific SPM may prevent autoimmunity (Gao et al., 2015; Rathod et al., 2016) and link resolution to T cell responses (Chiurchiu et al., 2016). In obese women, supplementation with n-3 PUFA increases systemic resolvins and upregulation of resolvin receptors (Polus et al., 2016), suggesting that, in humans, failed resolution can be rescued. It appears that sex differences in SPM are age, gender and organ site dependent.

## Resolution Pharmacology: New Mediators and Pharmacologic Agents Targeting Resolution

There are hundreds of endogenous mediators involved in the initiation of acute inflammation, hence there are likely hundreds of endogenous molecules that stimulate the resolution response. Along with lipid mediators, peptides and proteins, as well as gases, stimulate resolution. For example, prostaglandin $E_2$ and $PGD_2$, in addition to their roles in initiation, have precise roles in activating the production of proresolving mediators and resolution of leukocyte traffic by stimulating expression of human 15-LOX type I (Buckley et al., 2014; Levy et al., 2001). Glucocorticoids stimulate efferocytosis by macrophages (Gilroy et al., 2004) and regulate expression and function of annexin A1. Annexin A1

increases neutrophil apoptosis, blocks endothelial adhesion and transmigration of these cells and stimulates the macrophage phagocytosis of neutrophils (Perretti and D'Acquisto, 2009; Perretti et al., 2015) actions in resolution stimulated by binding and signaling via the lipoxin $A_4$ receptor ALX. Specific miRNA mediate actions of SPM in resolution (Recchiuti and Serhan, 2012). In human macrophages, miRNA-181b regulates ALX/FPR2 receptor-evoked resolution responses (Pierdomenico et al., 2015).

Endogenous gases such as hydrogen sulfide and carbon monoxide have a role in resolution. $H_2S$ promotes resolution of inflammation (Flannigan et al., 2015) via actions on microbiota and mucosal barriers (Motta et al., 2015). Inhaled low-dose carbon monoxide (CO) protects from acute lung injury, reduces pro-inflammatory mediator production (Shinohara et al., 2014) and shifts to SPM production in baboons (Dalli et al., 2015b), shortening the resolution of pneumonia. In addition, a number of candidate proresolver proteins were identified (Bannenberg et al., 2005). More recently, erythropoietin was shown to promote resolution, shortening the resolution interval in vivo in mice (Luo et al., 2016). Novel hybrid compounds of cyclin-dependent kinase inhibitors ($CDK_i$) and nitric oxide (NO), such as NO-releasing *R*-roscovitine hybrid derivatives, possess pro-resolution actions (Montanaro et al., 2013), giving promise for new therapeutics as pro-resolving agents. Also, inhibitors of leukotriene $A_4$ hydrolase ($LTA_4$) block $LTB_4$ production and stimulate lipoxin $A_4$ biosynthesis via leukotriene $A_4$ conversion to lipoxin $A_4$ and show superior therapeutic action over inhibitors of 5-lipoxygenase and 5-lipoxygenase-activating protein (FLAP) to promote LX-mediated resolution (Rao et al., 2007). Identification of new members of the resolution mediators can provide unique opportunities to address inflammation and infection in many diseases that may be caused by failed resolution mechanisms, including lung (Basil and Levy, 2016; El Kebir et al., 2012; Felton et al., 2014), diabetes, renal disease (Borgeson and Godson, 2012), neurodegenerative diseases, brain injuries (Zhu et al., 2015; Harrison et al., 2015; Liu et al., 2012) and vascular diseases (Fredman et al., 2016; Hasturk et al., 2015; Ho et al., 2010; Kang and Lee, 2016).

## Summation

SPM are mediators of resolution responses in vivo in preclinical models and in human phagocytes (neutrophils and macrophages) that help to regulate the inflammatory response and directly impact tissue regeneration and resolution in primitive model organisms (Dalli et al., 2015c; Dalli and Serhan, 2012). Given that SPMs are agonists of resolution it appears that they do not evoke either unwanted side effects such as immunosuppression. Hence the concept of using agonists to stimulate natural resolution circuits and programs is worthy of rigorous testing in human indications either standing alone or in combination with other traditional therapies for a given outcome. Consider, for example, that specific SPMs together with antibiotics lower the amount of antibiotics needed to clear infections (Chiang et al., 2012). The enhanced host innate response can possibly reduce the potential for increases in antibiotic resistance by lowering the amounts of antibiotic exposure (Chiang et al., 2012), which may also reduce viral burden (Imai, 2015). SPM can also help to increase the effectiveness of adjuvants and antibody production (Ramon et al., 2012) that can normalize differentially expressed proresolution pathways in humans (Morris et al., 2010). The available evidence from extensive pre-clinical animal models, human SPM production in

vivo and the limited results of randomized clinical trials in humans suggest it is important to consider stimulating resolution pathways as a new therapeutic direction for excessive inflammation and infection. The goal of resolution pharmacology is to stimulate the host innate response, so to expedite microbial clearance, limit collateral tissue damage and stimulate tissue regeneration. This might be achieved with personalized nutrition via profiling of resolution pathway and SPM in tissues.

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## Abbreviations

| | |
|---|---|
| **CTR** | conjugate in tissue regeneration |
| **DHA** | docosahexaenoic acid |
| **eicosanoid** | arachidonic acid-derived carbon-20-containing structure |
| **EPA** | eicosapentaenoic acid |
| **GPCR** | G protein-coupled receptor |
| **HDHA** | hydroxy-docosahexaenoic acid |
| **HEPE** | hydroxy-eicosapentaenoic acid |
| **HETE** | hydroxy-eicosatetraenoic acid |
| **HpETE** | hydroperoxy-eicosatetraenoic acid |
| **LC-MS-MS** | liquid chromatography tandem mass spectrometry |
| **LM** | lipid mediators |
| **LOX** | lipoxygenase |
| **$LTB_4$** | leukotriene $B_4$, (5*S*, 12*R*-dihydroxy-eicosa-6*Z*, 8*E*, 10*E*, 14*Z*-tetraenoic acid) |
| **LX** | lipoxin |
| **$LXA_4$** | lipoxin $A_4$ (5*S*, 6*R*, 15*S*-trihydroxy-eicosa-7*E*, 9*E*, 11*Z*, 13*E*-tetraenoic acid) |
| **$LXA_5$** | lipoxin $A_5$ (5*S*, 6*R*, 15*S*-trihydroxy-eicosa-7*E*, 9*E*, 11*Z*, 13*E*, 17*Z*-pentaenoic acid) |
| **$LXB_4$** | lipoxin $B_4$: (5*S*, 14*R*, 15*S*-trihydroxy-eicosa-6*E*, 8*Z*, 10*E*, 12*E*-tetraenoic acid) |
| **Maresin** | macrophage-derived resolution mediator of inflammation |

| | |
|---|---|
| **MaR1** | maresin 1 (7*R*, 14*S*-dihydroxy-docosa-4*Z*, 8*E*, 10*E*, 12*Z*, 16*Z*, 19*Z*-hexaenoic acid) |
| **PD** | protectin |
| **PD1** | protectin D1 (10*R*, 17*S*-dihydroxy-docosa-4*Z*, 7*Z*, 11*E*, 13*E*, 15*Z*, 19*Z*-hexaenoic acid), also known as neuroprotectin D1 (NPD1) |
| **Rv** | resolving |
| **RvD1** | Resolvin D1 (7*S*, 8*R*, 17*S*-trihydroxy-docosa-4*Z*, 9*E*, 11*E*, 13*Z*, 15*E*, 19*Z*-hexaenoic acid) |
| **RvD2** | Resolvin D2 (7*S*, 16*R*, 17*S*-trihydroxy-docosa-4*Z*, 8*E*, 10*Z*, 12*E*, 14*E*, 19*Z*-hexaenoic acid) |
| **RvD3** | Resolvin D3 (4*S*, 11*R*, 17*S*-trihydroxy-docosa-5*Z*, 7*E*, 9*E*, 13*Z*, 15*E*, 19*Z*-hexaenoic acid) |
| **RvD5** | Resolvin D5 (7*S*, 17*S*-dihydroxy-docosa-4*Z*, 8*E*, 10*Z*, 13*Z*, 15*E*, 19*Z*-hexaenoic acid) |
| **RvE1** | Resolvin E1 (5*S*, 12*R*, 18*R*-trihydroxy-eicosa-6*Z*, 8*E*, 10*E*, 14*Z*, 16*E*-pentaenoic acid) |
| **RvE2** | Resolvin E2 (5*S*, 18*R*-dihydroxy-eicosa-6*E*, 8*Z*, 11*Z*, 14*Z*, 16*E*-pentaenoic acid) |
| **RvE3** | Resolvin E3 (17*R*,18*R*-dihydroxy-eicosa-5*Z*, 8*Z*, 11*Z*, 13*E*, 15*E*-pentaenoic acid) |
| **SPM** | specialized pro-resolving mediator (Rv, MaR, PD) |

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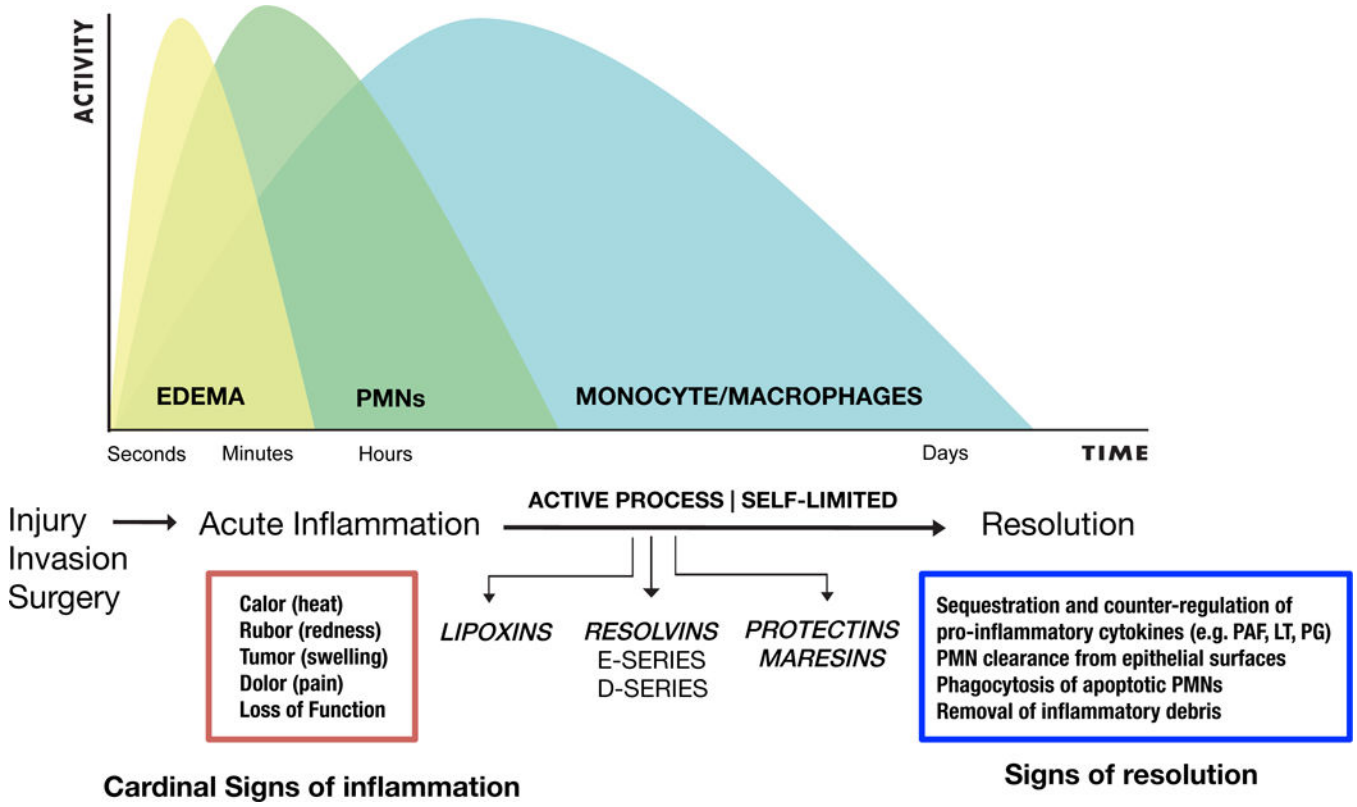


**Figure 1. The ideal outcome of inflammation: complete systems approach to mapping resolution**

Injury, infection or surgery initiate acute inflammation that is normally a host-protective mechanism. First event in acute inflammation is edema formation, followed by infiltration of PMN, and then monocyte and macrophages that clear PMN leading to resolution. Using the systems approach to map resolution, we demonstrated temporal biosynthesis of SPM in the resolution phase of self-limited inflammation. These SPM are a super-family of endogenous mediators, first identified in resolving exudates. They promote resolution of inflammation, wound healing and reduce organ fibrosis, leading to homeostasis. Based on these findings, we proposed the sign of resolution as listed. Identification and structure elucidation of these SPM provided the first evidence that resolution of inflammation is an active process.

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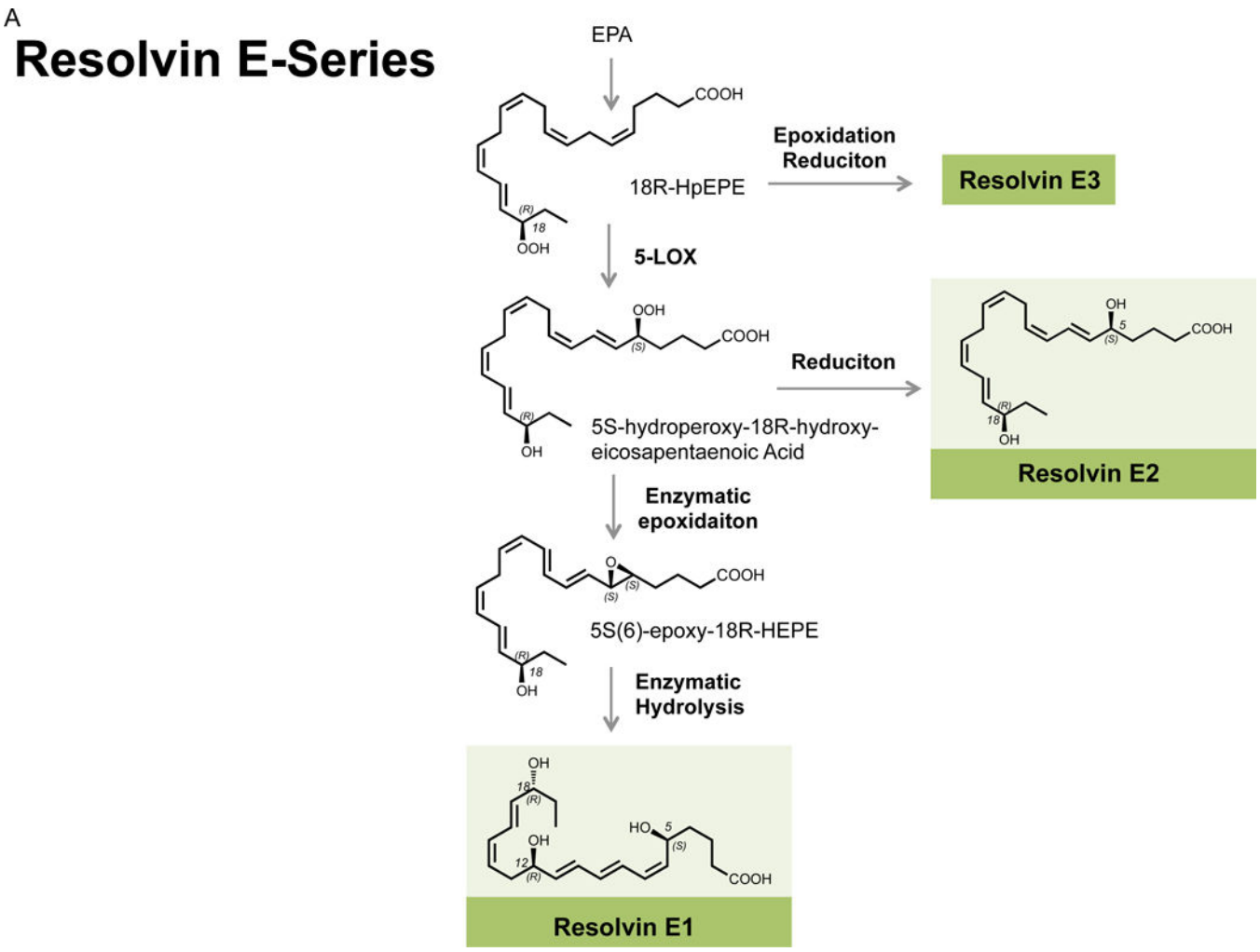

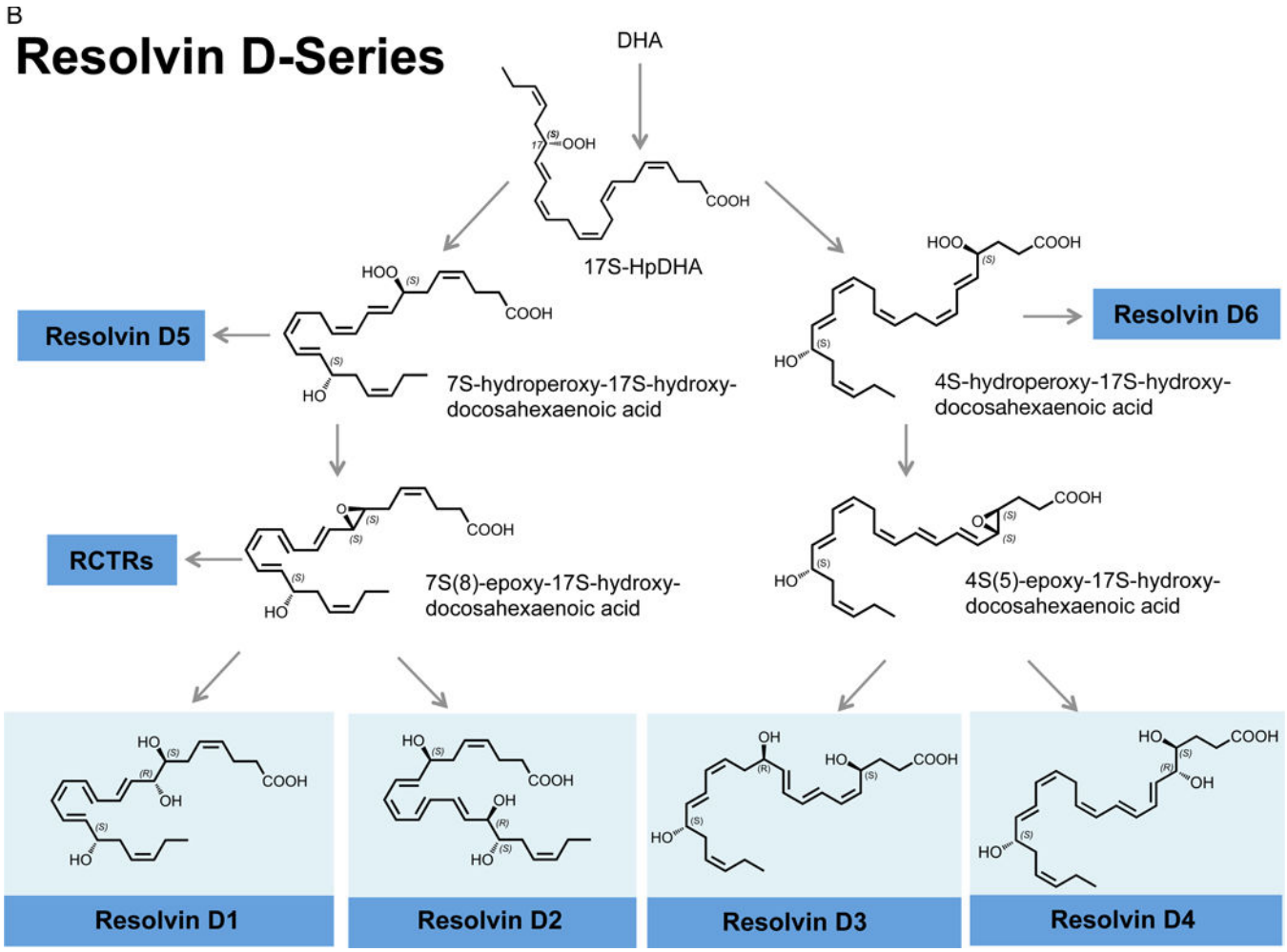

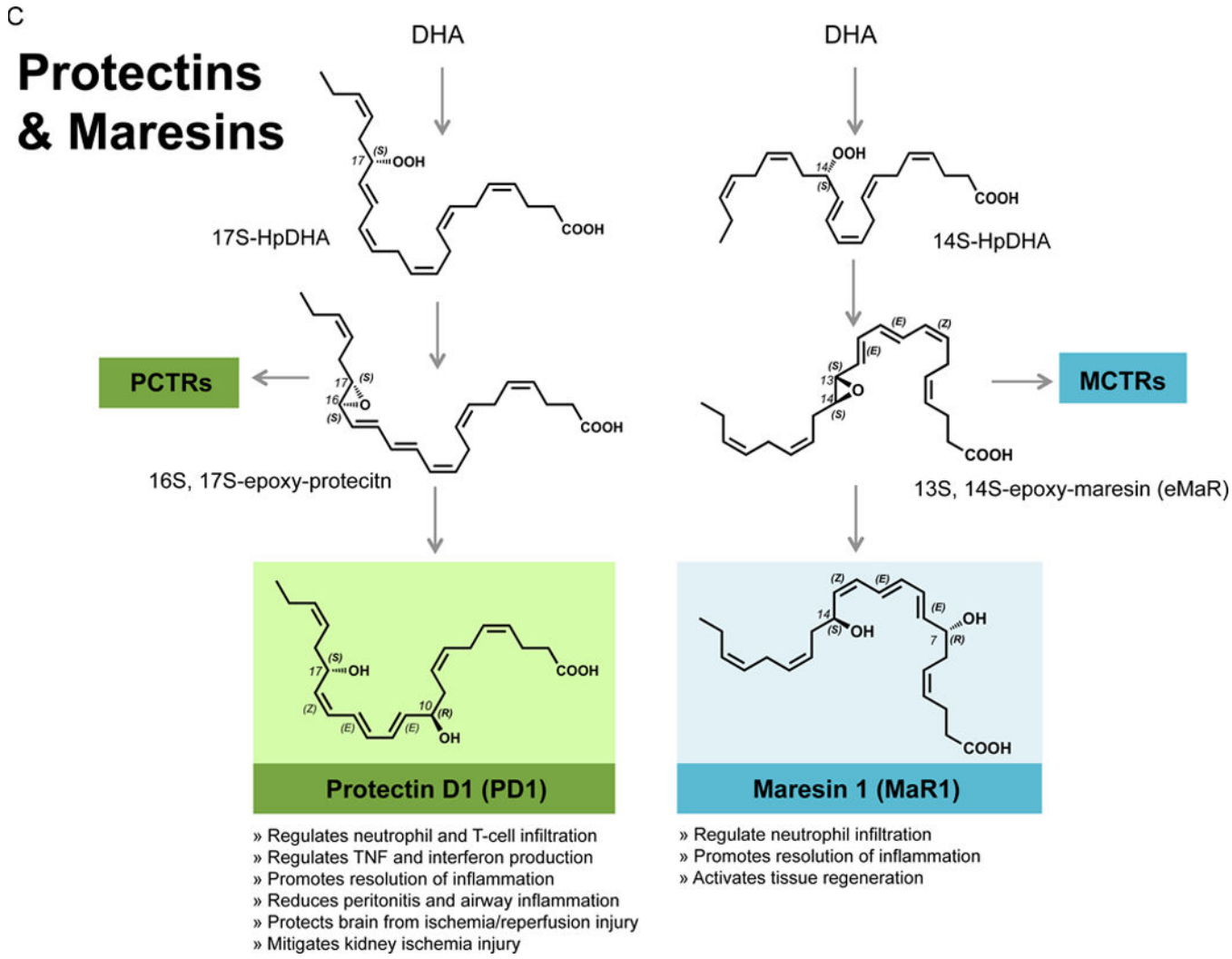


**Figure 2. SPM Biosynthetic Routes**

(A) Biosynthesis of E-series resolvins is initiated with molecular oxygen insertion at carbon-18 position of EPA, which is converted to bioactive E-series members resolvin E1, resolvin E2 and resolvin E3.

(B) Resolution metabolome also activates 17-lipoxygenation of DHA; 17*S*-HpDHA is converted to resolvin-epoxide intermediates by the leukocyte 5-lipoxygenase that are transformed to resolvins D1-D6, which each carry potent actions.

(C) 17-HpDHA is also precursor to 16,17-epoxide-protectin intermediate that is converted to protectin D1/neuroprotectin D1 and related protectins. Maresins are produced by macrophages via initial lipoxygenation at carbon-14 position by lipoxygenation and insertion of molecular oxygen, producing a 13*S*,14*S*-epoxide-maresin intermediate that is enzymatically converted to maresin family members. The stereochemistry of each bioactive SPM is established, and SPM biosynthesis in murine exudates and human tissues confirmed. See refs. (Serhan and Petasis, 2011; Winkler et al., 2016; Aursnes et al., 2015; Tungen et al., 2014; Dalli et al., 2016b) for original reports, total organic synthesis and stereochemical assignments and the text for further details.

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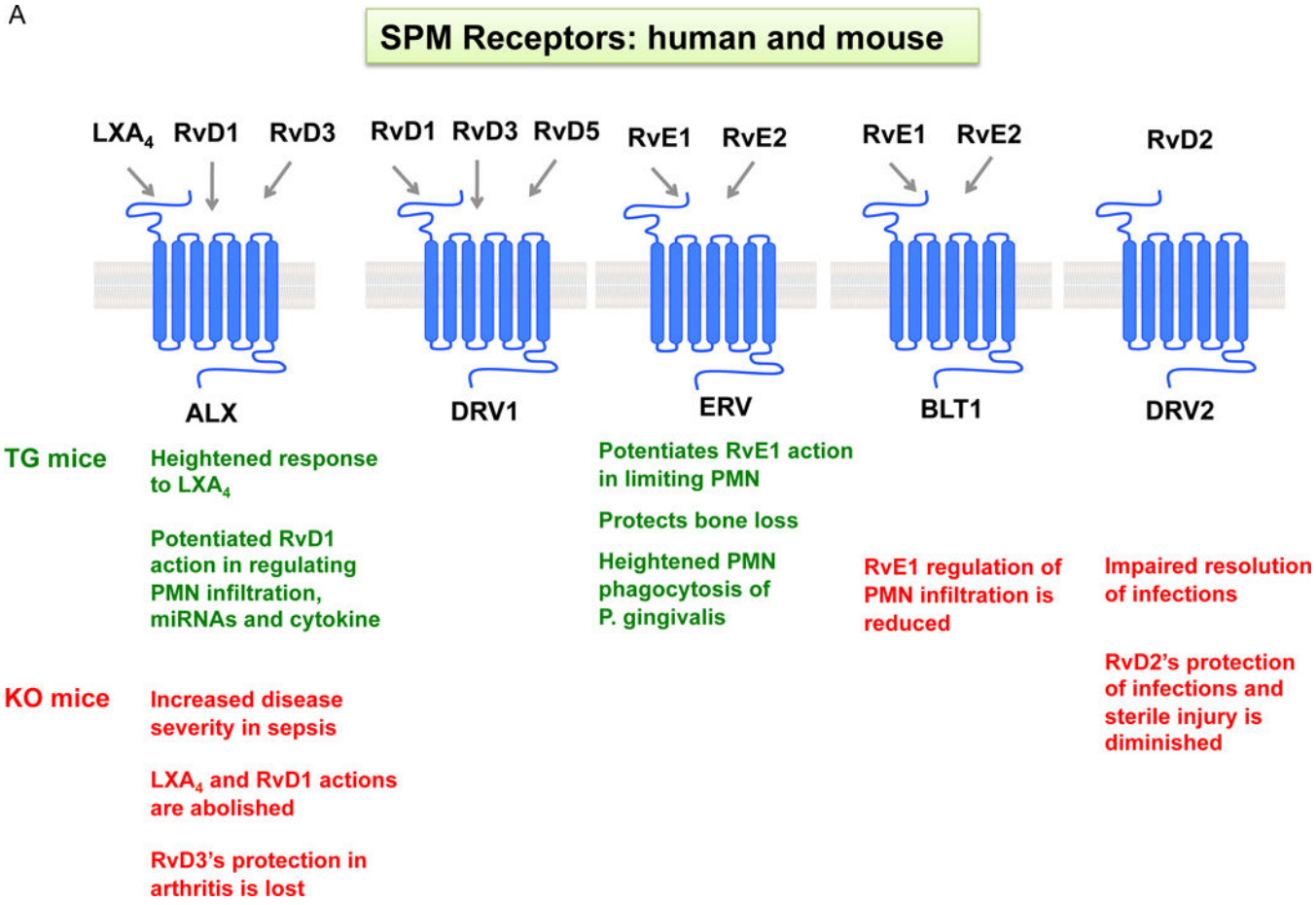

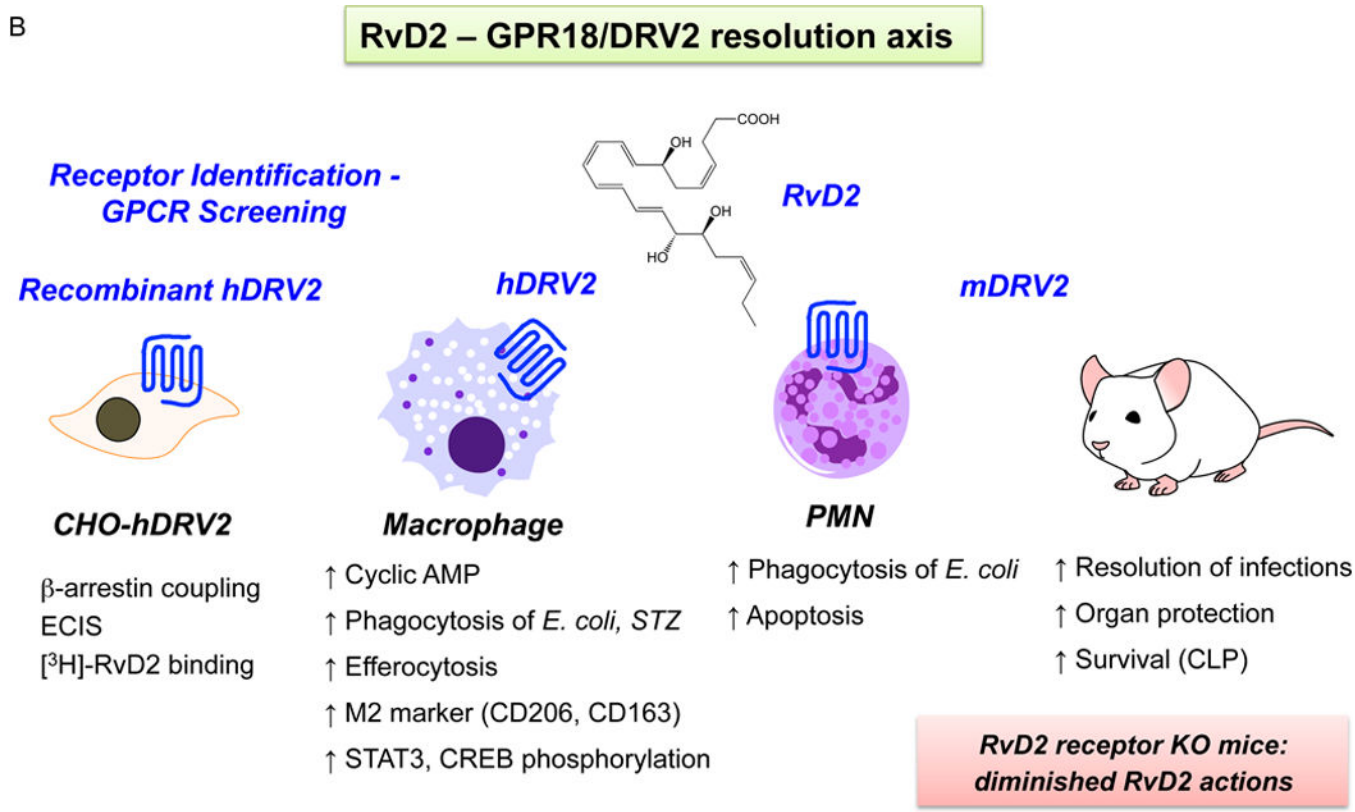


**Figure 3. SPM receptors: Pro-resolving GPCRs**

(A) Specific SPM receptors in human and mouse. $LXA_4$ and RvD1 both activate ALX receptor. RvD1 also activates DRV1, which was an orphan receptor GPR32 with human cells. RvE1 functions as an agonist for ChemR23/ERV and an antagonist for $LTB_4$ receptor BLT1 on PMN. RvD2 activates DRV2/GPR18 to control phagocyte functions in human and mouse. These receptors contribute to the SPM's pro-resolving actions on select cell types and also in vivo as demonstrated using transgenic and KO mice for ALX, ERV, BLT1 and DRV2.

(B) RvD2-GPR18/DRV2 resolution axis. A novel RvD2 receptor, namely DRV2/GPR18, was identified by GPCR screening. RvD2 directly binds to and activates recombinant DRV2. RvD2 activates endogenous human and mouse GPR18 to stimulate macrophage and PMN functions. In addition, RvD2 accelerates resolution of bacterial infections, improves survival in sepsis, and gives organ protection in sterile injury. These actions were diminished in DRV2 KO mice.

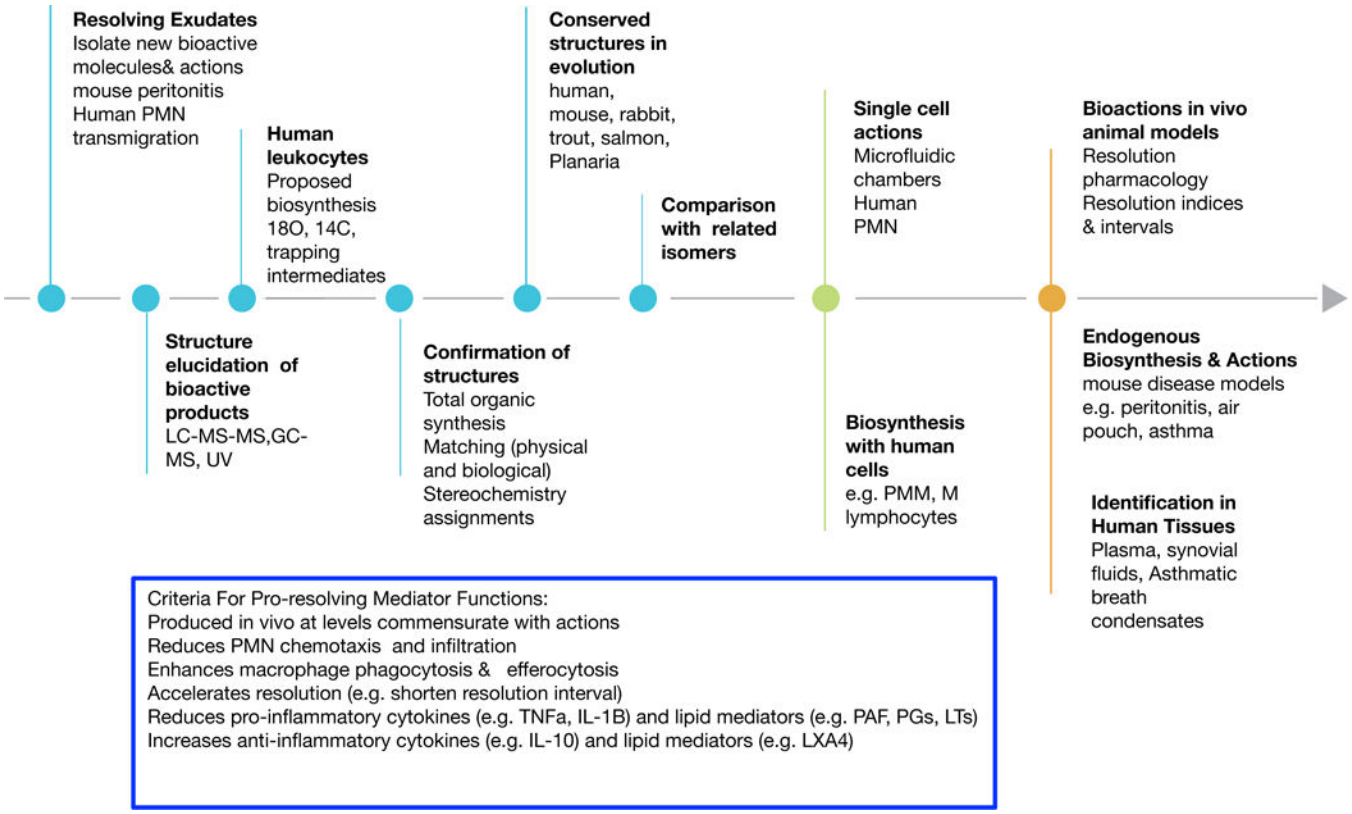


**Figure 4. Steps to Human Translation: Structure and functional elucidation of SPM**

Illustration of the key steps on structures and function of SPM and steps towards human translation. SPM were first isolated from resolving exudates, and reduced PMN infiltration and transmigration; their structures were elucidated and the biosynthesis was reconstructed with human cells. Their structures were then confirmed using synthetic material for matching and stereochemistry assignments. SPM structures proved to be highly conserved and are produced not only in mouse and human tissues but also in fish and planaria. Their actions on the single-cell level were demonstrated using microfluidic chamber with only one drop of human blood. In addition, we established bioactions in vivo animal models and define resolution indices. SPM production was documented in human cells, tissues and organs using mass spectrometry-based profiling. Criteria for pro-resolving mediators are listed in the box. These steps focusing on structure and function elucidation of SPM provided molecular basis for resolution pharmacology towards human translation.

## Table 1

Resolution indices: Actions of SPM and pharmacological agents

| Animal model | Compounds/conditions | Actions on resolution indices | Reference |
|---|---|---|---|
| Zymosan peritonitis | Zymosan<br>$LXA_4$<br>RvE1<br>PD1 | Define resolution indices with 1 mg zymosan<br>Reduces $\psi_{max}$<br>Reduces $\psi_{max}$, accelerates $T_{max}$ and $T_{50}$<br>Reduces $\psi_{max}$, accelerates $T_{max}$ and $T_{50}$, shortens Ri | (Bannenberg et al., 2005) |
| Zymosan peritonitis | $LXA_4$, RvE1, PD1 (administrated at peak of inflammation) | Accelerate $T_{50}$ shortens Ri | (Schwab et al., 2007) |
| Zymosan peritonitis | Lidocaine<br>Isoflurane | Increases $\psi_{max}$, delays $T_{max}$ and $T_{50}$<br>Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Chiang et al., 2008) |
| Zymosan peritonitis | o-[9, 12]-benzo-ro6-epi-$LXA_4$ | Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Sun et al., 2009) |
| Allergic airway inflammation | RvE1<br>RvD1, AT-RvD1 | Shortens Ri<br>Shortens Ri | (Haworth et al., 2008)<br>(Rogerio et al., 2012) |
| Zymosan peritonitis | Zymosan | Define resolution indices with 10 mg zymosan | (Navarro-Xavier et al., 2010) |
| Zymosan peritonitis | AT-RvD1 nanoparticles | Reduces $\psi_{max}$ accelerates $T_{50}$ shortens Ri | (Norling et al., 2011) |
| Zymosan peritonitis | RvD1 (ip) | Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Recchiuti et al., 2011) |
| *E. coli* peritonitis | RvD1 Ciprofloxacin | Reduces $\psi_{max}$ accelerates $T_{50}$ shortens Ri | (Chiang et al., 2012) |
| Allergic airway inflammation | TLR7 KO mice<br>R484 (TLR7 ligand) | Prolongs Ri<br>Shortens Ri | (Koltsida et al., 2013) |
| Zymosan peritonitis | Carbon Monoxide | Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Chiang et al., 2013) |
| Zymosan peritonitis | Ac2-26 nanoparticles | | (Fredman et al., 2015) |
| Lung inflammation (LPS)<br>*E. coli* lung infection | AT7519 (cyclin-dependent kinase inhibitor) | Shortens Ri | (Lucas et al., 2014) |
| Zymosan peritonitis | Vagotomy<br>Netrin-1<br>RvD1 | Increases $\psi_{max}$, delays $T_{50}$,. prolongs Ri<br>Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri<br>Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Mirakaj et al., 2014) |
| Zymosan peritonitis | Aged mice<br>DHA (SPM precursor) Monocytes +DHA&EPA<br>RvD3 | Increases $\psi_{max}$, delays $T_{50}$,. prolongs Ri<br>Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri<br>Accelerate $T_{50}$, shortens Ri<br>Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Arnardottir et al., 2014) |
| *E. coli* peritonitis | MCTR1,2,3<br>RvTs | Accelerate $T_{50}$, shortens Ri | (Dalli et al., 2014; Dalli et al., 2015a; Dalli et al., 2016a) |
| Zymosan peritonitis | RvD1 (oral) | Reduces $\psi_{max}$, accelerates $T_{50}$, shortens Ri | (Recchiuti et al., 2014) |

| Animal model | Compounds/conditions | Actions on resolution indices | Reference |
|---|---|---|---|
| Zymosan peritonitis | Melanocortin analog AP1189 | Accelerate $T_{50}$, shortens Ri | (Montero-Melendez et al., 2015) |
| Zymosan peritonitis | Human milk isolates RvD2, MaR1 | Reduces $\psi_{max}$, Accelerate $T_{50}$, shortens Ri | (Arnardottir et al., 2016a) |
| Arthritis | 17R-RvD1 | Accelerate $T_{50}$, shortens Ri | (Norling et al., 2016) |
| S. aureus skin infection (air pouch) | RvD4 | Reduces $\psi_{max}$, Accelerate $T_{50}$, shortens Ri | (Winkler et al., 2016) |
| Zymosan peritonitis | Erythropoietin | Accelerate $T_{50}$, shortens Ri | (Luo et al., 2016) |
| *E. coli* +vagotomy | PCTR1 | Reduces $\psi_{max}$, Accelerate $T_{50}$, shortens Ri | (Dalli et al., 2017a) |

## Table 2

SPM actions in infections

| *Infection* | Model | Actions | References |
|---|---|---|---|
| ***$LXA_4$ and 15-epi-$LXA_4$*** | | | |
| *Porphyromonas gingivalis* | Rabbit periodontitis | Reduces leukocyte infiltration and bone destruction | (Serhan et al., 2003) |
| *Toxoplasma gondii* | Mouse | Reduces IL-12 and mortality | (Aliberti et al., 2002b) (Aliberti et al., 2002a) |
| *Mycobacterium tuberculosis* | Mouse | Infected 5-LOX -/- mice have significantly lower bacterial loads than those from WT mice, and this enhancement in the resistance to M. tuberculosis was prevented by a stable $LXA_4$ analog. | (Bafica et al., 2005) |
| *Mycobacterium marinum* | Zebrafish | Reduces TNF-alpha in infected zebrafish | (Tobin et al., 2012) |
| *H5N1 influenza viruses* | Mouse | | (Cilloniz et al., 2010) |
| *Trypanosoma cruzi* | Mouse toxoplasmosis | Reduces parasitaemia and increases survival | (Molina-Berrios et al., 2013) |
| *Plasmodium berghei* ANKA strain | Mouse cerebral malaria | Prevents early mortality | (Shryock et al., 2013) |
| Sepsis | Mouse CLP | Increases survival and reduces bacterial load | (Walker et al., 2011; Wu et al., 2015) |
| ***Resolvins*** | | | |
| *Candida albicans* | Mouse candidiasis | RvE1 stimulates clearance of the fungus from circulating blood | (Haas-Stapleton et al., 2007) |
| *Sepsis* | Mouse CLP | RvD2 reduces mortality | (Spite et al., 2009) |
| *Escherichia coli* | Mouse pneumonia | RvE1 protects pneumonia and reduces mortality | (Seki et al., 2010) (El Kebir et al., 2012) |
| *Herpes simplex virus* | Mouse keratitis | RvE1 reduces lesion severity | (Rajasagi et al., 2011) |
| *Escherichia coli* | Mouse peritonitis | RvD1 reduces mortality<br>RvD1 and RvD5 protect hypothermia and enhance antibiotic effectiveness | (Chiang et al., 2012) |
| *Staphylococcus aureus* | Mouse skin infection | RvD1 and RvD5 reduce bacterial titers and enhance antibiotic effectiveness | (Chiang et al., 2012) |
| Sepsis | Mouse double-injury model of burn and sepsis | RvD2 increases survival | (Kurihara et al., 2013) |
| ***Protectins*** | | | |
| H5N1 influenza viruses | Mouse | PD1 improves the survival of severe influenza and reduces viral replication | (Morita et al., 2013) |
| ***SPM Conjugates in tissue regeneration*** | | | |
| *Escherichia coli* | Mouse peritonitis | MCTR1, 2, 3 increase bacterial clearance and efferocytosis<br>PCTR1 promotes E. coli clearance | (Dalli et al., 2014; Dalli et al., 2016a) (Ramon et al., 2016) |
| | Mouse peritonitis with vagotomy | PCTR1 reduces PMN infiltration, bacterial titers, enhances efferocytosis | (Dalli et al., 2017a) |
| ***13-series resolvins (RvTs)*** | | | |
| *Escherichia coli* | Mouse peritonitis | RvTs protect hypothermia, increase survival, enhance efferocytosis | (Dalli et al., 2015a) |

**Table 3**

Human Tissue SPM Identification and Profiling via LC-MS-MS

| | |
|---|---|
| Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue | (Colas et al., 2014) |
| Determination of omega-6 and omega-3 PUFA metabolites in human urine; reduced SPM in chronic pulmonary disease | (Sasaki et al., 2015) |
| Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation | (Mas et al., 2012) |
| The human urine metabolome | (Bouatra et al., 2013) |
| Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment | (Markworth et al., 2013) |
| High levels of anti-inflammatory and pro-resolving lipid mediators lipoxins and resolvins; declining levels in human milk during the first month of lactation | (Weiss et al., 2013) |
| Human milk SPM stimulate resolution of inflammation | (Arnardottir et al., 2016a) |
| Metabolomic profiling of regulatory lipid mediators in sputum from adult cystic fibrosis patients | (Yang et al., 2012) |
| Resolution of inflammation is altered in Alzheimer's disease Brain and cerebrospinal fluid reduced SPM and their receptors | (Wang et al., 2015) |
| Plasma metabolomics in human pulmonary tuberculosis disease: a pilot study | (Frediani et al., 2014) |
| Human arthritis, supplementation of n-3 increases SPM; RvE2 correlates with reduced pain | (Barden et al., 2016b) |
| Human arthritis synovial exudates; resolvin D1 and resolvin D3 | (Norling et al., 2016) |
| Human arthritis synovial exudates | (Arnardottir et al., 2016b) |
| Intensive care unit sepsis patients: eicosanoids and SPM | (Dalli et al., 2017b) |
| Randomized controlled trial in chronic kidney disease: increase SPM on n-3 supplementation | (Mas et al., 2015) |
| D-series resolvin precursor 17-HDHA increases in maternal and cord blood in late pregnancy | (Mozurkewich et al., 2016) |
| Human carotid atherosclerotic plaques | (Fredman et al., 2016) |
| 13-series resolvins increase in plasma after exercise | (Dalli et al., 2015a) |
| SPM increase in CVD patients taking Lovaza | (Elajami et al., 2016) |
| MCTRs and PCTRs identified in human serum, lymph nodes, and plasma | (Dalli et al., 2016b) |
| Human emotional tears contain SPM, where levels were higher in males than in females | (English et al., 2017) |