Published in final edited form as:
Curr Top Med Chem. 2011 ; 11(6): 629–647.

Novel Anti-Inflammatory -- Pro-Resolving Mediators and Their Receptors

Charles N. Serhan[*], **Sriram Krishnamoorthy**, **Antonio Recchiuti**, and **Nan Chiang**
Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Abstract

Resolution of inflammation, an actively coordinated program, is essential to maintain host health. It involves effective removal of inflammatory stimuli and the spatio-temporal control of leukocyte trafficking as well as chemical mediator generation. During the active resolution process, new classes of small, local acting endogenous autacoids, namely the lipoxins, D and E series resolvins, (neuro)protectins, and maresins have been identified. These specialized pro-resolving lipid mediators (SPM) prevent excessive inflammation and promote removal of microbes and apoptotic cells, thereby expediting resolution and return to tissue homeostasis. As part of their molecular mechanism, SPM exert their potent actions *via* activating specific pro-resolving G-protein coupled receptors. Together these SPM and their receptors provide new concepts and opportunities for therapeutics, namely promoting active resolution as opposed to the conventionally used enzyme inhibitors and receptor antagonists. This approach may offer new targets suitable for drug design for treating inflammation related diseases, for the new terrain of *resolution pharmacology*.

Keywords

G-protein coupled receptor; inflammatory exudates; leukocytes; mediator lipidomics; omega-3 fatty acids; resolvins

Introduction

Many of the currently used anti-inflammatory therapies are directed towards the inhibition of enzymes and/or antagonism of pro-inflammatory receptors. Selective cyclooxygenase inhibitors and anti-tumor necrosis factor α (TNF-α) are examples of this approach to treatment. Research in the author's laboratory has focused on profiling self-limited inflammation, which uncovered novel mechanisms that terminate the local acute inflammatory response as well as stimulate resolution and return of the tissue to homeostasis [1, 2]. Identification of these biochemical and cellular processes indicate that resolution of acute inflammation, once considered a passive process, is an active programmed tissue process; reviewed in Refs. [3, 4]. Hence, rather than targeting inhibition or antagonism of

Address correspondence: Prof. Charles N. Serhan, Director, Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesia, Perioperative and Pain Medicine, Harvard Institutes of Medicine, 77 Avenue Louis Pasteur, Boston, MA 02115 Telephone: +1-617-525-5001, Fax: +1-617-582-6141, cnserhan@zeus.bwh.harvard.edu.

Disclosure of conflict of interest

C.N.S. is inventor on patents assigned to Brigham and Women's Hospital and Partners Health Care on the composition of matter, uses, and clinical development of anti-inflammatory and proresolving lipid mediators. These are licensed for clinical development. C.N.S. retains founder stock in Resolvyx Pharmaceuticals.

inflammation, our research efforts address the potential use of endogenous agonists of resolution to stimulate naturally resolving inflammation.

This systems approach with self-limited exudates now opens a new understanding of the mechanisms underlying inflammatory disease as well as a new discipline, namely resolution pharmacology. Essential omega-3 fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are precursors to a new genus of potent lipid mediators (LM) that are both pro-resolving and anti-inflammatory (specialized pro-resolving mediators: SPM) and serve a physiologic role defining programmed resolution. This mini-review highlights the biosynthesis and actions of this novel genus of endogenous SPM that govern resolution of acute inflammation.

New Principles in Resolution – Endogenous Chemical Mediators of Resolution

The acute inflammatory reaction in response to infection or unwanted tissue damage is characterized by the classic cardinal signs of inflammation (heat, redness, swelling and pain), and in experimental settings *in vivo* the temporal relationships are well established, e.g., edema and the accumulation of leukocytes, specifically polymorphonuclear leukocytes (PMN), followed by monocytes and macrophages [5, 6]. These events in self-limited or resolving inflammatory reactions are coupled with release of local factors that prevent further or excessive trafficking of leukocytes allowing for resolution [1, 2]. Early in the inflammatory response, pro-inflammatory mediators such as prostaglandins and leukotrienes play an important role [7]. The progression from an acute to chronic inflammation as in many widely occurring human diseases such as arthritis, periodontal disease [8] and cardiovascular disease [9] is commonly viewed as an excess of pro-inflammatory mediators. Although mononuclear cells can sometimes contribute to pro-inflammatory responses, they are also critical in wound healing, tissue repair and remodeling in a non-phlogistic fashion [10]. Thus, it is highly plausible that defects associated with mounting endogenous pro-resolving circuits and local autacoids could underlie some of the pathologic events in chronic inflammation.

The complete resolution of an acute inflammatory response and the return of the local tissues to homeostasis is necessary for ongoing health. Removal of leukocytes from tissues involved in the inflammatory response without leaving remnants of the host defenses and combat between leukocytes, invading microbes, and/or other initiators of inflammation is an ideal outcome. We have focused on the question “How is the acute inflammatory response regulated?” since it was widely believed that simple dilution of pro-inflammatory mediators is sufficient to “burn out” inflammation, with the subsequent responses ending passively [5, 10].

Pro-resolving Lipid Mediators and Anti-Inflammation

Lipoxins and aspirin-triggered lipid mediators

SPM are a recently uncovered genus of endogenously biosynthesized chemical mediators identified in exudates and consists of four distinct new chemical families: lipoxins, resolvins, protectins and the recently identified maresins, which are involved in acute inflammation. Each of these families is actively biosynthesized in the resolution phase of acute inflammation and the mediators are potent agonists that control the duration and magnitude of inflammation [2, 11, 12]. They are also potent chemoattractants, but via a non-inflammatory mechanism: for example, lipoxins from arachidonate activate mononuclear cell recruitment without stimulating release of pro-inflammatory chemokines or activation of pro-inflammatory gene pathways [3]. They also stimulate the uptake of apoptotic PMNs

[13] and activate endogenous anti-microbial defense mechanisms [14] as well as clearance on mucosal surfaces [15]. These actions are agonistic in that they stimulate specific cell surface receptors; via acting on separate cell populations they stimulate overall resolution of inflammation.

Lipoxin A_4 (LXA_4) and LXB_4, which are lipoxygenase derived eicosanoids, are anti-inflammatory and were the first pro-resolving mediators identified, as their appearance signals the resolution of acute contained inflammation [16]. Lipoxins are derived enzymatically from arachidonic acid (AA), an omega-6 fatty acid that is released and mobilized during inflammation [7]. In human systems, they are biosynthesized, for example, during cell–cell interactions involving mucosal, i.e., epithelial cells of the gastrointestinal tract or bronchial tissue, interactions with leukocytes; within the vasculature platelet–leukocyte interactions are a main source [3]. Aspirin has an unexpected impact within resolution as it “jump-starts” the process by triggering endogenous biosynthesis of pro-resolving lipid mediators [17, 18].

During local contained inflammation, the first line of host defense, namely the neutrophils, die at the site and can undergo cell death by apoptosis as well as necrosis. As part of resolution, LX signal macrophages to enhance their uptake of the remains of these cells [13]. LX are highly potent anti-inflammatory mediators that are formed and act in picogram to nanogram amounts with human tissues and in animal disease models [16]. LX have the specific pro-resolution actions of limiting PMN recruitment and adhesion. They essentially serve as braking signals for PMN-mediated tissue injury. [see ref. 16 and references within].

Resolvins and protectins: Novel Chemical Mediators from Omega-3 Precursors

Resolvins and protectins are two distinct families of local mediators identified in the resolving exudates of acute inflammation. They were initially identified using a systems approach with LC-MS-MS-based lipidomics and informatics and then complete structural elucidation of the bioactive mediators and related compounds was achieved [1, 2, 18–20]. The term resolvins or *resolution-phase interaction products* refers to endogenous bioactive mediators biosynthesized from the major omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) namely the E series (RvE) and D series (RvD) resolvins [2]. Aspirin-triggered forms of resolvins are produced by a COX-2-dependent pathway in the presence of aspirin. A growing body of evidence indicates that resolvins possess potent anti-inflammatory and immunoregulatory actions that include blocking the production of pro-inflammatory mediators and regulating trafficking of leukocytes [reviewed in Ref. 4]. Specifically, resolvins stop PMN infiltration in vivo and transmigration [2, 21]. They also reduce cytokine expression by isolated microglia cells [19]. We also established the stereochemistry and actions of RvD1 and aspirin-triggered (AT)-RvD1, RvE1 and PD1 (*vide infra*) as well as demonstrated the stereoselective basis for their potent actions [18, 20, 21]. Both RvD1 and its 17*R*-AT form (0–1000 nM) stop PMN transendothelial migration in a concentration-dependent manner [21]. The potency of these compounds is noteworthy, with ~50% reduction in PMN transmigration at 10 nM. RvD1 actions were studied in several inflammatory disease models and confirmed to be potent and stereoselective (Table 1).

Protectins are also biosynthesized from DHA via a distinct pathway. Protectins are distinguished by the presence of their conjugated triene containing structure [20]. The name “protectins” was coined from the observed anti-inflammatory [19] and protective actions. The prefix *neuro*protectin gives the tissue location of its production, such as neuroprotectin D1 [20, 22]. Like resolvins, the protectins also stop PMN infiltration [19, 20]. They are biosynthesized by and act on glial cells and reduce cytokine expression [19]. In mouse models, NPD1 reduces the severity of retinal and corneal injury [22] and stroke damage [23], and improves wound healing in the cornea [24].

Programmed Resolution of Inflammation

To study self-limited inflammation using an unbiased systems approach, the murine dorsal air pouch was ideal because it permitted isolation of contained inflammatory exudates [1, 2]. This system also enabled direct LM-lipidomics–informatics (bioactive products, as well as their inactive precursors and further metabolites), proteomics, and cellular composition of the resolving exudate. Importantly, it was possible to establish by direct comparisons when and where different local mediators were biosynthesized and activated for resolution, namely temporal spatial differential analyses [3, 25]. We used the murine dorsal air pouch as well as peritonitis to determine the formation and roles of endogenous LXA_4 in the resolution of acute inflammation [26].

Upon initiation of inflammation with TNF-α, there was a typical acute-phase response characterized by rapid PMN infiltration preceded by generation of both local prostaglandins and leukotrienes. Unexpectedly, the eicosanoids then underwent what we termed earlier a “class switch.” As the exudate evolved, the eicosanoid profiles switched and the lipid mediators made within this milieu changed with time [26]. Indeed, leukotrienes (potent chemo-attractants) were deactivated and the transcriptional regulation of enzymes required for LX and resolvin production was activated. Resolvin biosynthesis by exudates utilizes plasma edema to carry the omega-3 DHA and EPA for conversion by exudates [27]. This in turn chemoattracts mononuclear cells and stimulates macrophages to ingest apoptotic neutrophils within these contained inflammatory exudate site. Interestingly, the lipid mediator class switch in exudates was driven in part by COX-derived prostaglandins E_2 and D_2, which regulate transcription of enzymes involved in lipoxin biosynthesis [26]. Along with Sir John Savill we introduced the concept that “alpha signals omega,” namely the beginning signals the end in inflammation [10].

DHA is well established to play an important role in neuronal systems and, along with AA, is also one of the major polyunsaturated fatty acid (PUFA) component found in the retina. The other major omega-3 PUFA present in marine fish oils is EPA. In order to determine whether EPA, DHA, and AA regulate inflammation *in vivo*, we studied disease models to compare the effects of omega-3 and -6 PUFAs in wild-type mice with mice that overexpress the *C. elegans fat-1* gene. This gene converts omega-6 PUFA into omega-3 resulting in elevated tissue levels of omega-3 PUFA within the *fat-1* overexpressing mice. A protective action against pathological angiogenesis was found in retina when there was a lower ratio of omega-6:omega-3 PUFA [28]. Wild-type mice lacking the *fat-1* transgene had extensive vaso-obliteration and severe retinal neovascularization compared with *fat-1* mice. In mice fed omega-3 PUFA, there were markers of neuroprotectin D1 (NPD1) and RvE1 biosynthesis. In mice without omega-3 PUFA supplementation, administration of RvD1, RvE1, or NPD1 gave protection from vaso-obliteration and neovascularization [28]. These *fat-1* mice are also protected from colitis [29]. In addition to exudates, murine bone marrow also synthesizes resolvins and protectins, which are enhanced with feeding EPA and DHA [30]

SPM play a protective role in peritonitis. For example, zymosan A was injected into mice to initiate peritonitis [20] and PD1 was protective [20, 21, 25]. RvE1 and PD1 each blocked >85% of further PMN migration into the site [20]. When both PD1 and RvE1 were injected together to determine whether their actions were synergistic or additive, RvE1 (10 ng) reduced PMN infiltration, although less than that with PD1 (10 ng). In combination, the reduction was greater, suggesting an additive action. Along these lines, inflammatory bowel disorders, like colitis, are characterized by relapsing inflammation. In a well-studied experimental colitis model, RvE1 protects against bowel inflammation in mice challenged with an intrarectal antigenic hapten, 2,4,6-trinitrobenzene sulfonic acid (TNBS), to induce

colitis [31]. As little as 1 μg RvE1 dramatically reduced mortality, weight loss, and histologic severity of colitis. Finally, RvE1 is protective in rabbit periodontal disease, where it appears to stimulate regeneration [32]. These findings exemplify the anti-inflammatory and anti-fibrotic actions of SPM in several widely used laboratory disease models (see Table 1).

Pro-Resolving GPCRs

ALX/FPR2 Specific Binding with Lipoxin A_4 (LXA_4): An Agonist of Anti-Inflammation and Resolution

Lipoxin A_4 is a potent anti-inflammatory and pro-resolving lipid mediator biosynthesized from arachidonic acid. It has a unique structure and belongs to a class of conjugated tetraene-containing eicosanoids that possess stereoselective properties and display potencies in the picomolar to nanomolar range in many mammalian systems [for complete reviews, see ref. 16]. The complete structure of LXA_4 is 5S, 6R,15S-trihydroxy-7,9,13-trans-11-eicosatetraeoic acid. The actions of LXA_4 differ sharply from those of many other eicosanoids that are, for the most part, pro-inflammatory. The in vivo agonist and anti-inflammatory and pro-resolving actions of this eicosanoid and related compounds have now been independently confirmed and extended by many investigators worldwide [3, for recent reviews, see refs. 33, 34, and 35, 36, 37].

LXA_4 is an endogenous lipid mediator thatdisplays multi-level control of processes relevant in acute inflammation via specific and selective actions demonstrated for several isolated cell types and by interaction with specific receptors [3]. These receptors and sites of LX action were recently subject to review and respond to LXA_4 depending on its tissue and location of formation [see ref. 33 for detailed review]. LXA_4 directly interacts, for example, with human FPRL1 (*ALX/FPR2*) and cysLT1, as well as signals to regulate BLT1, cytokine-chemokine receptors (e.g. TNF) and growth factor receptors (i.e., VEGF) in human leukocytes, vascular cells and mucosal epithelial cells, each contributing to regulate the resolution of inflammation [16]. In nanogram amounts, LXA_4 stops PMN infiltration *in vivo* [38, 39] and blocks human PMN transmigration across mucosal epithelial cells and vascular endothelial cells [40, 41]. In general terms, it is now well appreciated that LXA_4 actively "turns down" many endogenous processes that can amplify local acute inflammation; these include regulating leukocyte infiltration, TNF-stimulated cytokine and chemokine production, expression of chemokine receptors, adhesion molecules and production of reactive oxygen species as well as leukotriene formation and their actions [reviewed in refs. 3, 33]. To address the mechanism and sites of action for LXA_4, radiolabeled [11,12-^{3}H]-LXA_4 was synthesized almost twenty years ago from a synthetic [11,12]-acetylenic precursor Fig. (1A). Selective reduction of the carbon 11–12 positioned triple bond in an atmosphere enriched with ^{3}H gas and Lindlar catalyst gave labeled [11,12-^{3}H]-LXA_4, which we isolated by reverse phase HPLC and separated from isomerization products as well as unwanted reaction products that included excess reduced species of thefour conjugated double bond system in LXA_4. The RP-HPLC isolation of labeled [11,12-^{3}H]-LXA_4 was carried out routinely before each binding experiment and characterized using reported physical properties of the labeled LXA_4. This qualification of the [^{3}H]-LXA_4 was critical for confident analyses and interpretation of isolated cell-based binding experiments [42].

Using freshly isolated [^{3}H]-LXA_4 for each experiment, specific LXA_4 binding sites were first identified in isolated human PMN [43] and provided a mechanistic basis for the specific LXA_4 functions and stimulated actions with PMN. Intact human PMN give specific [11,12-^{3}H]-LXA_4 binding at Kd=0.7 nM that was also found to be inducible in the promyelocytic lineage (HL-60) cells differentiated with either retinoic acid or dimethylsulfoxide (DMSO). LXA_4 stimulates rapid (within seconds) phospholipase

activation in these cells that directly correlates with the induction time course of specific LXA_4 binding [44, 45]. In parallel experiments, a second approach was undertaken with GPCR that were known to be induced within the same time frame, namely 3 to 5 days. These receptors were screened for both [^{3}H]-LXA_4 binding and functional activation, namely ligand–receptor dependent increases in GTPase activity. High affinity specific [^{3}H]-LXA_4 binding was observed with one of the GPCR cloned earlier [46] from myeloid lineages, namely FPRL1; now renamed FPR2 [47]. When the action and ligands involve lipoxin A_4 or its mimetics/analogs, the receptor is denoted ALX/FPR2; for peptide agonists alone, FPR2 is proposed [47].

Labeled LXA_4 binding with ALX/FPR2 has high affinity and selectivity compared to other eicosanoid heteroligands including lipoxin B_4, leukotriene B_4, leukotriene D_4 and PGE_2. The plasmid DNA of this orphan receptor (denoted pINF114) at that time was transfected into Chinese Hamster Ovary (CHO) cells and gave a Kd ~1.7 nM for [^{3}H]-LXA_4 determined by Scatchard plot analysis [48]. This Kd value for recombinant human FPR2 is comparable to those obtained with the endogenous LXA_4 specific binding sites present on peripheral blood leukocytes. The human PMN has a remarkable Kd = 0.7 and 0.8 nM obtained with isolated plasma membrane fractions as well as granule membrane-enriched fractions. In addition, hFPR2-transfected CHO cells give functional responses with LXA_4. This proved to be very useful in verifying that binding indeed conferred with cellular functions. In CHO cells expressing the recombinant receptor, LXA_4 stimulates both GTPase activity as well as the release of esterified arachidonic acid, which are inhibited by pertussis toxin. These agonist actions of LX obtained *in vitro* are in line with the anti-inflammatory properties of endogenous LXA_4.

Human ALX/FPR2 was subsequently identified and cloned in several leukocyte sub-populations including monocytes [49], T cells [50], and macrophages as well as resident cells such as synovial fibroblasts [51] and intestinal epithelial cells [52]. Northern blot analysis demonstrated that the human ALX mRNA is ~2.1 Kb [39], and chromosome mapping reveals that the gene encoding human ALX is located on chromosome 19q [53]. These reconstituted LXA_4 functional responses linked with specific LXA_4 binding and assignment of these functional ligand-receptor pairs (LXA_4-ALX) [48]. This systematic screening of orphan receptors employed above takes into account both function and specific binding of receptors that are induced during myeloid cell differentiation. This was also the first identification of high affinity receptors for lipoxygenase pathway generated mediators.

Structure-activity relationships have been carried out at the level of both the ligand LXA_4 and the receptor ALX/FPR2. Each of the actions of LXA_4 proved to be stereoselective in that changes in potencies are associated with double bond isomerization and alcohol chirality (R or S) as well as dehydrogenation of alcohols and reduction of double bonds. Elimination of the carbon 15 position alcohol from LXA_4, denoted 15-deoxy-LXA_4, is essentially inactive in vivo and does not stop either PMN transmigration or reduce adhesion [54]. LXA_4 is enzymatically inactivated by conversion to 15-oxo-LXA_4 and 13, 14-dihydro-LXA_4 [55]. These further LXA_4 metabolites are essentially biologically inactive in stopping PMN transmigration and functional responses that are the key features of LXA_4 actions [56]. These pharmacophores for the anti-inflammatory action of the ligand LXA_4, namely the carbon 15-hydroxyl group and 13, 14-double bond, are also required for their interaction with specific cell surface receptors. The biologically inactive metabolic products and synthetic compounds tested, including 15-oxo-LXA_4, 15-deoxy-LXA_4, 11-trans-LXA_4 and 13, 14-dihydro-LXA_4, do not effectively bind to ALX/FPR2. This contrasts with the active LX ligands that include native LXA_4, which carries a 15*S*-configuration alcohol that originates from the lipoxygenase biosynthetic pathway and the aspirin triggered 15*R*- or 15-epi-LXA_4 (also denoted ATL or aspirin-triggered LXA_4) biosynthesized in the presence of

aspirin [3, 16]; each exhibits stereospecific receptor binding to both the human and murine ALX/FPR2 [39]. Of interest, the carboxy-methyl ester of LXA_4 is a partial ALX/FPR2 antagonist and does not effectively regulate NF-κB activity [57]. The LXA_4 methyl ester also blocks PMN transmigration across both epithelial and endothelial cells, but is less potent in reducing PMN chemotaxis with isolated cells. This highlights the point that LXA_4 methyl ester is rapidly hydrolyzed to its corresponding free acid *in vivo* and/or in the presence of PMN and other cell types such as epithelial cells that possess the esterase activity. Thus, the pharmacologic additions of LXA_4 and/or LX analogs as carboxy methyl esters are pro-ligands or pro-drugs that require local conversion to their corresponding carboxylic acids to stimulate ALX/FPR2-mediated bioactions including intracellular, phosphorylation and gene regulation.

Since rhodopsin structure is more complete than our understanding of GPCR such as ALX/FPR2 and its ligand interactions, it is instructive to compare the retinal chromophore of rhodopsin to fMLP and ligands. In this regard, 3-D modeling of the dimensions and spatial volumes for retinal, fMLP and LXA_4 have shown that they are remarkably similar in the 3-D spatial volumes [58]. Hence, similarities among the 3-D spatial volumes of ligands should reflect complementary sites in respective receptors as well as illustrate the spatial 3-D similarities among peptide vs. non-peptide or even lipid mediator ligands that might otherwise not seem obvious from their chemical structures.

The first ALX/FPR2 agonist identified for was micromolar fMLF [46]. The mouse ALX/FPR cDNA was cloned from a spleen cDNA library [39] and, when expressed in CHO cells, gave specific [^{3}H]-LXA_4 binding, Kd=1.5 nM determined by Scatchard plot analysis and LXA_4-initiated GTPase activity. Northern blot analysis showed that the mouse ALX mRNA is ~1.4 Kb. An orthologue of ALX was also isolated from rat leukocyte which displayed both specific radioligand binding and LXA_4-dependent inhibition of TNF-α mediated NF-κB activity [59].

Human [48], mouse [39] and rat [59] ALX/FPR2 cDNA all are characterized by an open reading frame of 1053 nucleotides which encodes a protein products that consists of 351 amino acids. To evaluate the structural contributions of the major domains from the receptor side via interactions with LXA_4 or anti-inflammatory and proinflammatory peptide ligands, chimeric receptors were constructed from ALX/FPR2 and BLT_1 receptors with opposing functions. These chimeras demonstrated that the seventh transmembrane and adjacent regions of the receptor are essential for LXA_4 recognition (see below), and additional regions including extracellular loops are required for high affinity binding for peptide ligands such as the MMK-1 and MHC peptides. A single GPCR can recognize and function with specific chemotactic peptides as well as non-peptide ligands such as LXA_4. But, clearly, these ligands act with different affinities and/or at separate interaction sites within the receptor [60]. Of interest, conserved N-glycosylation sites are present on Asn-4 and Asn-179 of the human receptor, and bacterial and viral infection are known to interfere with normal N-glycosylation of the host cells [61–63]. In this regard, deglycosylation of ALX does not dramatically alter LXA_4 recognition, but significantly lowers the affinity for peptide ligands [60]. Thus, N-glycosylation is an essential post-translational modification for ligand specificity of this receptor and may play a pivotal role in switching or changing this receptor's functional attributes at local host defense sites.

Several conserved motifs and amino acid residues important for post-translational modification have been identified in this receptor. These residues are Ser-236, Ser-237 and Tyr-302, which are essential for human ALX phosphorylation and signaling. Site-directed mutagenesis of these residues and expression of the wild-type and mutated receptors in CHO and HL-60 cells demonstrated that mutation of ALX at either serine Ser-236 and 237 or

tyrosine Tyr-302 phosphorylation sites gives sustained activation of both phospholipases PLA_2 and PLD. This contrasts with the transient activation of the wild-type receptor [64]. These structure-function studies (i.e. receptor chimera, glycosylation and phosphorylation) not only identify the key domains/residues important for ligand recognition and downstream signaling, but also further substantiate that LXA_4 is the highly stereospecific ligand for this receptor.

In addition to reports of anti-inflammatory ligands such as the annexin derived peptides that act at this and related receptors [65, 66], another compelling line of evidence comes from mice transgenic for the human (ALX/FPR2) receptor [67–69] and isolated cell types overexpressing this receptor that signal to down-regulate epithelial IL-8 expression and inflammation [41]. The overexpression of the human receptor in mice leukocytes demonstrates a gain-of-function in phenotype with three different transgenic lines. Each of the transgenics overexpressing this receptor showed lower amplitude of acute inflammation and a response of considerably shorter duration. Thus, mice overexpressing this receptor showed quicker resolution times of inflammation and a heightened sensitivity to the anti-inflammatory actions of a metabolically stable ligand, the ATL analog, in vivo compared to nontransgenic littermates. This higher potency displayed by the ATL mimetic in transgenic mice provides evidence for both an anti-inflammatory-pro-resolving role of this receptor in vivo as well as evidence for in vivo agonist LXA_4 ligand-receptor interactions.

Another independent line of investigation comes with the medicinal chemistry screening for human ALX/FPR2 agonists that identified non-peptide agonists with potent anti-inflammatory actions i.e. blocking human PMN chemotaxis in vitro and oral administration in mice reducing inflammation in vivo [70, 71]. The formation and endogenous counterregulatory actions of LXA_4 and its interactions with ALX/FPR have expanded in recent years to include reduced pain signaling and the presence of the receptor in neural tissues [72], a role in mucosal epithelial-based host-defense mechanisms with enhanced microbial killing [14], expedited ocular wound healing [73] and ocular edema [74]. Low-dose aspirin induces ALX expression in humans and triggers 15-epi-LXA_4 formation, which regulates neutrophil entry *in vivo* [75].

The intracellular signaling mechanisms sharply constrasts between peptide versus LXA_4 with ALX/FPR2. For example, fMLP relies heavily on Ca^{2+} mobilization for its amplification via this receptor, while LXA_4 signaling attenuates nuclear factor-κB (NF-κB) activation and blocks phosphorylation of p38 ERK and c-Jun N-terminal kinase (JNK) [50, 72, 76]. Also, low doses of LXA_4 in vivo enhance the expression of the receptor, suggesting a positive feed-forward loop in the physiology of this receptor and its responses to local lipid mediator ligands such as LXA_4 [77]. The ligand-dependent intracellular signal of this receptor is cell-type dependent [3, 49] and fascinating [33, 47], and warrants further studies to better understand the flexible and versatile ability of this receptor and its multiple ligand-dependent states of activation as well as its functional significance in human health and disease.

In order to determine the contributions of the major structural domains of ALX in interacting with either lipid or peptide ligands, chimeric receptors were constructed from receptors with opposing functions, namely ALX and BLT1. These chimeras reveal that the seventh transmembrane (TM) and adjacent regions of ALX are essential for LXA4 recognition, while additional regions of ALX (e.g. extracellular loops) are required for high affinity binding of selective peptide ligands (i.e. MMK-1 surrogate peptide and MHC binding peptide). These findings are the first to demonstrate that a single GPCR can recognize and function with specific peptides as well as lipid-derived ligands, LXA4. However these distinct classes of ligands (peptide vs. lipid-derived) clearly act with different

affinities and/or interaction sites within the receptor, ALX. Moreover, one of the ALX-BLT1 chimera encompassing the third extracellular loop and the seventh TM domain of ALX retains the specific binding to LXA4 but not LTB4 [60]. More recently, Damian et al produced heterodimers of this chimera and the wild type BLT1 to investigate GPCR trans-activation. Interestingly, ligand binding to one protomer in the heterodimer can induce cross-conformational changes in the other. However, these conformational changes are not associated with G protein trans-activation, i.e. agonist binding to one protomer cannot activate G proteins that are associated with the agonist-free protomer [78].

RvE1 GPCR Interactions

RvE1 generation in vivo in humans—RvE1 was originally found *in vivo* during resolution phase of inflammation in exudates from murine dorsal pouches treated with aspirin and EPA, and was generated during co-incubation of human endothelial cells and neutrophils [1]. RvE1 was monitored using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in healthy human volunteers given EPA and aspirin [18]. The plasma values ranged from 0.1 to 0.4 ng/ml for 6 healthy subjects. The formation of RvE1 is consistent with the scheme that endothelial cells expressing COX-2 treated with aspirin transform vascular EPA to produce and release 18*R*-HEPE [1]. One route of RvE1 biosynthesis can be evoked *in vivo*. Human leukocyte and endothelial cell interaction within the vasculature, rapidly converts 18*R*-HEPE to RvE1 via transcellular biosynthesis [18].

Identification of pro-resolving receptor candidates—The murine dorsal air pouch is a widely used experimental model to assess dermal inflammation [79]; it is anatomically characterized by a cavity and a lining composed of both fibroblast-like and macrophage-like cells [79]. Intrapouch application of TNF-α evokes leukocyte infiltration by stimulating local release of chemokines and chemoattractants that are often produced by fibroblasts and phagocytes via regulation of nuclear factor (NF)-κB transcription factors [80]. The systemic administration of RvE1 dramatically attenuated leukocyte recruitment, suggesting the receptor target for RvE1 was expressed in those cells, which counterregulate TNF-α induced NF-κB activation. RvE1 and LXA_4 have different structures [see Fig. (1)], are formed via different biosynthetic pathways and precursors (EPA vs arachidonate), yet they appear to share redundant beneficial actions that dampen excessive leukocyte recruitment [81] in the inflammatory milieu. Hence we reasoned that RvE1 receptors might share similar structural features to LOX-derived eicosanoid receptors such as ALX/FPR2 and the leukotriene $(LT)B_4$ receptor (BLT) [82].

Fig. (2A) (inset) illustrates a branch of the phylogenetic tree of G-protein coupled receptors (GPCRs) focused on human ALX/FPR2 with closely related receptors. Expression plasmids of each GPCR were introduced into HEK293 cells and the ability of RvE1 to inhibit TNF-α stimulated NF-κB activation was monitored by co-transfection with NF-κB response element-luciferase reporter plasmid. This permitted an unbiased analysis of the activation of the relevant post ligand-receptor “stop” signaling for downregulation of NF-κB activation as for example demonstrated with ALX-transfected cells and its ligands [83]. Among those screened [18], the orphan receptor denoted earlier as ChemR23 [84] was specifically activated by RvE1 and at 10 nM inhibited NF-κB activation, as shown in Fig. (2A). ChemR23 shares 36.4% identity with ALX in deduced amino acid sequences. Of note it contains a highly conserved domain in its second intracellular loop with 75% identity and within the seventh transmembrane region (69.5% identity with the corresponding regions in ALX).

RvE1 dose-dependently inhibited TNF-α induced NF-κB activation with an EC_{50} of ~1.0 nM in ChemR23 transfected cells compared to mock [see Fig. (2B)]. In this system, 1 mM

aspirin, a known inhibitor of NF-κB at high concentrations namely millimolar range [85], gave non-receptor dependent inhibition of 26.2±4.9% for the transfected cells. As shown in Fig. (2C), neither EPA nor 18*R*-HEPE at 100 nM, both metabolic precursors of RvE1, inhibited NF-κB in ChemR23 transfected cells. The double bond isomer of RvE1, 6-*trans*, 14-*trans*-RvE1, at 100 nM showed reduced potency for NF-κB inhibition that was essentially the same magnitude reduction *in vivo*. We also examined functional interactions between ChemR23 and G proteins using ligand-dependent binding of [^{35}S]-GTPγS, a hydrolysis resistant GTP analog. Specific [^{35}S]-GTPγS binding in isolated membranes obtained from cells expressing ChemR23 increased selectively with RvE1 in a concentration-dependent manner. These results indicate that RvE1 functions as a selective agonist of ChemR23 and counterregulates TNF-α stimulated NF-κB activation.

Radioligand Binding—RvE1 binding to ChemR23 was assessed with tritium-labeled RvE1, Fig. (1B), which was prepared by catalytic hydrogenation from synthetic diacetylenic RvE1. Integrity of the synthetic [^{3}H]-RvE1 was confirmed by HPLC; radioactive compound co-eluted with synthetic RvE1 beneath a single peak in HPLC. Its characteristic UV absorbance maxima at 271 nm and 234 nm were indicative of the conjugated triene and diene in RvE1 structure. Analysis of [^{3}H]-labeled RvE1 specific binding and Scatchard transformation is shown in Fig. (3). [^{3}H]RvE1 bound to an apparent single site on ChemR23 transfectants with high affinity (Kd=11.3±5.4 nM, Bmax=4200±1050 binding sites per cell). RvE1 biosynthesis precursors, EPA and 18*R*-HEPE, did not compete for specific [^{3}H]RvE1 binding. We also tested the synthetic peptide fragment (YHSFFFPGQFAFS) derived from chemerin that was recently reported to be a peptide ligand for this same receptor [86]. Chemerin peptide at 10 μM displaced specific [^{3}H]-RvE1 binding by ~70%, suggesting that RvE1 and chemerin share recognition sites on the same receptor ChemR23.

Expression, regulation and signaling properties of ChemR23—Profiling for tissue distribution of human ChemR23 was performed with dot blots containing mRNAs from human tissues that showed expression of ChemR23 such as cardiovascular system, brain, kidney, gastrointestinal tissues and myeloid tissues Fig. (4A). Also, a murine receptor counterpart was found in developing bone using *in situ* hybridization [87]. Among the human peripheral blood leukocytes, ChemR23 was abundantly expressed in monocytes, with lower amounts in neutrophils and T lymphocytes, findings consistent with the observation that this receptor is expressed in antigen-presenting cells (APC) such as macrophage and dendritic cells (DCs) [84, 86]. Both monocyte ChemR23 and COX-2 transcripts were highly upregulated by treatment with inflammatory cytokines such as TNF-α and IFN-γ, and ChemR23 showed delayed induction to that of COX-2. RvE1 increased phosphorylation of extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase both in peripheral blood monocytes and HEK-ChemR23 cells, but not in mock-transfected HEK293 cells. In addition, treatment of HEK-ChemR23 with pertussis toxin (PTX) abolished RvE1 dependent ERK activation and NF-κB inhibition, suggesting that the receptor couples to the G-proteins of Gαi/o class for the signal transduction [18].

Since RvE1 and chemerin peptide competed at the same receptor, it was of interest to determine whether there was a divergence between the responses initiated by these ligands. Chemerin-derived peptide activated ChemR23 as monitored by microphysiometry, evoking a response consistent with receptor-initiated increases in extracellular acidification rates (EAR) [18]. These results are in line with the reported properties of this peptide ligand [86]. RvE1 did not increase the EAR but selectively blocked the peptide-evoked responses. Consistently, the extent of G-protein activation evoked by chemerin peptide was 3 times as much as RvE1 whereas RvE1 was an order of magnitude more potent than chemerin peptide for inhibiting NF-κB activation. Thus, RvE1 interacts with ChemR23 and transmits signals via this receptor in a manner different than that of the chemerin-derived peptide.

RvE1-ChemR23 interaction blocks DC responses—Given expression of ChemR23 in antigen presenting cells (APCs), and since dietary ω-3 PUFA supplementation has an impact on APC function [88], we examined the activity of RvE1 on APC function using a microbial pathogen model. Injection of pathogen extract derived from *Toxoplasma gondii* (STAg) causes activation of splenic DCs to mobilize to T cell enriched areas where they produce high amounts of IL-12 [89]. Addition of increasing concentrations of RvE1 to isolated mouse splenic $CD11c^+$ DCs markedly inhibited IL-12p40 production by STAg within the nanomolar range [18]. We next carried out siRNA experiments to reduce ChemR23 in splenic DCs. Mouse receptor [87], which shares 80.3% identity with human ChemR23, was also present in splenic DCs. RvE1's action in regulating IL-12 production from DCs was eliminated by treatment with a siRNA specific for the mouse ChemR23. We confirmed that this siRNA treatment dramatically reduced ChemR23 mRNA expression in DCs and cell-surface expression of recombinant ChemR23 in HEK293 cells. Thus, RvE1's anti-inflammatory action is mediated via interaction with ChemR23. *In vivo* administration of RvE1 also blocked IL-12 production as well as DC migration into T cell areas of the spleen.

Specific Binding of [^{3}H]-RvE1 with Human PMN—[^{3}H]-RvE1 specific binding was demonstrated with membrane fractions isolated from human PMN. At 4° C, RvE1 binding to human PMN membrane fraction was specific with a Kd of 48.3 nM. We next examined the competition of [^{3}H]-RvE1 binding with several related eicosanoids. Among the compounds tested, [^{3}H]-RvE1 specific binding to human PMN was displaced by the homoligand RvE1 (Ki=34.3 nM), leukotriene (LT)B_4 (Ki=0.08 nM) and LTB_4 receptor 1 (BLT1) selective antagonist U-75302 (Ki=1.5 nM) [90], but not by the chemerin peptide [86], a ligand specific for another RvE1 receptor denoted ChemR23. For direct comparison, [^{3}H]-RvE1 to human ChemR23 was competed with RvE1 (Ki=330 nM) or chemerin peptide (K_i=429 nM), but not with LTB_4. These results demonstrate that RvE1 binding sites are pharmacologically distinct from ChemR23 on human PMN.

Since [^{3}H]-RvE1 binding on human PMN was displaced by LTB_4, we tested whether RvE1 binds to recombinant BLT1. HEK293 cells stably expressing human BLT1 (HEK-hBLT1) were prepared to determine RvE1 specific binding. [^{3}H]-RvE1 was specifically bound to HEK-hBLT1 with a Kd of ~45 nM. [^{3}H]-RvE1 specific binding was competed with LTB_4 (Ki=3 nM) or RvE1 (Ki=70 nM). Recombinant human LTB_4 receptor type 2 (BLT2) did not display specific binding for [^{3}H]-RvE1 at concentrations up to 10 nM. These results clearly indicated that RvE1 binds to BLT1 on human PMN.

RvE1 selectively interacts with BLT1—To examine whether the binding of RvE1 to BLT1 transduces intracellular signaling responses, we determined adenylyl cyclase activity by measuring the cAMP accumulation. LTB_4 inhibited 5 μM forskolin-activated adenylate cyclase activities in HEK-hBLT1 cells with EC_{50} of 0.015 nM, a value consistent with that reported previously [82]. In these experiments, RvE1 inhibited adenylate cyclase activities with EC_{50} of 3.2 nM. On the other hand, RvE1 gave no response with HEK-hBLT2 cells where LTB_4 gave signals (EC_{50}=11.5 nM), a value consistent with the previous report [90]. These results indicate that RvE1 selectively binds and activates BLT1 to modulate intracellular signaling pathways.

Arita et al. [91] reports the increase in intracellular calcium mobilization stimulated by LTB_4 or RvE1 in human PBMC. RvE1 at 100 nM increased intracellular calcium, but the maximum intensity of signal was only about one-third that of LTB_4. Importantly, prior exposure to RvE1 completely blocked LTB_4-induced intracellular Ca^2 response in a concentration dependent manner. Thus, RvE1 acts as a partial agonist to attenuate LTB_4-induced calcium signals in leukocytes.

Since LTB_4 induces pro-inflammatory cytokine and chemokine gene expression by activating the trancription factor, NF-κB [92], we next determined whether RvE1 could modulate LTB_4-BLT1 interaction induced NF-κB activation by using a luciferase reporter gene system. LTB_4 induced NF-κB activation in HeLa cells transfected with human BLT1 with EC_{50} of 0.36 nM. This induction was almost completely abolished by 100 ng/ml pertussis toxin (PTX), suggesting that BLT1 coupled to PTX sensitive G-proteins such as Gi/o to activate NF-κB transcription factor. RvE1 concentrations above 100 nM gave partial activations of NF-κB that were in a range comparable to that of the BLT1 antagonist U-75302. Importantly, LTB_4-dependent NF-κB activation was blocked by ~40–50% with RvE1 as low as 1 nM, which is an approximately 10-fold molar excess of LTB_4. These results clearly demonstrated that RvE1 upon interaction with BLT1 attenuates LTB_4-induced pro-inflammatory signals in a non-phlogistic manner.

BLT1-deficient mice and RvE1—We examined the functional outcome of RvE1-BLT1 interaction in vivo role in regulating leukocyte infiltration in zymosan A-induced peritonitis. In this model, zymosan activates resident macrophages and/or early accumulating neutrophils to produce LTB_4 from arachidonic acid, resulting in PMN influx in an autocrine loop of PMN accumulation during acute inflammation [93]. Intravenous administration of RvE1 at 100 ng dramatically blocked PMN infiltration by 38% at 2 h after zymosan injection in wild type mice. In contrast, RvE1 at 100 ng did not give statistically significant reduction in mice with targeted disruption of BLT1 (BLT1−/−). The early reduction of PMN influx in BLT1−/− mice, which represents BLT1-dependent PMN infiltrations, was noted as reported previously [93]. RvE1 reduced the numbers of infiltrating PMN in wild type mice to the levels of that obtained in BLT1−/− mice, indicating that intravenous administration of RvE1 at 100 ng blocked PMN migration by dampening BLT1 functions in vivo. Of interest, RvE1 at higher doses (1.0 μg i.v.) retained most of its anti-migratory actions in BLT1−/− mice, giving around 35% inhibition of PMN infiltration in both wild type and BLT1−/− mice. Together, these results demonstrate that intravenously administered RvE1 acts interacts with BLT1 and dampens BLT1 signals on leukocytes in addition to BLT1-independent mechanisms involving additional receptor(s) in vivo.

RvD1 Actions and its GPCRs

Single-cell (PMN) real-time monitoring: Direct Action of RvD1 and not DHA—The original structure elucidation of RvD1 demonstrated its presence and potent anti-inflammatory actions in self-resolving exudates and disease models [2, 21]. We questioned whether RvD1 or its precursor DHA has direct actions with human PMN. This is a central point because excessive PMN infiltration with a release of pro-inflammatory mediators is well appreciated in causing tissue damage.

To address this question, we used a *new* microfluidic chamber (MFC) in collaboration with **Drs. Irimia and Toner** [27]. This chamber permits PMN capture in < 5 minutes within 1 cubic μl from 1 drop of whole blood from a finger prick (upper photo). Microstructured valves within the chamber controls the timing of exposing PMN to chemotactic gradients. Images of human PMN before and during exposure to gradients are continuously monitored using a camera and recorded. PMN chemotaxing along IL-8 gradients show typical shape change and morphology during chemotaxis in a linear gradient. Before exposure to RvD1, individual PMN movements and distances were proportional to time. Uniform exposure to RvD1 [10 nM] rapidly and dramatically changed PMN shape and ceased chemotaxis demonstrating stopping of PMN migration. In contrast, its precursor DHA at equimolar concentrations did not stop PMN [27]. *Thus, RvD1 stops PMN as well as stimulates shape change.*

Resolvins are tissue protective—Ischemia followed by reperfusion is a well-recognized mechanism of organ injury and tissue damage that can be initiated by activated PMN. We studied murine hind-limb ischemia/reperfusion that models second organ injury and PMN mediated tissue damage observed in humans, for example, following tourniquet release of vessels in surgery involving extremities [5, 94]. On release of tourniquet occlusion, reflow is initiated and aberrantly activated PMN rapidly infiltrate murine secondary organs, causing damage [95]. We evaluated RvD1 in this system, to assess whether its ability to stop PMN in MFC could *translate* to reduction in PMN-mediated tissue damage. PMN accumulation in the lung was assessed by monitoring both tissue histology and a leukocyte marker myeloperoxidase [95]. Again, we directly compared actions of RvD1, DHA and RvE1. Neither *native* RvE1 nor DHA protected tissues from excessive PMN infiltration following reperfusion injury [27], whereas at 1 μg/mouse RvD1 sharply reduced ~50% leukocyte infiltration.

Since RvD1 undergoes local metabolic inactivation *in vivo* [21] as does RvE1 [96, 97], modifying their respective metabolic sites of inactivation within each molecule was undertaken to prepare stable mimetics. 17-(*R/S*)-methyl RvD1, 18-p-fluorophenoxy-17-epi-RvD1, and RvE1 analogs were prepared by total organic synthesis [21, 96, 97]. In work in progress, both RvD1 and related analogs sharply reduced PMN and MPO levels in tissues. The RvE1 analog, 19-*p*-fluorophenoxy-RvE1 at 100ng to 1 μg/mouse significantly reduced leukocyte infiltration while *native* RvE1 was *inactive* in this system. This differs from topical application, e.g. on the eye, where RvE1 is potent [27, 98]. Mouse lung enzymatically converts RvE1 to inactive 18-oxo-RvE1 [96, 97]. The 19-*p*-fluorophenoxy RvE1 analog prevents rapid inactivation and displays potent protective actions [27]. Thus, by reducing Rv metabolic inactivation beneficial SPM actions can be sustained.

Evidence for pro-resolving receptors—Since RvD1 limits PMN infiltration, reduces human PMN transendothelial migration [2, 21] and has pro-resolving actions enhancing MΦ phagocytosis [99], we prepared synthetic [^{3}H]-RvD1 [Fig (1B)] to address specific RvD1 binding and recognition.

RvD1 and RvE1 do not activate nuclear receptors—Because some nuclear receptors may evoke anti-inflammatory responses [100], we determined whether Rvs interact with specific nuclear receptors. To this end, HEK-293 cells were cotransfected with constructs of ligand binding domains for a panel of nuclear receptors linked to Gal4 DNA binding domain, along with a Gal4-responsive luciferase reporter. At anti-inflammatory doses [101] neither RvE1 nor RvD1 [1–100nM] *directly* activate PPAR-α, γ, and δ, or retinoid X receptor α (RXR) compared to known nuclear receptor agonists [102].

RvD1 regulates actin polymerization: PTX sensitivity—We tested whether RvD1 regulates actin polymerization, a key event in migration [103]. Human PMN incubated with RvD1 (10 nM) resulted in a decrease in actin polymerization. To assess whether GPCRs are involved in RvD1 signaling, PMN were treated with either pertussis toxin (PTX), or activated cholera toxin (CT). PTX diminished RvD1-dependent reduction in actin polymerization, whereas incubation with activated CT *did not*. These suggest RvD1 acts *via* a PTX-sensitive GPCR. Also, RvD1 (10 nM) significantly blocked actin reorganization and CD11b cell surface expression stimulated by LTB_4 [10 nM]. With human PMN, RvD1 did not stimulate intracellular Ca^{2+} nor activate cAMP suggesting that RvD1 signaling does not activate classic second messengers.

[^{3}H]-RvD1 specific binding—We next determined whether human phagocytes display specific RvD1 binding sites. To this end, tritium-labeled RvD1 ([^{3}H]-RvD1) was prepared by catalytic hydrogenation of synthetic [13, 14]-acetylenic RvD1-methyl ester (ME).

Following catalytic hydrogenation, ^{3}H-labeled RvD1 co-eluted with authentic RvD1-ME beneath a single HPLC peak and gave characteristic UV absorbance [2, 21]. [^{3}H]-RvD1-ME was saponified to obtain the corresponding carboxylic acid, [13,14]-[^{3}H] RvD1 and isolated immediately before each binding experiment. Representative saturation curves and Scatchard analysis are shown in Fig. (5A). These indicate that [^{3}H]-RvD1 specifically binds to human PMN with high affinity K_d = 0.17 nM ± 0.06 nM.

Competition binding was performed with RvD1, LXA_4, and the annexin 1-derived peptide Ac2-12, an anti-inflammatory peptide ligand that binds to ALX [104]. RvD1 displaced [^{3}H]-RvD1 binding to cells, defining 100% specific binding Fig. (5B). This specific binding was partially displaced ~ 60 % by LXA_4 while peptide $Ac_{2–12}$ did not compete Fig. (5B). In parallel, [^{3}H]-RvD1 gave specific binding with human monocytes [102]. These results demonstrated RvD1 high affinity and specific binding with human phagocytes.

Screening for RvD1 receptor candidates—Since the handling of [^{3}H]-RvD1 is costly and not without difficulties but required, we also sought functional screening systems that do not rely on radiolabel. To this end, since RvD1 counters the actions of TNF-α [2], we employed for initial screening a luciferase-based reporter system we constructed earlier for identification of RvE1 receptors [18]. This system utilized a luciferase-based reporter that measures NF-κB activated by TNF-α [102]. Human cDNA sequences of phylogenetically related GPCR were cloned into pcDNA3 vector and then co-transfected into HeLa cells together with a reporter vector consisting of NF-κB promoter sequence linked to the luciferase gene. Among these human GPCRs, RvD1 significantly reduced TNF-α stimulated NF-κB activity *only* in cells overexpressing ALX and the orphan GPR32. In contrast, cells transfected with other sequence-related GPCRs including BLT1, BLT2, GPR-1, FPR, and ChemR23 [102] *did not* respond to RvD1. These panels were screened because they are expressed on human leukocytes and appear to be functionally related for cell motility [105].

RvD1 directly acts at Human Orphan GPR32 and ALX—To monitor RvD1 receptor-ligand interactions, next we used a new GPCR-β-arrestin coupled system that is not signaling pathway dependent Fig. (6A) [106] because RvD1 does not simply mobilize Ca^{2+} [102]. Incubation of GPR32-β-arrestin cells with RvD1 gave dose-dependent increases for interactions of β-arrestin and the receptor $EC_{50} \sim 8.8 \times 10^{-12}$ M. In parallel, LXA_4 also gave dose-dependent increases in the activation of GPR32 ($EC_{50} \sim 3.4 \times 10^{-11}$ M) Fig. (6B). These findings, namely the K_d and EC_{50} values, are within the pg to ng range of RvD1 produced in vivo [107] and indicate that RvD1 and LXA_4 each directly activated GPR32. Recently, Amgen reported nitrosylated pyrazolone derivatives with agonistic actions on ALX receptor. The agonist denoted compound 43 is anti-inflammatory, potently reduces PMN migration and markedly inhibits mouse ear inflammation [70]. These results confirm our original identification of ALX-LXA_4 axis in inflammation using a separate medical chemistry approach [65].

As an ALX anti-inflammatory agonist *in vivo*, we prepared synthetic compound 43 as in ref. [70] and tested it with our β-arrestin cells stably expressing GPR32. Compound 43 also activated GPR32, $EC_{50} = 2.25 \times 10^{-12}$ M, Fig. (6B). In contrast, side-by-side comparisons indicated that RvD1 *did not* interact with ChemR23 compared to its lipid mediator ligand RvE1 [18], $EC_{50} \sim 1.3 \times 10^{-11}$ M. These findings demonstrate high selectivity of these SPM for specific GPCR. RvD1 directly activated ALX with an affinity $EC_{50} \sim 1.2 \times 10^{-12}$ M. This was comparable to the activation of ALX by LXA_4 $EC_{50} \sim 1.1 \times 10^{-12}$ M Fig. (6C). A receptor antagonist *tert*-butoxycarbonyl (*t*-Boc) peptide [108] blocked activation by both RvD1 and LXA_4 ($IC_{50} \sim 10^{-5}$ M). Again, using compound 43 with ALX stable β arrestin cells gave a dose-dependent activation with an EC_{50} of ~2.1×10^{-12} M Fig. (6C). For comparison, compound 43 was unable to activate β-arrestin system stably expressing ADP

receptor P2Y12, indicating selectivity of ligand GPCR interactions. These results suggest that RvD1 interacts with both ALX and GPR32.

Overexpression of GPR32 and ALX in human MΦ enhances RvD1-stimulated phagocytosis—Since complete resolution *requires* phagocytosis and clearance of apoptotic PMN by MΦ from the inflammatory milieu, which is enhanced by resolvins [3], we tested if RvD1 enhances human MΦ phagocytic activity. Human MΦ exposed to RvD1 enhanced their ability to ingest either zymosan or apoptotic human PMN in a dose-dependent manner. RvD1 activity peaked at 0.1–1.0 nM and proved time-dependent [102]. Next, we transiently transfected human MΦ with expression vectors for RvD1 candidate receptors, i.e. human ALX or GPR32. RvD1 showed an increase in phagocytosis of zymosan in MΦ transfected with mock further increased in MΦ overexpressing either GPR32 or ALX [102].

Protectin D1 specific binding

Retinal pigment epithelial (RPE) cells, derived from the neuroectoderm, biosynthesize the novel lipid mediator neuroprotectin D1 (NPD1) from docosahexaenoic acid (DHA) in response to oxidative stress or to neurotrophins, and in turn, elicits cytoprotection. Recently, we reported the identification of a 16,17-epoxide-containing intermediate in the biosynthesis of NPD1 in ARPE-19 cells from 17*S*-hydro-(peroxy)-docosahexaenoic acid. We prepared and isolated tritium-labeled NPD1 ([^{3}H]-NPD1) and demonstrated specific and high-affinity stereoselective binding to ARPE-19 cells (K_d = 31.3 ± 13.1 pmol/mg of cell protein). The stereospecific NPD1 interactions with these cells in turn gave potent protection against oxidative stress-induced apoptosis, and other structurally related compounds were weak competitors of NPD1 specific binding. This [^{3}H]-NPD1/PD1 also displayed specific and selective high affinity binding with isolated human neutrophils (K_d = ~25 nM). Neither resolvin E1 nor lipoxin A_4 competed for [^{3}H]-NPD1/PD1 specific binding with human neutrophils. Together, these results provide evidence for stereoselective specific binding of NPD1/PD1 with recognition sites on retinal pigment epithelial cells as well as human neutrophils. Moreover, they suggest the presence of specific receptors for this novel mediator in both the immune and visual systems [109].

Summation

The acute inflammatory response is a self-limited protective mechanism. In response to injury or infection, excessive or uncontrolled inflammatory responses can lead to chronic disorders. Neutrophil-derived pro-inflammatory mediators, including leukotrienes and prostaglandins, are produced from arachidonic acid and are made in the initial phase of contained acute inflammatory responses that they can amplify. Within contained inflammatory exudates, we found that neutrophils can change phenotypes with time to generate protective mediators, derived from essential polyunsaturated fatty acids, to promote resolution. There is an active catabasis to return tissues to a homeostatic health state from the inflammatory battle [25]. These protective mediators include the arachidonic acid-derived lipoxins as well as omega-3 EPA-derived resolvin E-series, DHA-derived resolvin-D series, protectins and maresins. These findings provide evidence that the resolution of acute inflammation is not a passive burning out of chemotactic signals, but rather an active, regulated process that orchestrates the clearance of inflammatory exudates. The endogenous production of pro-resolving lipid mediators, such as lipoxins, protectins and resolvins, as well as their administration *in vivo*, can accelerate the resolution of acute inflammation and the return to tissue homeostasis. The receptors for proresolving mediators and their signaling mechanisms in resolution can open new directions to treat inflammation-associated diseases.

Acknowledgments

The authors acknowledge the support of National Institutes of Health grants DE019938, GM038765 and DK074448. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Dental & Craniofacial Research, the National Institute of General Medical Sciences, the National Institute of Diabetes and Digestive and Kidney Diseases or the National Institutes of Health.

The authors thank Mary H. Small for expert assistance with manuscript preparation and the members of the Serhan laboratory and collaborators for their expertise and efforts in the reports referenced herein.

Abbreviations

APC	Antigen Presenting Cell
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GPCR	G-protein Coupled Receptor
LXA_4	Lipoxin A_4; 5*S*, 6*R*,15*S*-trihydroxytrihydroxy-7*E*,9*E*,11*Z*,13*E*-eicosatetraenoic acid
MΦ	macrophages
PD1	10R,17S-dihydroxy-4Z,7Z,11E,13E,15Z,19Z-docosahexaenoic acid
PMN	Polymorphonuclear leukocytes
RvD1	Resolvin D1; 7*S*, 8*R*,17*S*-trihydroxy-4*Z*,9*E*,11*E*,13*Z*,15*E*,19*Z*-docosahexaenoic acid
RvE1	Resolvin E1; 5S, 12R,18R-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid
SPM	Specialized Pro-resolving Mediators

References

1. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med. 2000; 192:1197–1204. [PubMed: 11034610]
2. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter pro-inflammation signals. J Exp Med. 2002; 196:1025–1037. [PubMed: 12391014]
3. Serhan CN. Resolution phases of inflammation: novel endogenous anti-inflammatory and pro-resolving lipid mediators and pathways. Annu Rev Immunol. 2007; 25:101–137. [PubMed: 17090225]
4. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008; 8:249–261.
5. Cotran, RS.; Kumar, V.; Collins, T., editors. Robbins Pathologic Basis of Disease. W.B. Saunders Co; Philadelphia: 1999. p. 1425
6. Majno, G.; Joris, I. Cells, Tissues, and Disease: Principles of General Pathology. Oxford University Press; New York: 2004. p. 1005
7. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. Science. 1987; 237:1171–1176. [PubMed: 2820055]
8. Van Dyke TE, Serhan CN. A novel approach to resolving inflammation. Sci Am Oral and Whole Body Health. 2006:42–45.

9. Hansson GK, Robertson AKL, Soderberg-Naucler C. Inflammation and atherosclerosis. Annu Rev Pathol Mech Dis. 2006; 1:297–329.
10. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. Nat Immunol. 2005; 6:1191–1197. [PubMed: 16369558]
11. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. Nature. 2007; 447:869–874. [PubMed: 17568749]
12. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: novel macrophage mediators with potent anti-inflammatory and pro-resolving actions. J Exp Med. 2009; 206:15–23. [PubMed: 19103881]
13. Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR. Cutting edge: Lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. J Immunol. 2000; 164:1663–1667. [PubMed: 10657608]
14. Canny G, Levy O, Furuta GT, Narravula-Alipati S, Sisson RB, Serhan CN, Colgan SP. Lipid mediator-induced expression of bactericidal/permeability-increasing protein (BPI) in human mucosal epithelia. Proc Natl Acad Sci USA. 2002; 99:3902–3907. [PubMed: 11891303]
15. Campbell EL, Louis NA, Tomassetti SE, Canny GO, Arita M, Serhan CN, Colgan SP. Resolvin E1 promotes mucosal surface clearance of neutrophils: a new paradigm for inflammatory resolution. FASEB J. 2007; 21:3162–3170. [PubMed: 17496159]
16. Serhan CN. Special Issue on Lipoxins and Aspirin-Triggered Lipoxins. Prostaglandins Leukot Essent Fatty Acids. 2005; 73(3–4):139–321. guest ed.
17. Chiang N, Bermudez EA, Ridker PM, Hurwitz S, Serhan CN. Aspirin triggers anti-inflammatory 15-epi-lipoxin A_4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci US A. 2004; 101:15178–15183.
18. Arita M, Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN. Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. J Exp Med. 2005; 201:713–722. [PubMed: 15753205]
19. Hong S, Gronert K, Devchand P, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autacoids in anti-inflammation. J Biol Chem. 2003; 278:14677–14687. [PubMed: 12590139]
20. Serhan CN, Gotlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. J Immunol. 2006; 176:1848–1859. [PubMed: 16424216]
21. Sun YP, Oh SF, Uddin J, Yang R, Gotlinger K, Campbell E, Colgan SP, Petasis NA, Serhan CN. Resolvin D1 and its aspirin-triggered 17*R* epimer: stereochemical assignments, anti-inflammatory properties and enzymatic inactivation. J Biol Chem. 2007; 282:9323–9334. [PubMed: 17244615]
22. Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG. Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc Natl Acad Sci US A. 2004; 101:8491–8496.
23. Marcheselli VL, Hong S, Lukiw WJ, Hua Tian X, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, Bazan NG. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. J Biol Chem. 2003; 278:43807–43817. [PubMed: 12923200]
24. Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Schwartzman ML. A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. J Biol Chem. 2005; 280:15267–15278. [PubMed: 15708862]
25. Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN. Molecular circuits of resolution: Formation and actions of resolvins and protectins. J Immunol. 2005; 174:4345–4355. [PubMed: 15778399]
26. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. Nat Immunol. 2001; 2:612–619. [PubMed: 11429545]
27. Kasuga K, Yang R, Porter TF, Agrawal N, Petasis NA, Irimia D, Toner M, Serhan CN. Rapid appearance of resolvin precursors in inflammatory exudates: novel mechanisms in resolution. J Immunol. 2008; 181:8677–8687. [PubMed: 19050288]

28. Connor KM, SanGiovanni JP, Lofqvist C, Aderman CM, Chen J, Higuchi A, Hong S, Pravda EA, Majchrzak S, Carper D, Hellstrom A, Kang JX, Chew EY, Salem NN Jr, Serhan CN, Smith LEH. Increased dietary intake of omega-3-polyunsaturated fatty acids reduces pathological retinal angiogenesis. Nat Med. 2007; 13:868–873. [PubMed: 17589522]
29. Hudert CA, Weylandt KH, Wang J, Lu Y, Hong S, Dignass A, Serhan CN, Kang JX. Transgenic mice rich in endogenous n-3 fatty acids are protected from colitis. Proc Natl Acad Sci US A. 2006; 103:11276–11281.
30. Poulsen RC, Gotlinger KH, Serhan CN, Kruger MC. Identification of inflammatory and pro-resolving lipid mediators in bone marrow and their profile alteration with ovariectomy and omega-3 intake. Am J Hematol. 2008; 83:437–445. [PubMed: 18429055]
31. Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, Blumberg RS, Serhan CN. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci US A. 2005; 102:7671–7676.
32. Hasturk H, Kantarci A, Goguet-Surmenian E, Blackwood A, Andry C, Serhan CN, Van Dyke TE. Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis in vivo. J Immunol. 2007; 179:7021–7029. [PubMed: 17982093]
33. Chiang N, Serhan CN, Dahlén SE, Drazen JM, Hay DWP, Rovati GE, Shimizu T, Yokomizo T, Brink C. The lipoxin receptor ALX: potent ligand-specific and stereoselective actions in vivo. Pharmacol Rev. 2006; 58:463–487. [PubMed: 16968948]
34. McMahon B, Godson C. Lipoxins: endogenous regulators of inflammation. Am J Physiol Renal Physiol. 2004; 286:F189–F201. [PubMed: 14707005]
35. Mitchell D, O'Meara SJ, Gaffney A, Crean JK, Kinsella BT, Godson C. The lipoxin A4 receptor is coupled to SHP-2 activation: implications for regulation of receptor tyrosine kinases. J Biol Chem. 2007; 282:15606–15618. [PubMed: 17403678]
36. O'Sullivan TP, Vallin KS, Shah ST, Fakhry J, Maderna P, Scannell M, Sampaio AL, Perretti M, Godson C, Guiry PJ. Aromatic lipoxin A4 and lipoxin B4 analogues display potent biological activities. J Med Chem. 2007; 50:5894–5902. [PubMed: 17960922]
37. Reville K, Crean JK, Vivers S, Dransfield I, Godson C. Lipoxin A4 redistributes myosin IIA and Cdc42 in macrophages: implications for phagocytosis of apoptotic leukocytes. J Immunol. 2006; 176:1878–1888. [PubMed: 16424219]
38. Takano T, Clish CB, Gronert K, Petasis N, Serhan CN. Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A_4 and novel lipoxin B_4 stable analogues. J Clin Invest. 1998; 101:819–826. [PubMed: 9466977]
39. Takano T, Fiore S, Maddox JF, Brady HR, Petasis NA, Serhan CN. Aspirin-triggered 15-epi-lipoxin A_4 and LXA_4 stable analogs are potent inhibitors of acute inflammation: Evidence for anti-inflammatory receptors. J Exp Med. 1997; 185:1693–1704. [PubMed: 9151906]
40. Colgan SP, Serhan CN, Parkos CA, Delp-Archer C, Madara JL. Lipoxin A_4 modulates transmigration of human neutrophils across intestinal epithelial monolayers. J Clin Invest. 1993; 92:75–82. [PubMed: 8326019]
41. Kucharzik T, Gewirtz AT, Merlin D, Madara JL, Williams IR. Lateral membrane LXA4 receptors mediate LXA4's anti-inflammatory actions on intestinal epithelium. Am J Physiol Cell Physiol. 2003; 284:C888–C896. [PubMed: 12456400]
42. Brezinski DA, Serhan CN. Characterization of lipoxins by combined gas chromatography and electron-capture negative ion chemical ionization mass spectrometry: formation of lipoxin A_4 by stimulated human whole blood. Biological Mass Spectrometry. 1991; 20:45–52. [PubMed: 1883860]
43. Fiore S, Ryeom SW, Weller PF, Serhan CN. Lipoxin recognition sites. Specific binding of labeled lipoxin A_4 with human neutrophils. J Biol Chem. 1992; 267:16168–16176. [PubMed: 1322894]
44. Nigam S, Fiore S, Luscinskas FW, Serhan CN. Lipoxin A_4 and lipoxin B_4 stimulate the release but not the oxygenation of arachidonic acid in human neutrophils: dissociation between lipid remodeling and adhesion. J Cell Physiol. 1990; 143:512–523. [PubMed: 2162850]
45. Fiore S, Romano M, Reardon EM, Serhan CN. Induction of functional lipoxin A_4 receptors in HL-60 cells. Blood. 1993; 81:3395–3403. [PubMed: 8389617]

46. Ye RD, Cavanagh SL, Quehenberger O, Prossnitz ER, Cocrane C. Isolation of a cDNA that encodes a novel granulocyte N-formyl peptide receptor. Biochem Biophys Res Commun. 1992; 184:582–589. [PubMed: 1374236]
47. Ye RD, Boulay F, Wang JM, Dahlgren C, Gerard C, Parmentier M, Serhan CN, Murphy PM. International Union of Basic and Clinical Pharmacology LXXIII. Nomenclature for the formyl peptide receptor (FPR) family. Pharmacol Rev. 2009; 61:119–161. [PubMed: 19498085]
48. Fiore S, Maddox JF, Perez HD, Serhan CN. Identification of a human cDNA encoding a functional high affinity lipoxin A_4 receptor. J Exp Med. 1994; 180:253–260. [PubMed: 8006586]
49. Maddox JF, Hachicha M, Takano T, Petasis NA, Fokin VV, Serhan CN. Lipoxin A_4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein linked lipoxin A_4 receptor. J Biol Chem. 1997; 272:6972–6978. [PubMed: 9054386]
50. Ariel A, Chiang N, Arita M, Petasis NA, Serhan CN. Aspirin-triggered lipoxin A_4 and B_4 analogs block extracellular signal-regulated kinase-dependent TNF-alpha secretion from human T cells. J Immunol. 2003; 170:6266–6272. [PubMed: 12794159]
51. Sodin-Semrl S, Taddeo B, Tseng D, Varga J, Fiore S. Lipoxin A_4 inhibits IL-1 beta- induced IL-6, IL-8, and matrix metalloproteinase-3 production in human synovial fibroblasts and enhances synthesis of tissue inhibitors of metalloproteinases. J Immunol. 2000; 164:2660–2666. [PubMed: 10679106]
52. Gronert K, Gewirtz A, Madara JL, Serhan CN. Identification of a human enterocyte lipoxin A_4 receptor that is regulated by IL-13 and IFN-gamma and inhibits TNF-alpha-induced IL-8 release. J Exp Med. 1998; 187:1285–1294. [PubMed: 9547339]
53. Bao L, Gerard NP, Eddy RL Jr, Shows TB, Gerard C. Mapping of genes for the human C5a receptor (C5AR), human FMLP receptor (FPR), and two FMLP receptor homologue orphan rceptors (FPRH1, FPRH2) to chromosome 19. Genomics. 1992; 13:437–440. [PubMed: 1612600]
54. Serhan CN, Maddox JF, Petasis NA, Akritopoulou-Zanze I, Papayianni A, Brady HR, Colgan SP, Madara JL. Design of lipoxin A_4 stable analogs that block transmigration and adhesion of human neutrophils. Biochemistry. 1995; 34:14609–14615. [PubMed: 7578068]
55. Serhan CN, Fiore S, Brezinski DA, Lynch S. Lipoxin A_4 metabolism by differentiated HL-60 cells and human monocytes: conversion to novel 15-oxo and dihydro products. Biochemistry. 1993; 32:6313–6319. [PubMed: 8518275]
56. Clish CB, Levy BD, Chiang N, Tai HH, Serhan CN. Oxidoreductases in lipoxin A_4 metabolic inactivation. J Biol Chem. 2000; 275:25372–25380. [PubMed: 10837478]
57. Fierro IM, Colgan SP, Bernasconi G, Petasis NA, Clish CB, Arita M, Serhan CN. Lipoxin A_4 and aspirin-triggered 15-epi-lipoxin A_4 inhibit human neutrophil migration: comparisons between synthetic 15 epimers in chemotaxis and transmigration with microvessel endothelial cells and epithelial cells. J Immunol. 2003; 170:2688–2694. [PubMed: 12594298]
58. Mills, JS.; Miettinen, HM.; Vlases, MJ.; Jesaitis, AJ. The *N*-formyl peptide receptor. In: Serhan, CN.; Ward, PA., editors. Molecular and Cellular Basis of Inflammation. Humana; Totowa, NJ: 1999. p. 215-245.
59. Chiang N, Takano T, Arita M, Watanabe S, Serhan CN. A novel rat lipoxin A_4 receptor that is conserved in structure and function. Br J Pharmacol. 2003; 139:89–98. [PubMed: 12746227]
60. Chiang N, Fierro IM, Gronert K, Serhan CN. Activation of lipoxin A_4 receptors by aspirin-triggered lipoxins and select peptides evokes ligand-specific responses in inflammation. J Exp Med. 2000; 191:1197–1207. [PubMed: 10748237]
61. Olofsson S, Khanna B, Lycke E. Altered kinetic properties of sialyl and galactosyl transferases associated with herpes simplex virus infection of GMK and BHK cells. J Gen Virol. 1980; 47:1–9. [PubMed: 6154121]
62. Villanueva MS, Beckers CJ, Pamer EG. Infection with Listeria monocytogenes impairs sialic acid addition to host cell glycoproteins. J Exp Med. 1994; 180:2137–2145. [PubMed: 7964488]
63. Kim JW, Cunningham JM. N-linked glycosylation of the receptor for murine ecotropic retroviruses is altered in virus-infected cells. J Biol Chem. 1993; 268:16316–16320. [PubMed: 8393858]
64. Kang Y, Taddeo B, Varai G, Varga J, Fiore S. Mutations of serine 235–237 and tyrosine 302 residues in the human lipoxin A_4 receptor intracellular domains result in sustained signaling. Biochemistry. 2000; 39:13551–13557. [PubMed: 11063592]

65. Perretti M, Chiang N, La M, Fierro IM, Marullo S, Getting SJ, Solito E, Serhan CN. Endogenous lipid- and peptide-derived anti-inflammatory pathways generated with glucocorticoid and aspirin treatment activate the lipoxin A_4 receptor. Nat Med. 2002; 8:1296–1302. [PubMed: 12368905]
66. Cash JL, Hart R, Russ A, Dixon JPC, Colledge WH, Doran J, Hendrick AG, Carlton MBL, Greaves DR. Synthetic chemerin-derived peptides suppress inflammation through ChemR23. J Exp Med. 2008; 205:767–775. [PubMed: 18391062]
67. Devchand PR, Arita M, Hong S, Bannenberg G, Moussignac RL, Gronert K, Serhan CN. Human ALX receptor regulates neutrophil recruitment in transgenic mice: Roles in inflammation and host-defense. FASEB J. 2003; 17:652–659. [PubMed: 12665478]
68. Levy BD, De Sanctis GT, Devchand PR, Kim E, Ackerman K, Schmidt BA, Szczeklik W, Drazen JM, Serhan CN. Multi-pronged inhibition of airway hyper-responsiveness and inflammation by lipoxin A_4. Nat Med. 2002; 8:1018–1023. [PubMed: 12172542]
69. Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD. Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. J Immunol. 2005; 174:5033–5039. [PubMed: 15814734]
70. Bürli RW, Xu H, Zou X, Muller K, Golden J, Frohn M, Adlam M, Plant MH, Wong M, McElvain M, Regal K, Viswanadhan VN, Tagari P, Hungate R. Potent hFPRL1 (ALXR) agonists as potential anti-inflammatory agents. Bioorg Med Chem Lett. 2006; 16:3713–3718. [PubMed: 16697190]
71. Frohn M, Xu H, Zou X, Chang C, McElvaine M, Plant MH, Wong M, Tagari P, Hungate R, Bürli RW. New ‘chemical probes’ to examine the role of the hFPRL1 (or ALXR) receptor in inflammation. Bioorg Med Chem Lett. 2007; 17:6633–6637. [PubMed: 17920884]
72. Svensson CI, Zattoni M, Serhan CN. Lipoxins and aspirin-triggered lipoxin stop inflammatory pain processing. J Exp Med. 2007; 204:245–252. [PubMed: 17242163]
73. Gronert K. Lipoxins in the eye and their role in wound healing. Prostaglandins Leukot Essent Fatty Acids. 2005; 73:221–229. [PubMed: 15979295]
74. Menezes-de-Lima O Jr, Kassuya CA, Nascimento AF, Henriques MG, Calixto JB. Lipoxin A_4 inhibits edema in mice: Implications for the anti-edematogenic mechanism induced by aspirin. Prostaglandins Oth Lipid Mediat. 2006; 80:123–135.
75. Morris T, Stables M, Hobbs A, de Souza P, Colville-Nash P, Warner T, Newson J, Bellingan G, Gilroy DW. Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol. 2009; 183:2089–2096. [PubMed: 19597002]
76. Jozsef L, Zouki C, Petasis NA, Serhan CN, Filep JG. Lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 inhibit peroxynitrite formation, NF-kappa B and AP-1 activation, and IL-8 gene expression in human leukocytes. Proc Natl Acad Sci US A. 2002; 99:13266–13271.
77. Bannenberg G, Moussignac RL, Gronert K, Devchand P, Schmidt B, Guilford WJ, Bauman JG, Subramanyam B, Perez HD, Parkinson JF, Serhan CN. Lipoxins and novel 15-epi-lipoxin analogs display potent anti-inflammatory actions after oral administration. Br J Pharmacol. 2004; 143:43–52. [PubMed: 15302682]
78. Damian M, Mary S, Martin A, Pin JP, Banères JL. G protein activation by the leukotriene B_4 receptor dimer: evidence for an absence of trans-activation. J Biol Chem. 2008; 283:21084–21092. [PubMed: 18490452]
79. Winyard, PG.; Willoughby, DA., editors. Inflammation Protocols. Humana; Totowa, NJ: 2003. p. 378
80. Tessier PA, Naccache PH, Clark-Lewis I, Gladue RP, Neote KS, McColl SR. Chemokine networks in vivo: involvement of C-X-C and C-C chemokines in neutrophil extravasation in vivo in response to TNF-α. J Immunol. 1997; 159:3595–3602. [PubMed: 9317159]
81. McMahon B, Mitchell S, Brady HR, Godson C. Lipoxins: revelations on resolution. Trends Pharmacol Sci. 2001; 22:391–395. [PubMed: 11478982]
82. Yokomizo T, Izumi T, Chang K, Takuwa T, Shimizu T. A G-protein-coupled receptor for leukotriene B_4 that mediates chemotaxis. Nature. 1997; 387:620–624. [PubMed: 9177352]
83. Gewirtz AT, Collier-Hyams LS, Young AN, Kucharzik T, Guilford WJ, Parkinson JF, Williams IR, Neish AS, Madara JL. Lipoxin A_4 analogs attenuate induction of intestinal epithelial proinflammatory gene expression and reduce the severity of dextran sodium sulfate-induced colitis. J Immunol. 2002; 168:5260–5267. [PubMed: 11994483]

84. Samson M, Edinger AL, Stordeur P, Rucker J, Verhasselt V, Sharron M, Govaerts C, Mollereau C, Vassart G, Doms RW, Parmentier M. ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. Eur J Immunol. 1998; 28:1689–1700. [PubMed: 9603476]
85. Kopp E, Ghosh S. Inhibition of NF-κB by sodium salicylate and aspirin. Science. 1994; 265:956–959. [PubMed: 8052854]
86. Wittamer V, Franssen J, Vulcano M, Mirjolet J, Le Poul E, Migeotte I, Brezillon S, Tyldesley R, Blanpain C, Detheux M, Mantovani A, Sozzani S, Vassart G, Parmentier M, Communi D. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. J Exp Med. 2003; 198:977–985. [PubMed: 14530373]
87. Methner A, Hermey G, Schinke B, Hermans-Borgmeyer I. A novel G protein-coupled receptor with homology to neuropeptide and chemoattractant receptors expressed during bone development. Biochem Biophys Res Commun. 1997; 233:336–342. [PubMed: 9144535]
88. Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med. 1989; 320:265–271. [PubMed: 2783477]
89. Aliberti J, Hieny S, Reis e Sousa C, Serhan CN, Sher A. Lipoxin-mediated inhibition of IL-12 production by DCs: a mechanism for regulation of microbial immunity. Nat Immunol. 2002; 3:76–82. [PubMed: 11743584]
90. Yokomizo T, Kato K, Terawaki K, Izumi T, Shimizu T. A second leukotriene B4 receptor, BLT2. A new therapeutic target in inflammation and immunological disorders. J Exp Med. 2000; 192:421–432. [PubMed: 10934230]
91. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B_4 receptor BLT1 and ChemR23 to regulate inflammation. J Immunol. 2007; 178:3912–3917. [PubMed: 17339491]
92. Huang L, Zhao A, Wong F, Ayala JM, Struthers M, Ujjainwalla F, Wright SD, Springer MS, Evans J, Cui J. Leukotriene B_4 strongly increases monocyte chemoattractant protein-1 in human monocytes. Arterioscler Thromb Vasc Biol. 2004; 24:1783–1788. [PubMed: 15271789]
93. Chiang N, Gronert K, Clish CB, O'Brien JA, Freeman MW, Serhan CN. Leukotriene B_4 receptor transgenic mice reveal novel protective roles for lipoxins and aspirin-triggered lipoxins in reperfusion. J Clin Invest. 1999; 104:309–316. [PubMed: 10430612]
94. Gelman S. The pathophysiology of aortic cross-clamping and unclamping. Anesthesiology. 1995; 82:1026–1060. [PubMed: 7717537]
95. Qiu FH, Wada K, Stahl GL, Serhan CN. IMP and AMP deaminase in reperfusion injury down-regulates neutrophil recruitment. Proc Natl Acad Sci USA. 2000; 97:4267–4272. [PubMed: 10760293]
96. Arita M, Oh S, Chonan T, Hong S, Elangovan S, Sun YP, Uddin J, Petasis NA, Serhan CN. Metabolic inactivation of resolvin E1 and stabilization of its anti-inflammatory actions. J Biol Chem. 2006; 281:22847–22854. [PubMed: 16757471]
97. Hong S, Porter TF, Lu Y, Oh SF, Pillai PS, Serhan CN. Resolvin E1 metabolome in local inactivation during inflammation-resolution. J Immunol. 2008; 180:3512–3519. [PubMed: 18292578]
98. Jin Y, Arita M, Zhang Q, Saban DR, Chauhan SK, Chiang N, Serhan CN, Dana MR. Novel anti-inflammatory and pro-resolving lipid mediators block inflammatory angiogenesis. Invest Ophthalmol Vis Sci. 2009; 50:4743–4752. [PubMed: 19407006]
99. Merched A, Ko K, Gotlinger KH, Serhan CN, Chan L. Atherosclerosis: Evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. FASEB J. 2008; 22:3595–3606. [PubMed: 18559988]
100. Barish GD, Atkins AR, Downes M, Olson P, Chong LW, Nelson M, Zou Y, Hwang H, Kang H, Curtiss L, Evans RM, Lee CH. PPARdelta regulates multiple proinflammatory pathways to suppress atherosclerosis. Proc Natl Acad Sci US A. 2008; 105:4271–4276.
101. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol. 2008; 153(Suppl 1):S200–S215. [PubMed: 17965751]

102. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, Petasis NA, Serhan CN. Resolvin D1 binds human phagocytes with evidence for pro-resolving receptors. Proc Natl Acad Sci US A. 2010; 107:1660–1665.

103. Naccache PH, Faucher N, Therrien S, Borgeat P. Calcium mobilization, actin polymerization and right-angle light scatter responses to leukotriene B4, 12(R)- and 12(S)-hydroxyeicosatetraenoic acid in human neutrophils. Life Sci. 1988; 42:727–733. [PubMed: 2828801]

104. Perretti M, D'Acquisto F. Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. Nat Rev Immunol. 2009; 9:62–70. [PubMed: 19104500]

105. Shimizu T. Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. Annu Rev Pharmacol Toxicol. 2009; 49:123–150. [PubMed: 18834304]

106. Olson KR, Eglen RM. Beta galactosidase complementation: a cell-based luminescent assay platform for drug discovery. Assay Drug Dev Technol. 2007; 5:137–144. [PubMed: 17355206]

107. Duffield JS, Hong S, Vaidya V, Lu Y, Fredman G, Serhan CN, Bonventre JV. Resolvin D series and protectin D1 mitigate acute kidney injury. J Immunol. 2006; 177:5902–5911. [PubMed: 17056514]

108. Perretti M, Getting SJ, Solito E, Murphy PM, Gao JL. Am J Pathol. 2001; 158:1969. [PubMed: 11395373]

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

110. Ishida T, Yoshida M, Arita M, Nishitani Y, Nishiumi S, Masuda A, Mizuno S, Takagawa T, Morita Y, Kutsumi H, Inokuchi H, Serhan CN, Blumberg RS, Azuma T. Resolvin E1, an endogenous lipid derived from eicosapentaenoic acid, prevents dextran sulfate sodium-induced colitis. Inflamm Bowel Dis. 2010; 16:87–95. [PubMed: 19572372]

111. Dornelles FN, Santos DS, Van Dyke TE, Calixto JB, Batista EL Jr, Campos MM. In vivo up-regulation of kinin B1 receptors after treatment with Porphyromonas gingivalis lipopolysaccharide in rat paw. J Pharmacol Exp Ther. 2009; 330:756–763. [PubMed: 19561153]

112. González-Périz A, Horrillo R, Ferré N, Gronert K, Dong B, Morán-Salvador E, Titos E, Martínez-Clemente M, López-Parra M, Arroyo V, Clària J. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. FASEB J. 2009; 23:1946–1957. [PubMed: 19211925]

113. Haworth O, Cernadas M, Yang R, Serhan CN, Levy BD. Resolvin E1 regulates interleukin 23, interferon-gamma and lipoxin A4 to promote the resolution of allergic airway inflammation. Nat Immunol. 2008; 9:873–879. [PubMed: 18568027]

114. Aoki H, Hisada T, Ishizuka T, Utsugi M, Kawata T, Shimizu Y, Okajima F, Dobashi K, Mori M. Resolvin E1 dampens airway inflammation and hyperresponsiveness in a murine model of asthma. Biochem Biophys Res Commun. 2008; 367:509–515. [PubMed: 18190790]

115. Spite M, Summers L, Porter TF, Srivastava S, Bhatnagar A, Serhan CN. Resolvin D1 controls inflammation initiated by glutathione-lipid conjugates formed during oxidative stress. Br J Pharmacol. 2009; 158:1062–1073. [PubMed: 19422383]

116. Ariel A, Li PL, Wang W, Tang WX, Fredman G, Hong S, Gotlinger KH, Serhan CN. The docosatriene protectin D1 is produced by T_H2 skewing and promotes human T cell apoptosis via lipid raft clustering. J Biol Chem. 2005; 280:43079–43086. [PubMed: 16216871]

117. Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, Israel E, Haley KJ, Serhan CN. Protectin D1 is generated in asthma and dampens airway inflammation and hyper-responsiveness. J Immunol. 2007; 178:496–502. [PubMed: 17182589]

118. Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. J Clin Invest. 2005; 115:2774–2783. [PubMed: 16151530]

119. Hassan IR, Gronert K. Acute changes in dietary omega-3 and omega-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury and formation of

renoprotective docosahexaenoic acid-derived protectin D1. J Immunol. 2009; 182:3223–3232. [PubMed: 19234220]

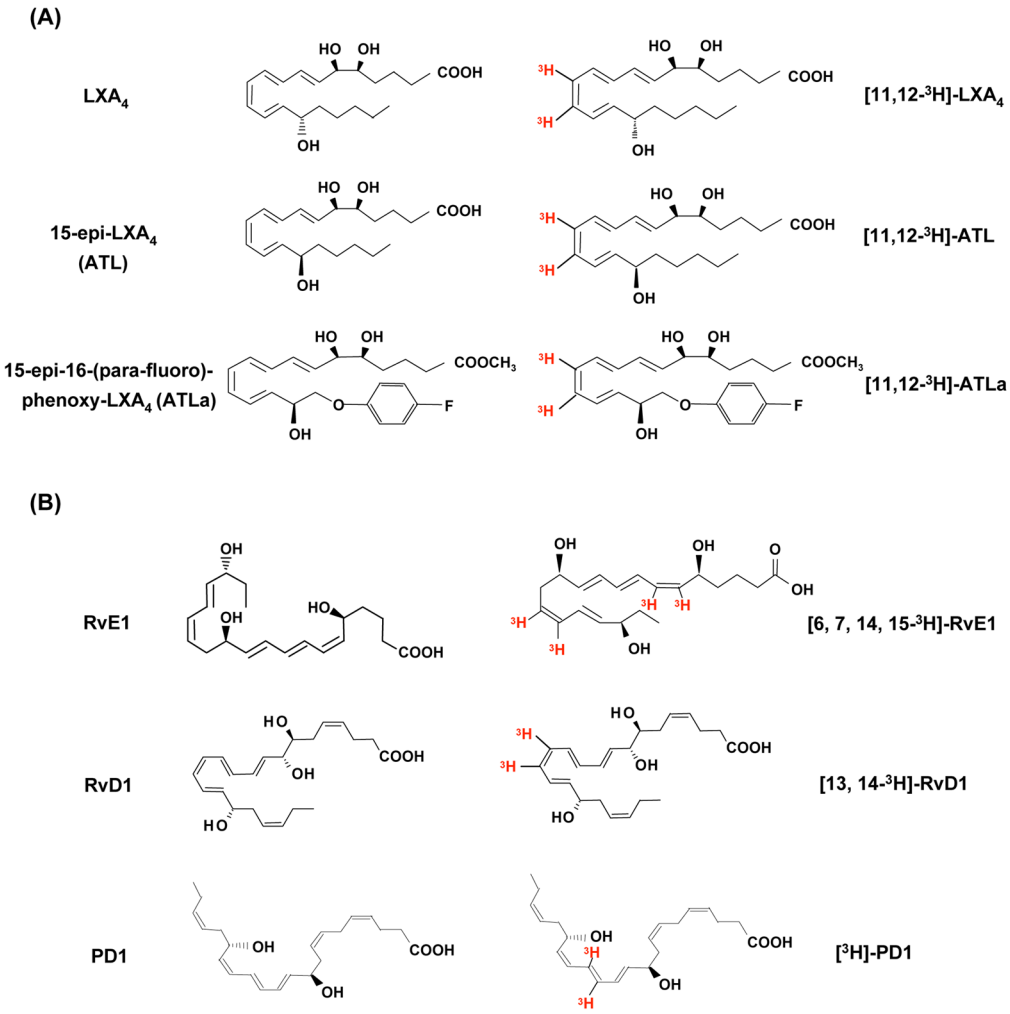

Figure 1.
Structures of LXA_4, ATL, ATLa (A), RvE1, RvD1, PD1 (B) and their tritiated radioligands.

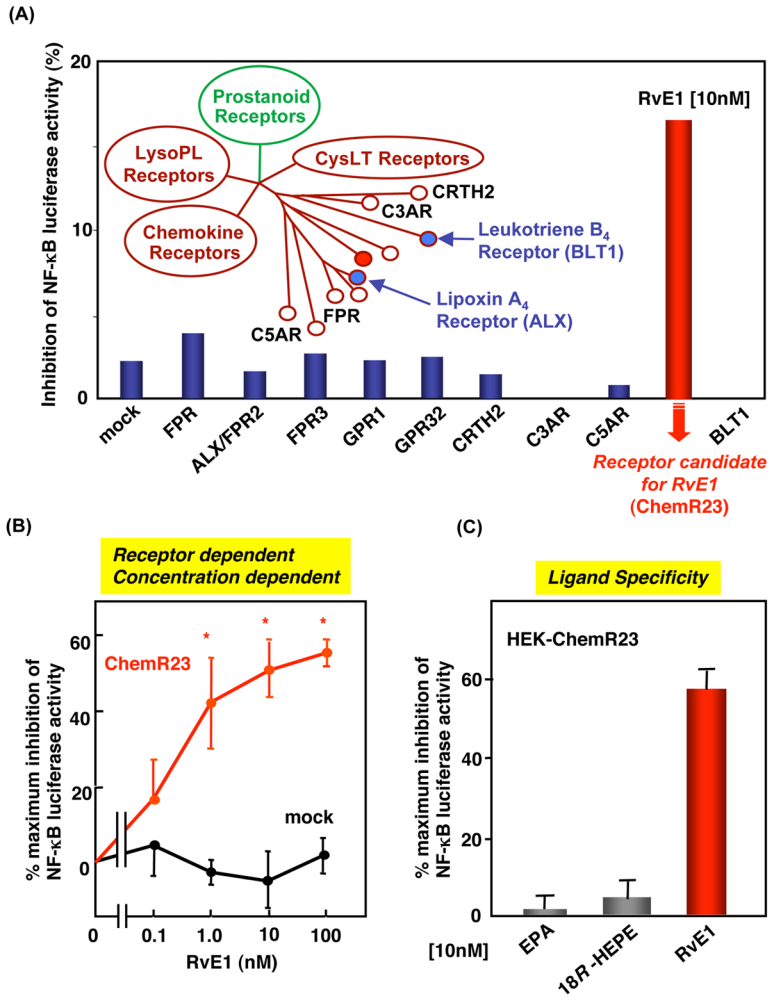

Figure 2.

Identification of RvE1 receptor.

(A) Functional screening for RvE1 receptors. HEK293 cells cotransfected with pNF-κB-luciferase and pcDNA3-GPCRs were exposed to RvE1 (10 nM) and TNF-α (inset) Phylogenetic tree representing amino acid sequence similarities between the human LXA_4 receptor (ALX), leukotriene B4 receptor (BLT1) and related GPCRs.

(B) Receptor dependence. RvE1 inhibits luciferase activity in a concentration-dependent manner on cells transfected with ChemR23 but not mock transfected cells.

(C) Ligand specificity. Cells transfected with pcDNA3-ChemR23 were exposed to 100 nM of each compound. RvE1 showed ~60%, while EPA and 18-HEPE do not have inhibition of NF-κB luciferase activity,

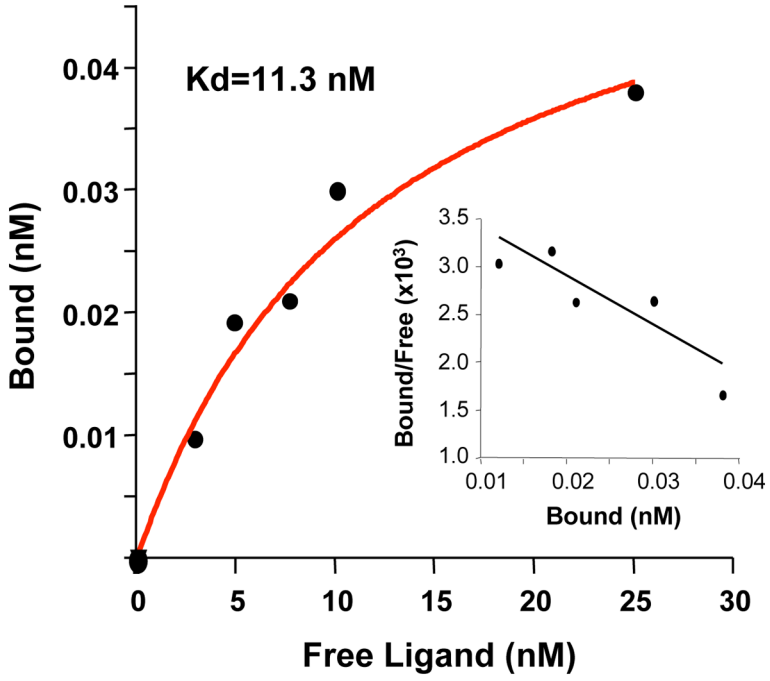

Figure 3.

[^{3}H]-RvE1 binding to ChemR23.

Saturation binding. Human ChemR23–transfected CHO cells (10^6 cells) were incubated with indicated concentrations of [^{3}H]-RvE1 in the presence or absence of 10 μM of unlabeled RvE1. (Inset) Scatchard plot.

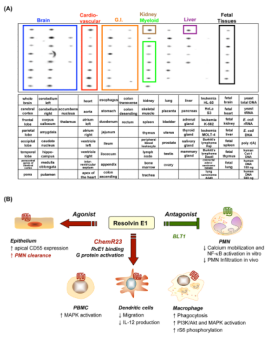

Figure 4.

(A) Tissue distribution of human ChemR23 determined by dot blot hybridization

(B) Mechanisms of action for RvE1. In a ChemR3-dependent manner, RvE1 activates MAPK in monocytes, rS6 phosphorylation in macrophages, and reduces IL-12 production and migration in dendritic cells. RvE1 also induces expression of an anti-adhesive molecule CD55 on the apical surface of mucosal epithelium, promoting clearance of PMN across mucosal surface. In addition, RvE1 directly interact with BLT1 on human PMN, inhibiting calcium mobilization, NF-κB activation in vitro and PMN infiltration in vivo. Therefore RvE1 gave multi-level and cell-type specific actions, serving as an agonist for ChemR23 on mononuclear and dendritic cells as well as an antagonist for BLT1 signals on PMN.

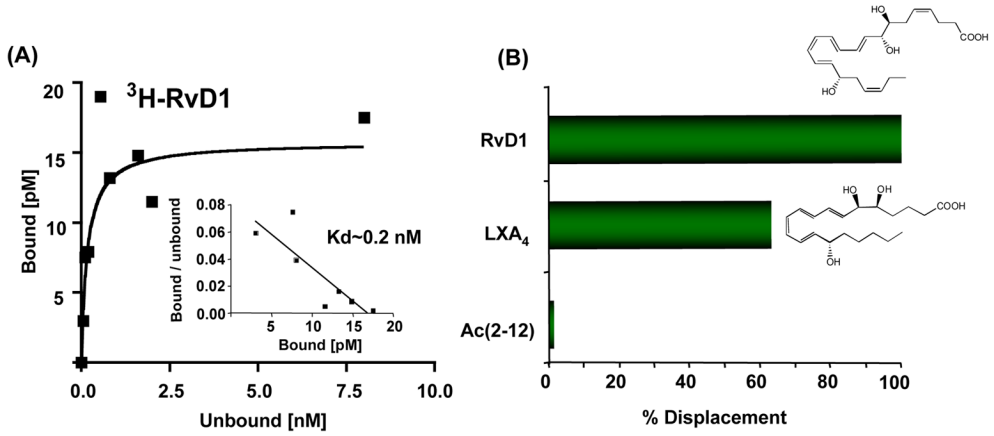

Figure 5.

[^{3}H]-RvD1 binding to human PMN.

(A) Saturation binding. Human PMN (5×10^6 cells) were incubated with indicated concentrations of [^{3}H]-RvD1 in the presence or absence of 10 μM of unlabeled RvD1. (Inset) Scatchard plot.

(B) Competition binding. Human PMN were incubated with 10 nM of [^{3}H]-RvD1 in the presence of 10 μM of LXA$_4$, or Ac2-12 peptide. Results are expressed as percent competition of [^{3}H]-RvD1–specific binding.

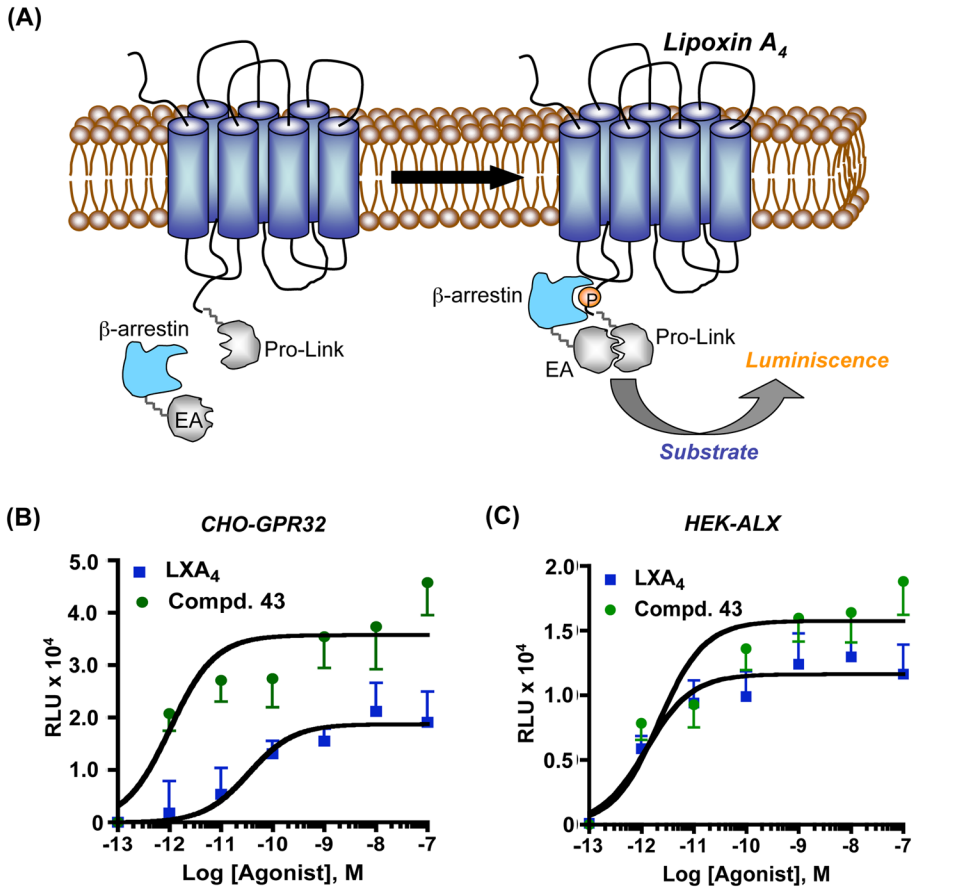

Figure 6.

(A) Ligand-dependent receptor activation for monitoring receptor ligand interactions. This system is engineered by stably co-expressing the target GPCR (ALX or GPR32) tagged with the β-gal Pro-Link peptide with β-arrestin linked to the β-gal EA fragment. In the presence of ligand, activated GPCR interacts with β-arrestin, bringing to proximity the EA and Pro-Link fragments, forming a functional enzyme, whose activity can be measured by adding the substrate and generating a chemiluminescent signal. (B) Dose response activation curves of LXA_4 and compound 43 with β-arrestin cells stably overexpressing GPR32. Results are mean ± SEM (n = 4 – 8). (C) Dose response activation curves of LXA_4 and compound 43 with β-arrestin cells stably overexpressing ALX. Results are mean ± SEM (n = 4 – 7). (RLU – Relative Luminiscence Unit).

Table 1

Resolvins and Protectins in Animal Disease Models*

Disease model	Species	Action(s)	References
Resolvin E1			
Periodontitis	Rabbit	Reduces neutrophil infiltration; prevents connective tissue and bone loss; promotes healing of diseased tissues; regenerates of lost soft tissue and bone	[32]
Peritonitis	Mouse	Stops neutrophil recruitment; regulates chemokine/cytokine production Promotes lymphatic removal of phagocytes	[11, 18, 25]
Dorsal air pouch	Mouse	Stops neutrophil recruitment	[1]
Retinopathy	Mouse	Protects against neovascularization	[28]
Colitis	Mouse	Decreases neutrophil recruitment and proinflammatory gene expression; improves survival; reduces weight loss	[31]
	Mouse	Reduces weight loss, colon shortening and thickening, and PMN infiltration	[110]
Bacterial infection (P. gingivalis)	Rat	Reduces edema formation and PMN infiltration. Reduces kinin B1 receptor and TNF-α expression	[111]
Corneal injury	Mouse	Reduces PMN and MF infiltration. Regulates IL-1β, TNF-α, and VEGF Protects against eye neovascularization	[98]
Insulin resistance and Fatty Liver Disease	Mouse	Protects against hepatic steatosis. Reduces macrophage infiltration in adipose tissue. Increases adiponectin, GLUT-4, IRS-1, and PPAR-γ	[112]
Asthma	Mouse	Reduces airway leukocyte infiltration and expedites airway inflammation resolution. Reduces IL-23, 27, 6, and LTB_4 release. Increases IFN-g and LXA4 Improves lung function	[113]
	Mouse	Reduces airway leukocyte infiltration and expedites airway inflammation. Reduces IgG E and IL-13	[114]
Resolvin D1			
Peritonitis	Mouse	Stops neutrophil recruitment	[19, 21]
Dorsal skin air pouch	Mouse	Stops neutrophil recruitment	[2, 19]
Kidney ischemia-reperfusion	Mouse	Protects from ischemia-reperfusion-induced kidney damage and loss of function; regulates macrophages and protects from fibrosis	[107]
Retinopathy	Mouse	Protects against neovascularization	[28]
Corneal injury	Mouse	Reduces PMN and MF infiltration. Regulates IL-1β, TNF-α, and VEGF Protects against eye neovascularization	[98]
Hind limb ischemia/second organ injury	Mouse	Reduces neutrophil infiltration into the lungs	[27]
Peritonitis	Mouse	Reduces neutrophil infiltration	[115]
Protectin D1			
Peritonitis	Mouse	Stops neutrophil recruitment; regulates chemokine/cytokine production Promotes lymphatic removal of phagocytes Regulates T-cell migration	[11, 18, 25, 116]
Asthma	Mouse	Protects from lung damage, airway inflammation and airway hyperresponsiveness	[117]
	Human	Protectin D1 is generated in humans and appears to be diminished in asthmatics	[117]

Disease model	Species	Action(s)	References
Kidney ischemia-reperfusion	Mouse	Protects from ischemia-reperfusion-induced kidney damage and loss of function; regulates macrophages and is anti-fibrotic	[107]
Retinopathy	Mouse	Protects against neovascularization	[28]
Ischemic stroke	Rat	Stop leukocyte infiltration, inhibits NF-κB and cyclooxygenase-2 induction	[23]
Alzheimer's disease	Human	Diminished protectin D1 production in human Alzheimer's disease	[118]
Insulin resistance and Fatty Liver Disease	Mouse	Increase adiponectin	[112]
Ischemic renal injury	Mouse	Reduces kidney neutrophil infiltration. Increases hemeoxygenase-1 expression	[119]

* The actions of each of the main resolvins and protectins, i.e., RvE1, RvD1 and PD1, were confirmed with compounds prepared by total organic synthesis (see text and cited references for further details).