Pro–resolving lipid mediators are leads for resolution physiology

Charles N. Serhan

Advances in our understanding of the mechanisms that bring about the resolution of acute inflammation have uncovered a new genus of pro–resolving lipid mediators that include the lipoxin, resolvin, protectin and maresin families, collectively called specialized pro–resolving mediators. Synthetic versions of these mediators have potent bioactions when administered in vivo. In animal experiments, the mediators evoke anti–inflammatory and novel pro–resolving mechanisms, and enhance microbial clearance. Although they have been identified in inflammation resolution, specialized pro–resolving mediators are conserved structures that also function in host defence, pain, organ protection and tissue remodelling. This Review covers the mechanisms of specialized pro–resolving mediators and omega–3 essential fatty acid pathways that could help us to understand their physiological functions.

Excessive inflammation is widely appreciated to be a unifying component in many chronic diseases, including vascular diseases, metabolic syndrome and neurological diseases, and thus is a public health concern. Understanding endogenous control points within the inflammatory response could provide us with new perspectives on disease pathogenesis and treatment approaches. Barrier break, trauma and microbial invasion create a need for the host to neutralize invaders, clear the site, and remodel and regenerate tissue. The acute inflammatory response is protective, providing a terrain in which lipid mediators — such as eicosanoids (prostaglandins and leukotrienes) — produced from the essential fatty acid arachidonic acid, as well as many cytokines and chemokines — have crucial roles in the initial response. Interactions among prostaglandins, leukotrienes and pro-inflammatory cytokines amplify inflammation, the signs of which can be reduced by pharmacological inhibition and receptor antagonists. However, given that excessive inflammation contributes to many widely occurring diseases, improvements are needed in treatment and in our understanding of the mechanisms involved.

Pathologists divide the acute inflammatory response into initiation and resolution (Fig. 1). Resolution was considered to be a passive process. With the identification of mediators with pro-resolving capacity that could be biosynthesized from omega-3 (or n-3) essential fatty acids (EFA), evidence emerged that resolution of self-limited acute inflammation might be an active, programmed response that is ‘turned on’ in animal models, and not simply a process of passive dilution of chemoattractants. For a metabolite to fulfil the role of mediator, it must be produced in sufficient amounts in vivo to evoke bioactions. The omega-3 fatty acids EPA and DHA, which are found in marine oils, have long been thought to have anti-inflammatory properties, whereby they compete with arachidonic acid, reducing pro-inflammatory eicosanoids. The molecular mechanism by which this occurs is unclear, and the evidence is inconclusive as to whether omega-3 EPA and DHA are beneficial for human health and as treatments for disease. Resolving inflammatory exudates use omega-3 fatty acids (Fig. 1) to produce structurally distinct families of signalling molecules — resolvins, protectins and maresins, collectively termed specialized pro–resolving mediators (SPMs). This realization prompted new interest in resolution pathways and the innate immune mechanisms for homeostasis. SPMs are agonists with the potential to stimulate key cellular resolution events, namely limiting polymorphonuclear neutrophil infiltration and enhancing macrophage clearance of apoptotic cells, as shown in pre-clinical studies using animal models. This Review addresses the role of novel lipid–derived SPMs in resolution that also function in host defence, pain and tissue regeneration, which could help us to understand the role of SPM pathways in human resolution physiology.

Cellular events in resolution of acute inflammation

During the initiation phase, leukocytes traffic from circulation to the site of trauma or microbial invasion, forming inflammatory exudates — conventionally these are considered to be the ‘battlefields’ where most resolution occurs. The first responders, neutrophils, swarm like bees to defend the body, moving along chemotactic gradients (for example, increasing levels of the leukotriene LTB4) and exiting vessels governed by prostaglandins (such as PGE2 and PGI2) that act on vascular cells and blood flow (Fig. 1). These lipid mediators, along with many cytokines, chemokines and complement components (C5a and C3b), stimulate the chemotaxis of neutrophils into tissues to phagocytize and neutralize invaders. Many therapeutic agents block or antagonize the initiation steps of acute inflammation (for example, prostaglandin biosynthesis inhibitors or chemokine receptor antagonists). At a cellular level, the main events of resolution are the cessation of neutrophil influx and efferocytosis (macrophage clearance of debris, including apoptotic neutrophils). The protective acute inflammatory response evolved to permit the repair of injured tissues and eliminate invading organisms. Ideally, it is a self–limited process, leading to complete resolution that enables a return to homeostasis (Fig. 1). By studying self-limited inflammation in animal models, and using a systems approach to investigate resolving exudates, novel bioactive products derived from essential fatty acids were uncovered. The bioactions of these products include limited neutrophil influx in vivo, reduced human neutrophil transmigration and counter-regulation of cytokines such as tumour necrosis factor–α (TNF–α) in mice. Each bioactive product...
was systematically evaluated, and found to be biosynthesized from EPA and DHA by separate pathways in human neutrophils and macrophages.

**Elucidation of pro-resolving lipid–derived mediators**

The anti-inflammatory process is not equivalent to pro-resolution, which involves SPMs acting as agonists to stop further neutrophil influx and the activation of nonphlogistic responses by macrophages and resolution programs (Fig. 1). The key to understanding this difference was the identification of novel families of autacoids and forms of these molecules that could potentially be triggered by aspirin, providing evidence that, in animal models, resolution is actively orchestrated by lipid mediators (Fig. 2 and Box 1). Harnessing the pathways that stimulate resolution will be a challenge for the future.

Omega-3 fatty acids are a widely taken dietary supplement, but less than 25% of these supplements are prescribed by health-care providers. Given the mixed results on the benefit of these supplements in clinical trials, it is crucial for public health that the mechanisms that underlie their requirement are established.

Using a systems approach with resolving mouse exudates was key to elucidating SPM actions and pathways. Biosynthesis with human leukocytes and complete stereochemistry of each major resolvin (RvE1, RvD1, RvD2, RvD3 and RvD5), protectin and maresin was achieved, and their role in governing neutrophil influx, resolution macrophages and reducing pro-inflammatory mediators (PAF, LTB4 and prostaglandins) seems to be fundamental in all organs.

**Resolvin D3 and the maresin pathway**

Within self-limited exudates, RvD3 has a unique time frame compared with RvD1 and RvD2. In mouse peritonitis, RvD3 appears late in resolution, suggesting it has a specific role. The complete stereochemistry of RvD3 was recently established, confirming its role in anti-inflammatory and pro-resolving actions. Macrophage biosynthesis of maresin Mra1 and its potent pro-resolving and tissue regenerative actions involve an active 13S,14S-epoxide-maresin intermediate, stimulating a macrophage phenotype switch from M1 to M2 (Fig. 2) involving an active 13S,14S-epoxide-maresin intermediate, stimulating a macrophage phenotype switch from M1 to M2 (Fig. 2).

**Resolution agonists and resolution disrupters**

Several lessons have emerged from recent studies. Prostaglandins are central to vascular responses, permitting neutrophils and monocytes to leave post-capillary venules (a process called diapedesis). Their production through COX-1 and COX-2 (also known as prostaglandin G/H synthase 1 and 2) is crucial for initiation and timely resolution. The potent anti-inflammatory and anti-inflammatory responses that depend on tissue location. PG_E2 enhances LTβR-mediated neutrophil extravasation and tissue injury that is blocked, for example, by topical administration of synthetic lipoxin A4 (LXA4) and its aspirin-triggered epimer 15-epi-LXA4 (ref. 28), illustrating both a pro-inflammatory PGE2 signal to adaptive immunity. Liquid chromatography with tandem mass spectrometry (LC–MS–MS)-based profiling demonstrated the temporal switch from an environment with high levels of non-phlogistic monocytes. The resolving macrophages then clear apoptotic neutrophils in a process called efferocytosis (stimulated by resolvins and protectins). After this has taken place normal structure and homeostasis can be restored. Signs of resolution include sequestration of pro-inflammatory cytokines, clearance of neutrophils from epithelial surfaces, phagocytosis of apoptotic neutrophils and removal of inflammatory debris and microbial invaders. Failed resolution can lead to increased levels of prostaglandins and leukotrienes, chronic inflammation (which can be inhibited by resolvins) and fibrosis. SPMs counter-regulate pro-inflammatory chemical mediators, reducing the magnitude and duration of inflammation, and stimulate re-epithelialization, wound healing and tissue regeneration in model organisms. In addition to the release of omega-3 substrates from phospholipid stores, these substrates can enter exudate as a result of oedema from peripheral blood, as shown in mice. SPMs enhance efferocytosis, stimulate signs of resolution and signal to adaptive immunity.

---

**Figure 1 | Lipid mediators in the acute inflammatory response, resolution and other outcomes.** Lipid mediators have pivotal roles in the vascular response and leukocyte trafficking, from initiation to resolution. Lipoxins, resolvins, protectins and maresins, collectively called specialized pro-resolving mediators (SPMs), are produced during this self-limited response (Fig. 2). After barrier break, tissue injury or trauma, eicosanoids are crucial in initiating the cardinal signs of inflammation (redness, heat, pain and swelling). As part of the vascular response, leukocytes traffic to the site of injury. The prostaglandins PGE2 and PGD2 (involved in vasodilation) and the leukotriene LTB4 (involved in chemotaxis and adhesion) stimulate the migration of neutrophils to the tissue. As part of the neutrophil–monocyte sequence, lipid mediator (LM) class switching from eicosanoids to lipoxins signals ‘stop’ and begins the end of the acute inflammatory response. Lipoxins and resolvins stimulate the recruitment of non-phlogistic monocytes. The resolving macrophages then clear apoptotic neutrophils in a process called efferocytosis (stimulated by resolvins and protectins). After this has taken place normal structure and homeostasis can be restored. Signs of resolution include sequestration of pro-inflammatory cytokines, clearance of neutrophils from epithelial surfaces, phagocytosis of apoptotic neutrophils and removal of inflammatory debris and microbial invaders. Failed resolution can lead to increased levels of prostaglandins and leukotrienes, chronic inflammation (which can be inhibited by resolvins) and fibrosis. SPMs counter-regulate pro-inflammatory chemical mediators, reducing the magnitude and duration of inflammation, and stimulate re-epithelialization, wound healing and tissue regeneration in model organisms. In addition to the release of omega-3 substrates from phospholipid stores, these substrates can enter exudate as a result of oedema from peripheral blood, as shown in mice. SPMs enhance efferocytosis, stimulate signs of resolution and signal to adaptive immunity.
of prostaglandins and LTB₄, to one with high levels of lipoxins, a process known as lipid-mediator class switching, in mouse exudates (Fig. 1 and Box 1). PGE₂ or PGD₂ added to isolated human neutrophils increases 15-lipoxygenase type 1 translation from messenger RNA stores in a cyclic-AMP-dependent manner, increasing lipoxin biosynthesis (identified using MS–MS spectra)²⁶.

Inhibition of COX-2 delays resolution because prostaglandins have crucial roles in resolution and because they are initiators of lipid-mediator class switching (Fig. 1), as shown in animal disease models in vivo¹⁶,¹³,¹⁹. When mapping resolution, it became apparent that initiation signals the end of inflammation⁴ and that leukocyte traffic in pus permits prostanoids to signal the biosynthesis of other resolution mediators (Fig. 1 and Fig. 2). For example, disruption of physiological lipid-mediator class switching has deleterious consequences in mouse models of arthritis⁹.

To pinpoint the crucial steps and mechanisms of SPM action within inflammation resolution, it was important to introduce quantitative indices¹⁸,³⁰ that enabled the assessment of resolution in vivo²¹,²⁷,³¹. Resolution indices identified agents that stimulate as well as those that disrupt or delay resolution (resolution interval); for example, COX-2 and lipoxygenase inhibitors¹⁸,²⁷,³². Specific SPMs shorten the resolution interval by limiting neutrophil recruitment and stimulating both macrophage efferocytosis (Fig. 1) and bacterial killing³¹,³³,³⁴, demonstrating the neutrophil–monocyte sequence and the macrophage responses needed for tissue regeneration²³.

Glucocorticoids, specific cyclin-dependent kinase inhibitors, statins, annexin peptides and aspirin all enable resolution¹¹,³⁵,³⁶. In the same way there are many mediators in the initiation of inflammation, there are also many endogenous mediators and drugs that have an impact on resolution¹³,¹⁸,²⁰.

Although both aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) inhibit prostanoid biosynthesis, aspirin is an irreversible inhibitor that acetylates COX-1 and COX-2, whereas other NSAIDs are reversible inhibitors¹². Aspirin acetylation of COX-2 modifies the catalytic domain, blocking prostaglandin biosynthesis¹², but it remains active, producing 15R-HETE from arachidonic acid, 18R-HEPE from EPA and 17R-HDHA from DHA in cells carrying COX-2. These products can be transformed by human neutrophils in vitro to aspirin-triggered lipoxins, aspirin-triggered resolvins⁷,⁸ and aspirin-triggered protectins³⁷. Each has a potent effect, stopping human neutrophil migration and enhancing macrophage clean up, improving resolution in mice. Whether aspirin or statins enhance the production of aspirin-triggered SPMs in humans remains to be established using mass spectral-based identification. And whether aspirin favours resolution in humans, in whom distinct resolution-phenotypes have emerged, is of considerable interest²⁷,³⁸. In mice, intravascular LX₄ is produced by platelet–neutrophil aggregates during ischaemia, which reduces vascular inflammation. Aspirin triggers 15-epi-LXA₄, which was identified using LC–MS–MS, and this was less effective in mice deficient for the lipoxin receptor ALX,
**BOX 1**

**Resolution omega-3 metabolome**

Bioactive products of omega-3 were originally isolated from mouse resolving exudates. The structure of these products was elucidated, and the biosynthesis of each new omega-3 family from EPA and DHA was recapitulated with isolated human neutrophils and macrophages in vitro. Biosynthesis of E-series resolvins is initiated by the molecular oxygen insertion at the carbon-18 position of EPA produced by hypoxic human endothelial cells and acetylated COX-2, or p450. Microbial p450 (ref. 7) (Fig. 2, left) also produces 18-HEPE, which is converted to bioactive E-series members by human neutrophils. Both aspirin-dependent and independent formation occurs in human peripheral blood. The resolution metabolome also activates 17-lipoxygenation of DHA by hydrogen abstraction; 17S-HpDHA is converted to resolvin-epoxide-containing intermediates (Fig. 2, centre) that are transformed to resolvins D1–D4 by human neutrophils, each identified using methods such as mass spectrometry of bioactive products. The 17S-HpDHA intermediate is also a precursor to 16,17-epoxide-protectin, which is converted to protectin D1/neuroprotectin D1 and related structures by human leukocytes (neutrophils and T cells), neural cells and retinal-pigmented epithelial cells. Maresins isolated from human and mouse macrophages are produced by initial lipoxygenation with molecular oxygen insertion at the carbon-14 position to form the hydroperoxide intermediate, which is rapidly converted to 13S,14S-epoxide-maresin and enzymatically converted to maresin-1 (ref. 24) (Fig. 2, right). Lipoxigenase mechanisms involve hydrogen abstraction and molecular oxygen insertion at specific carbon positions that are predominantly in the S configuration. Aspirin, by COX-2 acetylation and p450 enzymes, contributes to the biosynthesis of R-configuration alcohols in lipoxins, resolvins and protectins. The stereochemistry of each bioactive specialized pro-resolving mediator family member (Fig. 2) has been determined, and the biosynthesis and potent pro-resolving and anti-inflammatory actions in murine exudates and human tissues has been confirmed (see ref. 17 for original reports and refs 10, 20 for reviews).

**New mechanisms in local SPM biosynthesis**

Microparticles are membrane-derived vesicles produced by a range of cell types that contribute to human pathologies. Microparticles from self-resolving exudates show anti-inflammatory and pro-resolving capacity in mice. Resolution microparticles enhance efferocytosis and carry pro-resolving signals, including hydroxy-SPM intermediates esterified in phospholipids. Secreted PLA₂ (sPLA₂) prompts the release of pro-resolving signals from microparticles for trans-cellular conversion by human macrophages in vitro. Because nanomedicines are useful for local targeting and delivery, resolution microparticles, and their ability to shorten the resolution interval in mouse peritonitis, were used as a basis for biomimicry to construct humanized nanoparticles containing an LXA₄ analogue, or aspirin-triggered-RvD1 (ref. 40). These nano-pro-resolving medicines (NPRMs), carrying SPMs or SPM analogues, enhance wound healing of human keratinocytes and are protective in a mouse model of temporomandibular joint disease characterized by inflammation-induced bone loss (Fig. 1 and Box 2).

Microparticles can also transfer substrate and intermediates to macrophages during efferocytosis to enhance SPM biosynthesis, which was demonstrated by transfer of deuterium labels from precursors to SPMs (identified using LC–MS–MS). Myeloid cells at different stages have agonist- and phenotype-specific lipid mediator profiles. For example, human neutrophils from healthy peripheral blood predominantly produce LTB₄, whereas apoptotic neutrophils produce PGE₂, LXB₄, and RvE2 signals.

Both M1 and M2 macrophages have specific markers and pathways that are specialized to the functions of that subpopulation in inflammation and resolution. Human M2 macrophages have more of the enzymes needed for cell-type-specific lipid mediators. M2 cells produce SPM profiles with lower levels of LTB, and prostaglandins than M1 cells. Both cell types engulf apoptotic neutrophils, changing their lipid mediator signature profiles. In M2 cells, LTB₄ is downregulated and SPMs are increased, suggesting that M1 and M2 subpopulations produce functional lipid mediator signatures that can affect both physiological and pathophysiological states. In addition, group IID sPLA₂ was identified as a resolving sPLA₂ that was expressed in dendritic cells and macrophages that release substrates with the capacity for producing RvD1 and PGJ₂, as identified by mass spectrometry in mouse lymphoid tissue in vivo. The functional contributions of this resolving sPLA₂ to the inflammatory response in humans is unknown.

The role of eosinophils is well appreciated in parasitic infections and allergic responses. In patients with severe asthma, protectin D1 (PD1) is present in exhaled breath condensates, and is lower in eosinophils from these patients. Human eosinophils produce PD1, which reduces adhesion molecules (CD11b and L-selectin), eotaxin-1 and chemotaxis at nanomolar concentrations, without affecting degradation, superoxide generation or cell survival. Eosinophils also stimulate resolution in mouse peritonitis through SPMs initiated by mouse eosinophils. LC–MS–MS–lipidomics identified LXA₄, RvD5, 17-HDHA and PD1 from eosinophils, and RvE3 in vivo, which limit neutrophil infiltration and regulate macrophages. Hence, by their ability to produce SPMs, eosinophils might contribute to resolution. In support of this, eosinophil depletion has been found to lead to failed resolution, which is rescued by PD1 or eosinophil restoration in mice. Thus, cellular traffic to sites of inflammation has a dynamic impact on lipid mediator signatures and specific SPM metabolomes activated within the local milieu.

**SPM cellular actions in disease models**

SPMs increase survival in diverse mouse models; for example, inflammation and tissue injury in the airway, skin and eye that result from collateral damage are improved with exogenous SPMs. The SPM nanomolar doses required to stop ongoing inflammation and promote resolution rely on G-protein-coupled receptors (GPCRs). Several SPM receptors have been identified using GPCR screening, labelled ligands for specific binding (stereospecific nanomolar dissociation constant) and functional cellular responses. In general, SPMs do not utilize intracellular calcium mobilization in neutrophils for signal transduction but instead activate phosphorylation. RvE1 specifically binds to the receptors ChemR23 (ref. 47) and BLT1 to evoke pro-resolving responses. RvE1 activation of ChemR23 enhances macrophage phagocytosis by phosphoprotein-mediated signalling. RvE1 blocks LTβR binding and promotes, through BLT1, apoptosis of neutrophils for their clearance by macrophages, whereas LTβR–BLT1 signals neutrophil survival. The blocking of survival signals to neutrophils by RvE1 marks an important difference from the process that occurs in the innate response, in which neutrophils must undergo timely apoptosis for clearance (Fig. 2).

RvD1 binds and activates the human receptor GPR32, and binds to both the human and murine forms of the LXA₄ receptor (called ALX/FPR2). Transgenic mice overexpressing human ALX/FPR2 require
B O X 2
Omega-3 pro-resolving mediator structure, function and steps towards human translation

A number of steps have led to the possibility of assessing SPM function in humans (Box 2 Fig.). The molecules derived from omega-3 and their bioactions have been identified in the resolving inflammatory exudates of mice. Studies have reported the structure of these molecules, their actions with human neutrophils and their proposed biosynthesis from EPA and DHA using 18-oxygen, 14-carbon and trapping of transient intermediates. Confirmation of the bioactive structures and total organic synthesis is necessary to assign complete stereochemistry to each specialized pro-resolving mediator (SPM) family member required the development of a strategy to match the biologically active isolated product with those prepared by total organic synthesis. NMR (nuclear magnetic resonance) confirmed the geometry of the conjugated double bond systems present in SPMs; for example, RvE1 (ref. 47), RvD1, RvD2 (ref. 34) or MaR1 (refs 23, 24) (ref. 17 describes the matching criteria). The resolving functions of SPMs are separate from those of anti-inflammatory mediators of PGE2; to serve as an SPM, the omega-3 product must be biosynthesized at the in vivo levels that are commensurate with the SPM’s bioactions. For many of the SPMs, this criterion has been achieved and confirmed with commercial resolvins (see ref. 10 for a review).

SPMs have been identified as conserved structures that are present in trout (PD1 and RvD1)88, salmon (RvD1 and RvD2)117 and planaria (RvE1 and MaR1)23. Microfluidic chambers that permit visualisation of cell–cell interactions between leukocyte subpopulations (human neutrophils and monocytes) and distinguish phagocytic behaviour are ideal for screening SPMs and humanized nano-pro-resolving medicines (NPRMs)40,56. This single-cell screening permits the optimization of enrichment of microparticles with SPMs, the production of NPRMs and the viewing of neutrophil-monocyte interactions56,57, which are essential for appreciating the signals that occur during the neutrophil-monocyte sequence (Fig. 1).

Using microfluidic chambers, single human neutrophils were assayed within about 5 minutes of their capture from whole blood (minimizing the isolation time reduces potential artefacts). Nanomolar concentrations of each SPM prevents human neutrophil migration to interleukin-8 (IL-8), whereas at equimolar doses the precursor (DHA) is not active56,57. Resolution indices permit the quantitative assessment of SPM actions in animal models18,30; this is essential for defining the SPMs within the integrated response of the host to acute inflammatory challenges. Demonstration and identification of omega-3 SPMs in human tissues is required so that we can appreciate their potential roles in humans. RvE1 and RvE2 (refs 47, 61) were identified in the peripheral blood of healthy volunteers, some of whom had been given EPA supplements, using LC–MS–MS multi-reaction monitoring. RvD1, RvD2 (ref. 111), PD1 and 17-HDHA were identified in the exhaled breath condensates of people with asthma42; and additional SPMs were identified in human adipose tissue118; the brains of people with Alzheimer’s disease96,101, and patients with multiple sclerosis11,19 and rheumatoid arthritis10 using mass spectral identification. Although, at present, the demonstration of omega-3 in human tissues is in the initial stages, with the capabilities of LC–MS–MS–based profiling11, SPMs could have potential as markers for nutritional status. The field is now set with tools for assessing SPM function in humans and the relationships of SPMs with nutrition and disease.
immunosuppressive actions of anti-inflammatory drugs3,2. Surprisingly, RvD2 protects mice from caecal ligation puncture (CLP)-induced sepsis5; it has potent actions, enhancing phagocytosis and bacterial killing. In self-limited Escherichia coli infections, resolution programs are activated in mice, and levels of host PD1, RvD1 and RvD1 are elevated4. When SPMs identified in vivo were added back to mouse phagocytes, human macrophages or neutrophils as synthetic SPMs, they enhanced bacterial phagocytosis and killing, as well as clearance33,34,61. Importantly, when the host is given SPMs that are known to act on the host, lower antibiotic doses are needed to clear infections33.

Exogenous LXA4 is also protective in rat CLP-induced sepsis, reducing bacterial burden and pro-inflammatory mediators through a macrophage nuclear-factor-κB (NF-κB)-mediated mechanism, which reduces systemic inflammation62. Aspirin-triggered LXA4 increases E. coli phagocytosis in a phosphatidylinositol-3-OH kinase (PI(3)K)- and scavenger receptor-dependent manner, and ALX/FPR2 is upregulated in patients with Crohn’s disease and enhances bacterial clearance63. Mycobacterium tuberculosis infections are susceptible to modulation of LTA hydrolase. Besides altering LTR, this may also engage resolution programs by activating LTR-, LXA4 production, regulating host responses in zebrafish, mice and possibly in humans34,65. Given the problem of rising antibiotic resistance, activation of resolution programs could provide new antimicrobial approaches to lower our exposure to antibiotics33.

The herpes simplex virus causes oculocutaneous infections that lead to stromal keratitis with viral-initiated immunopathology. RV1 and PD1 are both potent and topically active in mouse models of the infection, reducing pro-inflammatory mediators and stimulating IL-10 (refs 66, 67). Lethal dissemination of the HSV1 virus activated genes in mice that are associated with lipoxin biosynthesis, whereby sustained inflammation inhibited lipoxin-mediated anti-inflammatory host responses, permitting viral dissemination68. HSV1 activates the host resolution-metabolome increasing PD1 levels (identified by LC–MS–MS)69. Host proteomes show antiviral activity, blocking replication of the HSV1 influenza virus. During the time course of infection with the low-pathogenicity strain of influenza H3N2, anti-inflammatory mediators are produced that correlate with resolution and SPM-related pathway markers3. SPMs are also found in yeasts infections; for example, Candida albicans infections, in which RVe1 enhances yeast killing and clearance in mice71. These results support the idea that treating the infected host with host-directed pro-resolving molecules could provide new opportunities to reduce inflammation and enhance clearance by exploiting host–pathogen interactions33,61,72.

Chronic inflammatory disease models

Periodontal disease is a chronic inflammatory disease in which infection leads to neutrophil-mediated tissue injury around the tooth. Activated neutrophils from patients with periodontitis produce PGE2, LTB4, and LXA4, each identified using LC–MS–MS73. PGE2 in this tissue leads to bone loss. P. gingivalis elicits neutrophil recruitment in mouse air pouches along with COX-2 upregulation. LXA4, stable analogues reduced both neutrophil influx and COX-2 expression that was associated with the oral pathogen74. In addition, P. gingivalis increased COX-2 expression in the lung and heart of mice, and 16S ribosomal RNA of oral pathogen74. In murine models of arthritis, RvD1 and 17-HDHA reduce pain and tissue damage, proving more potent than either steroid or pain treatments75.

Unresolved inflammation, epithelial and microvascular injury can lead to excessive fibrosis (Fig. 1) that impairs organ function. Leukotrienes are profibrotic, and in people with scleroderma interstitial lung disease, LXA4 is present in the lungs at levels that seem unable to counter-regulate profibrotic factors76. In animals, an exogenous aspirin-triggered lipoxin analogue reduces pulmonary fibrosis induced by the antibiotic bleomycin77, and both LXA4 and benzo-LXA4 reduce renal fibrosis. Exogenous RVe1 and RVd1 protect from renal fibrosis, reducing collagen I and IV, α-SMA and fibroactin42. In addition, exogenous RVd1 reduces pro-inflammatory mediators that are generated in response to cigarette smoke and pulmonary toxicants43.

Organ regeneration and wound healing

In mice, exogenous SPMs have been found to stimulate wound healing in a range of studies. LXA4 stimulates re-epithelialization of the cornea in a gender-specific fashion in mice78; when applied to wounds, RVe1, RvD1 and RVd2 each stimulate murine dermal healing, reducing neutrophilic infiltration and stimulating re-epithelialization45; RvD1 and RVd2 also stimulate wound healing in diabetic mice51,86. Macrophages have a role in wound healing and organ regeneration, and the macrophage-derived maresin pathway stimulates tissue regeneration. This pathway (Fig. 2) is present in the flatworm Dugesia tigrina, in which RVe1 and MaR1 were each found to reduce regeneration times (speed of head-segment regrowth)79. Given the importance of tissue regeneration in trauma and infection, regulation of resolution programs could hold promise as a therapy.

SPMs in adaptive immunity

Lymphoid tissue such as mouse spleen produces RVd1, 17-HDHA, PD1 (ref. 87) and LXA4 (ref. 88) from endogenous sources, which was identified using mass spectrometry, suggesting these products are strategically positioned to act on lymphocytes (Fig. 1). Both exogenous 17-HDHA and RVd1 increase human B-cell immunoglobulin-M and immunoglobulin-γ, a response that was not shared by PD1. B-cell differentiation is enhanced by exogenous 17-HDHA towards the CD27 ‘CD38’ antibody-secreting cell phenotype80. PD1 is biosynthesized by human T-helper-2-skewed mononuclear cells by a 16(17)-epoxy-protectin intermediate (Fig. 2) and reduces T-cell migration, TNF-α and interferon-γ, promoting T-cell apoptosis81. Exogenous LXA4, RVe1 and PD1 each upregulate expression of the chemokine receptor CCR5 on leukocytes, facilitating their clearance and resolution82. Exogenous PD1 reduces CD4+ T-cell infiltration into cornea83, as does RVe1 (ref. 67), in herpes simplex viral infections. Exogenous RVd1 reduces CD11b+ leukocytes and CD4+ and CD8+ T lymphocytes in uveitis85. Exogenous RVe1 and RVd1 each regulate T-cell activation in choroid and retina, and are biosynthesized in this tissue, as identified by LC–MS–MS86. Exogenous RVe1 induces apoptosis of activated T cells by the induction of 2,3-dioxygenase in dendritic cells, representing a new functional subtype of dendritic cells that have a role in resolution87. Exogenous RVe1 has also been shown to reduce numbers of mouse CD4+ T cells and CD8+ T cells in atopic dermatitis88.

Neuroinflammation and pain

Mouse and human brains have the capacity to produce resolvins and protectins, as do human microglial cells, in which they reduce cytokine expression89,90. And the production of SPMs by the brain cells of trout
indicates that they are conserved from fish to humans. In ischaemic mouse brains, immunoreactive resolvins, protectins and their aspirin-triggered forms are produced; in these mice synthetic SPMs are protective, downregulating excess leukocyte infiltration and reducing local neuronal injury, COX-2 induction, and levels of IL-1β and NF-κB. Thus, in the brain, DHA could be a precursor to neuroprotective signalling pathways evoked by ischaemia reflow tissue injury. The DHA product 10,17-dihydroxy-protection is called neuroprotectin D1 (NPD1) when biosynthesized and activating in neural tissues and retinal epithelial cells owing to its potent actions to reduce neuroinflammation and protect neural cells.

DHA is enriched in the brain, synapses and the retina, in which it is known to have a protective role, but its role as a precursor to mediators in resolution and neuroprotection is still emerging. Synthetic NPD1 has a potent protective role in the nervous system, reducing stress pathways that lead to cell death and increase cell survival, and in several ocular models of important diseases (such as herpes and neovascularization in the eye). NPD1 targets microglia and macrophages. Human neutrophils biosynthesize 17R-NPD1/PD1 that is enhanced by aspirin in vitro, limiting neutrophil transmigration and enhancing macrophage efferocytosis. Synthetic aspirin-triggered NPD1 reduces brain oedema in penumbra and subcortical lesion size, and improves neurological scores.

In the brains of people with Alzheimer’s disease, NPD1 is reduced and the resolution pathway (SPM receptors and products) is diminished. LXA₄ and RvD1 are reduced in cerebrospinal fluid and hippocampus, which correlated with lower scores on the mini-mental state examinations in these patients. These findings provide further evidence that failed resolution of the inflammatory response could contribute to human disease. RvD1 added to macrophages from patients with Alzheimer’s in vitro reduces the macrophages’ pro-inflammatory phenotype and enhances phagocytosis of amyloid-β. This is consistent with the suggestion that resolvins promote clearance of amyloid-β deposition to reduce inflammation in Alzheimer’s. Hence, SPMs might have homeostatic roles in brain and peripheral tissues, whereby each SPM has selective functions to reduce neuroinflammation.

Inflammation can evoke persistent pain. ALX/FPR2 is expressed on spinal astrocytes, and local spinal delivery of synthetic LXA₄, LXB₄ or other lipids that reduce pro-inflammatory mediators can result in cognitive decline, which can occur after major surgery or critical illness and is a major public health concern. Systemic aspirin-triggered RvD1 prophylaxis helps to prevent memory decline in mice that have undergone surgery, protecting them from post-operative neuronal dysfunction. Whether SPM actions in mouse pain models translate to reducing human pain and improving cognition remains of interest.

Towards human translation

Because resolvins and protectins were identified in mouse exudates, it was essential to establish their biosynthesis by human leukocytes and in human tissues. Using mass spectrometry, RvE1 and RvE2 can be identified in human blood, provided EPA or DHA is available. RvD1 and RvD2 are found in human plasma and serum. The capacity of murine placenta to form resolvins (RvD1 and RvD2) and protectins (PD1 and 105,17S-dihDHA) is confirmed by mass spectrometry, and the levels of each were increased by dietary omega-3 (ref. 113). Strategically, SPMs are also present in human breast milk, and RvD1 and RvE1 were identified in milk of mothers in their first month of lactation using mass spectrometry. These identifications, made possible with LC–MS–MS and the availability of SPMs, could provide opportunities for the rigorous assessment of SPM functional roles in human physiology and their potential for therapy.

Evidence for lipid-mediator class switching in humans has recently been reported. LC–MS–MS lipidomic analysis of venous blood collected after strenuous resistance exercise identified lipid mediators in peripheral blood. Prostanoids in the initial post-exercise recovery phase were temporally followed by leukotrienes and p450-derived eicosanoids (EETs), as well as lipoxins, resolvins and protectins. The NSAID ibuprofen, which is widely used for muscle aches and pains, blocked exercise-induced prostanooids, reduced LTB₄ and both delayed and diminished the SPM response, as identified by mass spectrometry. This study of resistance exercise in humans illustrates the acute response of pro-inflammatory mediators, presumably from muscle, and the mediators’ potential link to resolution programs. However, the amount of resolvins and protectins reported are relative, and additional studies are needed to establish their levels in healthy exercising individuals.

In a 60-patient double-blind trial of infants with eczema, topical 15(R/S)-methyl-LXA₄ relieved severity and improved quality of life without apparent adverse events. In these infants, the lipoxin analogue was as effective as a topical steroid.

Looking forward

For autacoids, it is when and where that counts, and it is important to emphasize that the first response in acute inflammation is ubiquitous and mounts throughout the body. SPMs are agonists of resolution. Each stimulates cessation of neutrophil influx, efferocytosis and enhances phagocytosis for microbial containment — signs of resolution (Fig. 1). These steps are the defining SPM functions. Each SPM pathway has additional, non-redundant functions on target cell types. At the cellular and molecular levels, SPMs counter-regulate pro-inflammatory mediators (eicosanoids, chemokines, cytokines and adipokines); regulate specific miRNAs and cell traffic; and enhance microbial killing by receptor-mediated mechanisms in animal models in vivo and with human neutrophils and macrophages.

Results from preclinical disease models suggest that treatment of inflammation-associated disease might be possible with SPM agonists that stimulate resolution and protect organs from collateral damage (Box 2). RvE1, MaR1 and NPD1/PD1 are each in clinical development programs. An RvE1 mimetic is in clinical trials for ocular indications; RvE1, MaR1 and NPD1/PD1 are each in clinical development programs. An RvE1 mimetic is in clinical trials for ocular indications (http://www.auventx.com/auven/products/1x10045.php), and NPD1/PD1 is in clinical development for neurodegenerative diseases (http://www.anidapharma.com/lead-molecule.html); given their ability to be biosynthesized and acting in neural tissues and retinal epithelial cells owing to its potent actions to reduce neuroinflammation and protect neural cells.

Direct comparisons between synthetic LXA₄ and aspirin-triggered RvD1 mechanical hypersensitivity in a rat model of inflammation-induced pain indicate that both effectively reduce hypersensitivity and pro-inflammatory mediators from astrocytes. Local increases in pro-inflammatory mediators can result in cognitive decline, which can occur after major surgery or critical illness and is a major public health concern. Systemic aspirin-triggered RvD1 prophylaxis helps to prevent memory decline in mice that have undergone surgery, protecting them from post-operative neuronal dysfunction. Whether SPM actions in mouse pain models translate to reducing human pain and improving cognition remains of interest.
regulate inflammation resolution without immunosuppression, it is hopeful they will become therapies.

Because identifying SPMs in human tissues relies on LC–MS–MS-based approaches and internal standards that have only recently become available, at this point relatively few studies have demonstrated the presence of SPMs in human tissues (such as blood, milk, and adipose and brain tissue). Hence, evidence for SPM formation in humans is at a very early stage, and their functional importance in human health and diseases remains to be established. Whether SPMs have physiological actions in target tissues in humans, now that we know that they are produced in levels that show potent selectivity actions in animals, can now be addressed. This personalized metabolomics can be used to determine the relationship to resolution mechanisms. It is also important to determine, in healthy individuals, the omega-3 EFA supplementation and doses that might increase or diminish SPMs within specific tissues. Importantly, it is crucial to assess whether human diseases that are characterized by excessive inflammation result from failed resolution mechanisms because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, as could determining the relationship of SPMs to nutrition in humans.

SPMs have emerged from animal models as potential regulators in physiological pathways of resolution and unresolved inflammation that can affect infection, pain, obesity, organ protection, and inflammasomes. The SPMs produced by human apoptotic neutrophils and macrophages can be identified using LC–MS–MS profiling without omega-3 EFA supplementation; it is therefore now possible, with the sensitivity of LC–MS–MS, to determine individual human SPM profiles (personalized lipid mediator–SPM metabolomics). This personalized metabolomics can be used to determine the relationship to resolution mechanisms. It is also important to determine, in healthy individuals, the omega-3 EFA supplementation and doses that might increase or diminish SPMs within specific tissues. Importantly, it is crucial to assess whether human diseases that are characterized by excessive inflammation result from failed resolution mechanisms because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, as could determining the relationship of SPMs to nutrition in humans. New approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflammation is a fundamental process in all organs, new approaches for stimulating resolution and using SPMs as pathway markers could soon be possible, because of the absence of specific SPM pathways and whether these mechanisms can be rescued either by EPA or DHA, or by therapeutic versions of SPM mimetics. Given that resolution of inflation...
This article reports the identification of a secretory phospholipase A2 activated during contact hypersensitive reactions that is specifically involved in the generation of resolvin D1 and protectin D1.

In this article, the authors demonstrate that pro-resolving mediators such as resolvin E1 protect the eye from Herpes simplex virus infection and stimulates clearance of the virus. It also shows that resolvin E1 promotes phagocytosis of bacteria while inhibiting inflammatory cytokine production.

This systematic analysis of tuberculosis in infections in zebrafish identified evidence of a heritable genetic variant that impairs promoter activity.

In this article, the authors demonstrate that 17-HDHA and aspirin-triggered RvD1 can expedite healing of wounds encountered in diabetes.

In this article, the authors demonstrate that resolvin E1 displays anti-hyperalgesic properties in adjuvant-induced arthritis in rats.

In this article, the authors demonstrate that aspirin-triggered RvD1 has potent protective actions in adjuvant-induced arthritis and reduce pain in this model of arthritis.

This article reports that resolvins E1 and D1 inhibit intestinal fibrosis in the obstructed kidney via local fibroblast proliferation. It also shows that resolvins E1 and D1 in acute cigarette smoke-induced lung inflammation.

In this study, the authors demonstrate that 17-HDHA and aspirin-triggered RvD1 can expedite healing of wounds encountered in diabetes.

In this study, the authors demonstrate that aspirin-triggered RvD1 has potent protective actions in adjuvant-induced arthritis and reduce pain in this model of arthritis.
This article reports an exciting discovery demonstrating that RvD1 counter-regulates pro-inflammatory mediators produced during surgery-induced cognitive decline.

This article reports exciting new results indicating that strenuous exercise activates acute inflammation and pro-inflammatory eicosanoids, which then transition in humans to pro-resolving mediators present in peripheral blood following a time course consistent with lipid-mediator class switching and resolution of exercise-induced muscle stress or inflammation.

This article reports an important contribution demonstrating that resolvins and protectins are present in placenta and that their levels can be substantially increased with dietary supplementation.

This article is an important contribution demonstrating that resolvins and protectins are present in placenta and that their levels can be substantially increased with dietary supplementation.

This article reports new results indicating that strenuous exercise activates acute inflammation and pro-inflammatory eicosanoids, which then transition in humans to pro-resolving mediators present in peripheral blood following a time course consistent with lipid-mediator class switching and resolution of exercise-induced muscle stress or inflammation.

This article reports a paediatric clinical trial that was the first to demonstrate that the topical addition of an aspirin-triggered LXA4 stable analogue is safe and effective in reducing infantile eczema.

This article is the first description of NPD1 action in regulating neovascularization by targeting microglia.