REVIEW

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Pro–resolving lipid mediators are leads for resolution physiology

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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)[1,2] produced from the essential fatty acid arachidonic acid, as well as many cytokines and chemokines[3–5] — 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[1–3]. 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[6]. 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[7,8]. 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[9]. 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[5,6], as shown in pre-clinical studies using animal models[10]. 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[6]. The first responders, neutrophils, swarm like bees to defend the body, moving along chemotaxic gradients (for example, increasing levels of the leukotriene LTB_4)[11], and exiting venules governed by prostaglandins (such as PGE_2 and PGI_2) that act on vascular cells and blood flow[1] (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[3–6]. Many therapeutic agents block or antagonize the initiation steps of acute inflammation (for example, prostaglandin biosynthesis inhibitors or chemokine receptor antagonists)[1,3,12]. At a cellular level, the main events of resolution are the cessation of neutrophil influx and efferocytosis (macrophage clearance of debris, including apoptotic neutrophils)[4,5]. 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

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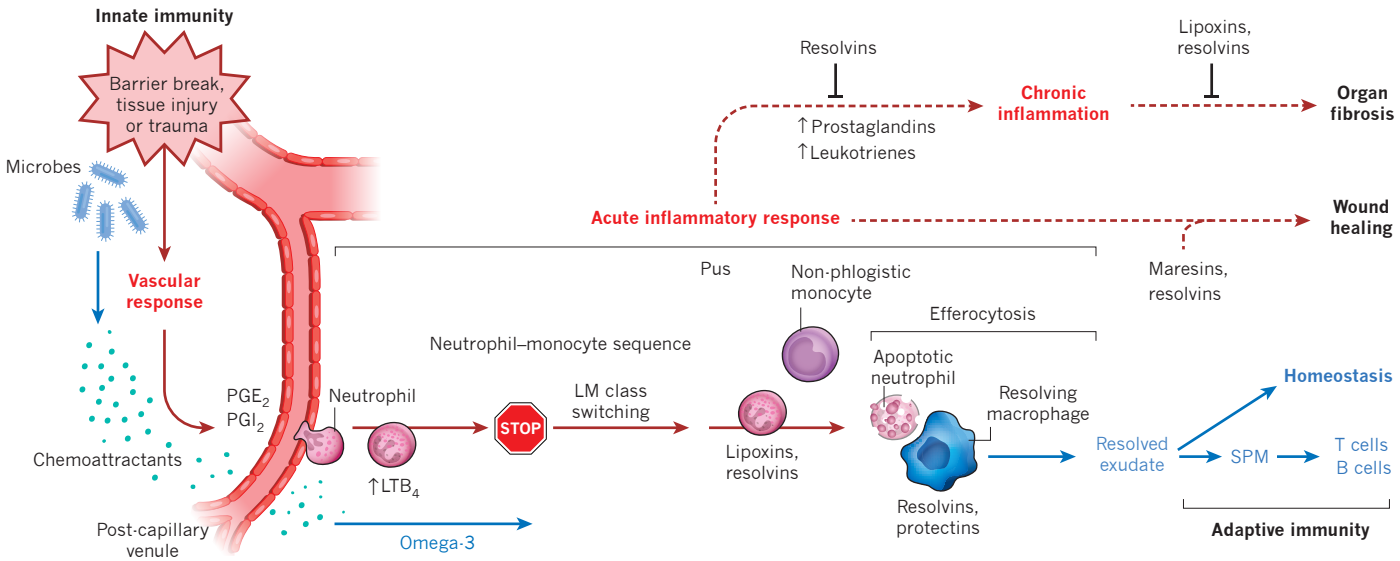


Figure 1 | Lipid mediators in the acute inflammatory response, resolution and other outcomes. Lipid mediators have pivotal roles in the vascular response and leukocyte trafficking, from initiation to resolution. Lipoxins, resolvins, protectins and maresins, collectively called specialized pro-resolving 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 PGE_2 and PGI_2 (involved in vasodilation) and the leukotriene LTB_4 (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[95], these substrates can enter exudate as a result of oedema from peripheral blood, as shown in mice[57]. SPMs enhance efferocytosis, stimulate signs of resolution and signal to adaptive immunity.

was systematically evaluated, and found to be biosynthesized from EPA and DHA by separate pathways in human neutrophils and macrophages *in vitro*[7,8,13].

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[14].

Omega-3 fatty acids are a widely taken dietary supplement, but less than 25% of these supplements are prescribed by health-care providers[15]. Given the mixed results[16] 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[4,8]. Biosynthesis with human leukocytes and complete stereochemistry of each major resolvin (RvE1, RvD1, RvD2, RvD3 and RvD5), protectin and maresin was accomplished by matching these mediators with those prepared by total organic synthesis, which also confirmed their potent bioactivity[10,17]. Resolvins and protectins control the magnitude and duration of inflammation in animal disease models[18], and can, for example, increase animal survival[10] (Fig. 2). The potent *in vivo* actions of RvD1 and RvD2 are reported in many pathologies, such as obesity and pathologies affecting the vascular[19], airway[20], dermal, renal and ocular systems, and in processes including pain, fibrosis and wound healing[10,21]. Their role in governing neutrophil influx, resolution macrophages and reducing pro-inflammatory mediators (PAF, LTB_4 and prostaglandins) seem 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[22], confirming its potent anti-inflammatory and pro-resolving actions[8]. Macrophage biosynthesis of maresin MaR1 and its potent pro-resolving and tissue regenerative actions[23] (Fig. 2) involve an active 13S,14S-epoxide-maresin intermediate, stimulating a macrophage phenotype switch from M1 to M2 (ref. 24) (Fig. 2). The switch towards the M2 phenotype is associated with reparative and anti-inflammatory macrophage functions[5,25].

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[26,27] (Fig. 1). PGE_2 and PGD_2 each evoke pro-inflammatory and anti-inflammatory responses that depend on tissue location[12]. PGE_2 enhances LTB_4-mediated neutrophil extravasation and tissue injury that is blocked, for example, by topical administration of synthetic lipoxin A_4 (LXA_4) and its aspirin-triggered epimer 15-epi-LXA_4 (ref. 28), illustrating both a pro-inflammatory PGE_2 function in mouse skin and the ability of 15-epi-LXA_4 mimetics to limit neutrophil infiltration and tissue injury. Liquid chromatography with tandem mass spectrometry (LC–MS–MS)-based profiling demonstrated the temporal switch from an environment with high levels

of prostaglandins and LTB_4 to one with high levels of lipoxins, a process known as lipid-mediator class switching, in mouse exudates (Fig. 1 and Box 1). PGE_2 or PGD_2 added to isolated human neutrophils increases 15-lipoxygenase type I 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 vivo*[18,26,29]. When mapping resolution, it became apparent that initiation signals the end of inflammation[4] 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 arthritis[29].

To pinpoint the crucial steps and mechanisms of SPM action within inflammation resolution, it was important to introduce quantitative indices[18,30] that enabled the assessment of resolution *in vivo*[21,27,31]. Resolution indices identified agents that stimulate as well as those that disrupt or delay resolution (resolution interval); for example, COX-2 and lipoxygenase inhibitors[18,27,32]. Specific SPMs shorten the resolution interval by limiting neutrophil recruitment and stimulating both macrophage efferocytosis (Fig. 1) and bacterial killing[31,33,34], demonstrating the neutrophil–monocyte sequence and the macrophage responses needed for tissue regeneration[23]. Glucocorticoids, specific cyclin-dependent kinase inhibitors, statins, annexin peptides and aspirin all enable resolution[31,35,36]. 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 resolution[5,18,20].

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 inhibitors[1,2]. Aspirin acetylation of COX-2 modifies the catalytic domain, blocking prostaglandin biosynthesis[1,2], 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 resolvins[7,8] and aspirin-triggered protectins[37]. 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 interest[27,38]. In mice, intravascular LXA_4 is produced by platelet–neutrophil aggregates during ischaemia, which reduces vascular inflammation. Aspirin triggers 15-epi-LXA_4, which was identified using LC–MS–MS, and this was less effective in mice deficient for the lipoxin receptor ALX,

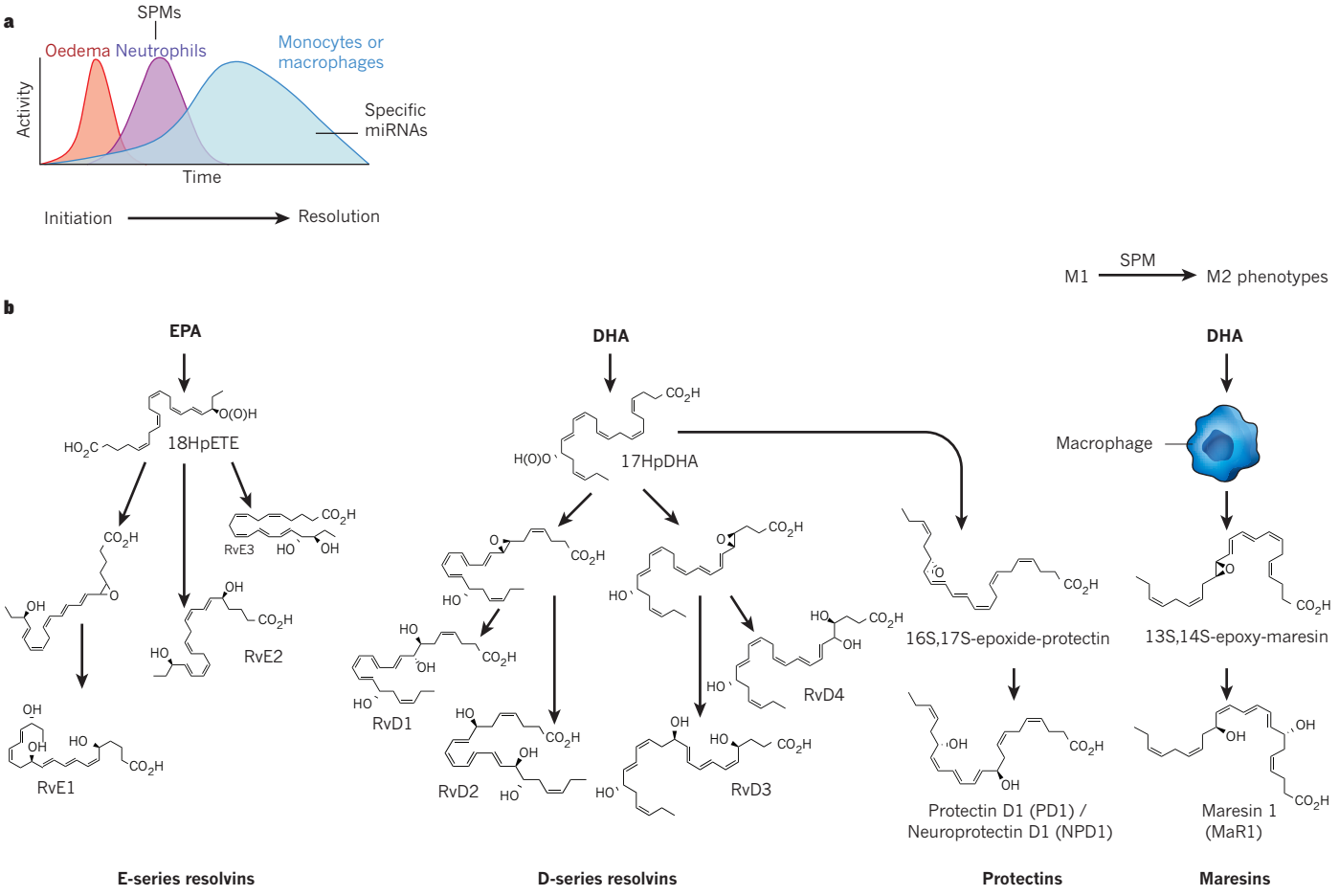


Figure 2 | Production of specialized pro-resolving mediators in resolving inflammatory exudates. **a**, A typical self-limited acute inflammatory response time course in experimental settings from initiation (time 0) to resolution: oedema; neutrophilic infiltration, including the temporal biosynthesis of specialized pro-resolving mediators (SPMs) that occurs in resolving exudates; and non-phlogistic recruitment of monocytes or macrophages, which is required for homeostasis, and the repair and regeneration of injured tissues (involving specific microRNAs regulated by SPM receptors). **b**, Resolution omega-3 metabolome. Biosynthesis of resolvins, protectins and maresins from EPA and DHA with the main bioactive structures from each family (Box 1; see refs 17 and 20 for details of the biosynthetic mechanisms and stereochemical assignments of the bioactive products). Each SPM stimulates macrophage switching to the M2 phenotype and is produced by human neutrophils, apoptotic neutrophils and macrophages[13].

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[8,20] and macrophages *in vitro*[23,24]. 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[47,61]. 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[8,97,98,112]. 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). Lipoxygenase 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[37]. 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).

providing *in vivo* evidence that aspirin can jump-start resolution circuits in mice[39].

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[40] in mice. Resolution microparticles enhance efferocytosis[13,40] and carry pro-resolving signals, including hydroxy-SPM-intermediates esterified in phospholipids[40]. Secreted PLA_2 ($sPLA_2$) prompts the release of pro-resolving signals from microparticles for trans-cellular conversion by human macrophages *in vitro*[13,40]. 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 LXA_4 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[40] (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)[13]. Myeloid cells at different stages have agonist- and phenotype-specific lipid mediator profiles. For example, human neutrophils from healthy peripheral blood predominantly produce LTB_4, whereas apoptotic neutrophils produce PGE_2, LXB_4 and RvE2 signals[13].

Both M1 and M2 macrophages have specific markers and pathways that are specialized to the functions of that subpopulation in inflammation and resolution[25]. Human M2 macrophages have more of the enzymes[41] needed for cell-type-specific lipid mediators. M2 cells produce SPM profiles with lower levels of LTB_4 and prostaglandins than M1 cells. Both cell types engulf apoptotic neutrophils, changing their lipid mediator signature profiles. In M2 cells, LTB_4 is downregulated and SPMs are increased[13], suggesting that M1 and M2 subpopulations[25,41] produce functional lipid mediator signatures that can affect both physiological and pathophysiological states[13]. In addition, group IID $sPLA_2$ was identified as a resolving $sPLA_2$ that was expressed in dendritic cells and macrophages that release substrates with the capacity for producing RvD1 and PGJ_2, as identified by mass spectrometry in mouse lymphoid tissue *in vivo*[42]. The functional contributions of this resolving $sPLA_2$ 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[43], and is lower in eosinophils from these patients[44]. Human eosinophils produce PD1, which reduces adhesion molecules (CD11b and L-selectin), eotaxin-1 and chemotaxis at nanomolar concentrations, without affecting degranulation, superoxide generation or cell survival. Eosinophils also stimulate resolution in mouse peritonitis through SPMs initiated by mouse eosinophils[45]. LC–MS–MS-lipidomics identified LXA_4, RvD5, 17-HDHA and PD1 from eosinophils, and RvE3 *in vivo* (Fig. 2), which limit neutrophil infiltration and regulate macrophages[44–46]. 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[10]. 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[47–49]. In general, SPMs do not utilize intracellular calcium (Ca^{2+}) 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[48]. RvE1 blocks LTB_4 binding and promotes, through BLT1, apoptosis of neutrophils for their clearance by macrophages[49], whereas LTB_4–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[5,49] (Fig. 2).

RvD1 binds and activates the human receptor GPR32, and binds to both the human and murine forms of the LXA_4 receptor (called ALX/FPR2). Transgenic mice overexpressing human ALX/FPR2 require

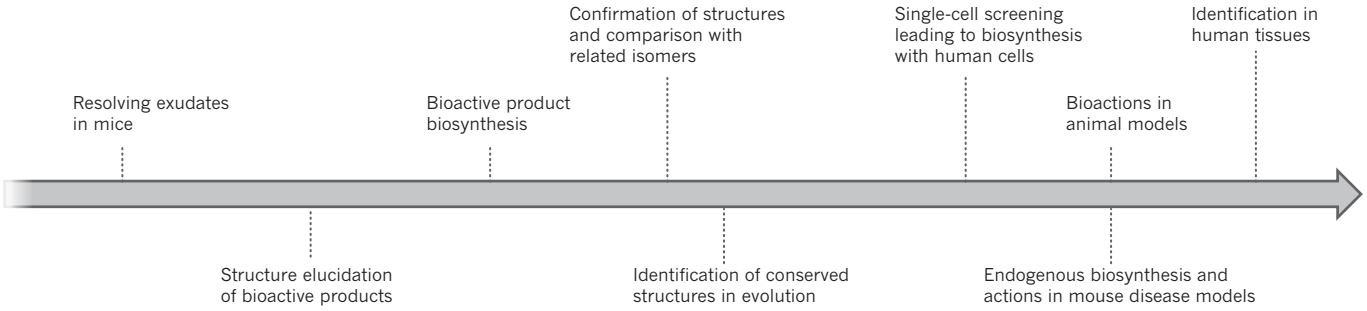
BOX 2

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[17]. Confirmation of the bioactive structures and total organic synthesis 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-inflammatories; 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 visualization of cell–cell interactions between leukocyte subpopulations (human neutrophils and monocytes) and distinguish phlogistic compared with non-phlogistic phagocyte 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 interactions[56,57], 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 active[56,57]. Resolution indices permit the quantitative assessment of SPM actions in animal models[18,30]; 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 asthma[43]; and additional SPMs were identified in human adipose tissue[118], the brains of people with Alzheimer's disease[96,101], and patients with multiple sclerosis[119] and rheumatoid arthritis[120] 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 profiling[13], 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.



less RvD1 than wild-type mice to stop inflammation[50], and in receptor-deficient mice, RvD1 seems to have no leukocyte-directed actions[51]. Resolution involves specific microRNAs (miRNAs) regulated by SPM receptors[50,52,53]. RvD1–GPR32 upregulates miR-208 and anti-inflammatory interleukin-10 (IL-10), and downregulates miR-219, decreasing LTB_4 levels through the regulation of 5-lipoxygenase[52]. This miRNA regulation is an example of SPM signalling with a sustained tissue impact.

SPM receptors are also rapid signallers. For example, recombinant RvD1–GPR32 blocks histamine H1 receptor-stimulated increases in intracellular Ca^{2+} in Chinese hamster ovary (CHO) cells by rapidly stimulating the phosphorylation of the H1 receptor, stopping Ca^{2+} mobilization[54]. This form of SPM signalling, which has been reported in conjunctival goblet cells and RvD1, is also functional in salivary glands[55] and is probably relevant in human neutrophils, which rapidly stop chemotaxis in microfluidic chambers and change shape on exposure to SPMs[56,57]. RvD3 and RvD5 can also activate recombinant GPR32 (refs 22, 33). Given the temporal production of RvD3 *in vivo*[22] (Fig. 2), these findings underscore that locally produced SPMs can affect different cell types and receptors with a spatio-temporal dependency.

In addition to RvD1 and LXA_4, ALX/FPR2 is activated by peptide pro-resolving mediators, such as annexin A1, as well as high concentrations of pro-inflammatory peptides[36]. This capacity of ALX/FPR2 involves ligand-biased receptor activation and heterodimerization of ALX/FPR2, with related FPRs dictating pro-inflammatory signalling and homodimerization of ALX/FPR2 determining pro-resolving signalling[36]. LXA_4 also enhances the activity of the ALX/FPR2 promoter, which is mutated in human cardiovascular disease[58].

Infection and resolution programs

Topical RvE1 and LXA_4 each reduce the severity of periodontal disease in rabbits by enhancing clearance of the causative organism *Porphyromonas gingivalis*[59,60]. Although the anti-inflammatory actions of SPMs were uncovered in sterile inflammation models[7,8], the relationship between resolution and infection is of interest because of the known eventual

immunosuppressive actions of anti-inflammatory drugs[12]. Surprisingly, RvD2 protects mice from caecal ligation puncture (CLP)-induced sepsis[34]; 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, RvD5 and RvD1 are elevated[33]. 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 clearance[33,34,61]. Importantly, when the host is given SPMs that are known to act on the host, lower antibiotic doses are needed to clear infections[33].

Exogenous LXA_4 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 inflammation[62]. Aspirin-triggered-LXA_4 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 clearance[63]. *Mycobacterium tuberculosis* infections are susceptible to modulation of LTA_4 hydrolase. Besides altering LTB_4, this may also engage resolution programs by activating LTB_4–LXA_4 production, regulating host responses in zebrafish, mice and possibly in humans[64,65]. Given the problem of rising antibiotic resistance, activation of resolution programs could provide new antimicrobial approaches to lower our exposure to antibiotics[33].

The herpes simplex virus causes ocular infections that lead to stromal keratitis with viral-initiated immunopathology. RvE1 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 H5N1 virus activated genes in mice that are associated with lipoxin biosynthesis, whereby sustained inflammation inhibited lipoxin-mediated anti-inflammatory host responses, permitting viral dissemination[68]. H5N1 activates the host resolution-metabolome increasing PD1 levels (identified by LC–MS–MS)[69]. Host protectins show antiviral activity, blocking replication of the H5N1 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 markers[70]. SPMs are also found in yeast infections; for example, *Candida albicans* infections, in which RvE1 enhances yeast killing and clearance in mice[71]. 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 interactions[33,61,72].

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 PGE_2, LTB_4 and LXA_4, each identified using LC–MS–MS[73]. PGE_2 in this tissue leads to bone loss. *P. gingivalis* elicits neutrophil recruitment in mouse air pouches along with COX-2 upregulation. LXA_4 stable analogues reduced both neutrophil influx and COX-2 expression that was associated with the oral pathogen[73]. In addition, *P. gingivalis* increased COX-2 expression in the lung and heart of mice, and 16S ribosomal RNA of *P. gingivalis* was present in these tissues, providing evidence of a role for this oral pathogen in the development of systemic inflammation. Transgenic rabbits overexpressing human 15-lipoxygenase type I produce 6–10 times the amounts of LXA_4, which was identified using LC–MS–MS, than wild-type-rabbits[59]; transgenic rabbits have less bone loss in periodontitis and markedly reduced neutrophil recruitment and vascular leakage through their skin on immune challenge compared with wild-type rabbits, suggesting that overexpression of lipoxin biosynthesis is protective and could be useful in controlling inflammation-mediated bone destruction[59].

Unexpectedly, overexpression of 15-lipoxygenase in these transgenic rabbits sharply reduces atherosclerotic lesions[74]. In 12/15-lipoxygenase transgenic mice, LC–MS–MS identified RvD1, PD1 and 17-HDHA with reduced PGE_2 from activated macrophages[75]. LXA_4, PD1 and RvD1 each reduced cytokines (for example, MCP-1) from endothelial cells and adhesion molecules (P-selectin and VCAM-1) but not ICAM-1. LXA_4, PD1 and RvD1 also enhanced uptake of apoptotic thymocytes, which could contribute to the anti-atherogenic role of this pathway in mice. This process can also be manipulated by diet to govern severity of atherogenic lesions[75,76]. RvE1 is protective in periodontal disease but, unlike LXA_4, exogenous RvE1 also stimulates bone regeneration in rabbit models of periodontitis[60,77]. In murine models of arthritis, RvD1 and 17-HDHA reduce pain and tissue damage, proving more potent than either steroid or pain treatments[78].

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, LXA_4 is present in the lungs at levels that seem unable to counter-regulate profibrotic factors[79]. In animals, an exogenous aspirin-triggered lipoxin analogue reduces pulmonary fibrosis induced by the antibiotic bleomycin[80], and both LXA_4 and benzo-LXA_4 reduce renal fibrosis[81]. Exogenous RvE1 and RvD1 protect from renal fibrosis, reducing collagen I and IV, α-SMA and fibronectin[82]. In addition, exogenous RvD1 reduces pro-inflammatory mediators that are generated in response to cigarette smoke and pulmonary toxicants[83].

Organ regeneration and wound healing

In mice, exogenous SPMs have been found to stimulate would healing in a range of studies. LXA_4 stimulates re-epithelialization of the cornea in a gender-specific fashion in mice[84]; when applied to wounds, RvE1, RvD1 and RvD2 each stimulate murine dermal healing, reducing neutrophilic infiltration and stimulating re-epithelialization[85]; RvD1 and RvD2 also stimulate wound healing in diabetic mice[21,86]. 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)[23]. 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 LXA_4 (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 phenotype[87]. 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 apoptosis[89]. Exogenous LXA_4, RvE1 and PD1 each upregulate expression of the chemokine receptor CCR5 on leukocytes, facilitating their clearance and resolution[90]. Exogenous PD1 reduces $CD4^+$ T-cell infiltration into cornea[66], as does RvE1 (ref. 67), in herpes simplex viral infections. Exogenous RvD1 reduces $CD11b^+$ leukocytes and $CD4^+$ and $CD8^+$ T lymphocytes in uveitis[91]. Exogenous RvE1 and RvD1 each regulate T-cell activation in choroid and retina, and are biosynthesized in this tissue, as identified by LC–MS–MS[92]. 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 resolution[93]. Exogenous RvE1 has also been shown to reduce numbers of mouse $CD4^+$ T cells and $CD8^+$ T cells in atopic dermatitis[94].

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 expression[8,95,96]. And the production of SPMs by the brain cells of trout

indicates that they are conserved from fish to humans[88]. In ischaemic mouse brains, immunoreactive resolvins, protectins and their aspirin-triggered forms are produced[97]; 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-protectin is called neuroprotectin D1 (NPD1) when biosynthesized and acting in neural tissues and retinal epithelial cells owing to its potent actions to reduce neuroinflammation and protect neural cells[98].

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[66] and neovascularization in the eye[99]) NPD1 targets microglia[8,98,99]. Human neutrophils biosynthesize 17R-NPD1/PD1 that is enhanced by aspirin *in vitro*, limiting neutrophil transmigration and enhancing macrophage efferocytosis[37]. Synthetic aspirin-triggered NPD1 reduces brain oedema in penumbra and subcortical lesion size, and improves neurological scores[100].

In the brains of people with Alzheimer's disease, NPD1 is reduced[96] and the resolution pathway (SPM receptors and products) is diminished[101]. LXA_4 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[101]. RvD1 added to macrophages from patients with Alzheimer's *in vitro* reduces the macrophages' pro-inflammatory phenotype and enhances phagocytosis of amyloid-β[102]. 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 LXA_4, LXB_4 or their metabolically stable analogues reduces inflammation-induced pain[103]. Many SPMs dampen pain; in mice, they have specific targets of action[104], as demonstrated with RvE1 and RvD1 for inflammatory pain involving both central and peripheral sites[105]. RvE1 administered intrathecally in mice is more potently analgesic than morphine or a COX-2 inhibitor. The RvE1 receptor ChemR23 is present in dorsal root ganglia, in which synthetic RvE1 regulates phosphorylation of ERK-dependent transient receptor potential vanilloid subtype-1 (TRPV1) inhibition and TNF-α-mediated hyperalgesia, centrally. In postsynaptic neurons, synthetic RvE1 inhibits glutamate and TNF-α stimulation of *N*-methyl-D-aspartic acid (NMDA) receptor and mechanical allodynia[105]. Synthetic RvD1, RvD2 and PD1/NPD1 each reduce pain by the inhibition of specific TRPV channels[106,107].

MaR1 inhibits TRPV1 in neurons and blocks capsaicin-induced inward current (the half-maximum inhibitory concentration is 0.49 nM), diminishing inflammatory and chemotherapy-evoked neuropathic pain in mice[24]. Both aspirin-triggered RvD1 and 17R-HDHA reduce adjuvant-induced arthritis in rats and associated pain[78], reduce NF-κB and COX-2 expression in the spinal cord, and, in arthritic joints, reduce TNF-α and IL-1β levels. In addition to leukocytes and microglia, SPM receptors are present on neuronal bodies, nerve terminals (skin and muscle) and synaptic terminals, on which they regulate specific transient receptor potential channels. For example, RvE1–ChemR23 interaction in dorsal root ganglion regulates TRPV1, but not by direct activation of channels like endocannabinoids[104] or other lipids that directly bind transient receptor potential channels; instead, each SPM activates a specific GPCR in the pico-nanomolar range to regulate channels involved in pain signalling.

Direct comparisons between synthetic LXA_4 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[108]. 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[109]. 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[7,8,95] (Box 2). Using mass spectrometry, RvE1 and RvE2 can be identified in human blood[47,61,110], provided EPA or DHA is available. RvD1 and RvD2 are found in human plasma and serum[111]. The capacity of murine placenta to form resolvins (RvD1 and RvD2) and protectins (PD1 and 10S,17S-diHDHA[112], also known as PDx) was 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[114]: LXA_4, RvD1 and RvE1 were identified in milk of mothers in their first month of lactation using mass spectrometry[114]. 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[115]. 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 prostanoids, reduced LTB_4, 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[115]. 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[116] of infants with eczema, topical 15(R/S)-methyl-LXA_4 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[33] and adipokines[22]); regulate specific miRNAs[53] and cell traffic; and enhance microbial killing by receptor-mediated mechanisms in animal models *in vivo* and with human neutrophils and macrophages[10,13,21].

Results from preclinical disease models[10] 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 (http://www.auventx.com/auven/products/rx10045.php), and NPD1/PD1 is in clinical development for neurodegenerative diseases (http://www.anidapharma.com/lead-molecule.html); given their ability to

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 selective actions in animals[10,19], can now be addressed (Box 2) — along with the impact of anti-inflammatories on SPM pathways — using LC–MS–MS-based lipid-mediator–SPM profiling of human tissues. The SPMs produced by human apoptotic neutrophils and macrophages can be identified using LC–MS–MS profiling[13] 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.

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[21] and inflammatory diseases (Box 2) beyond the roles of their omega-3 EFA precursors in intermediary metabolism and membrane dynamics. Identification of SPM bioactive metabolomes and an appreciation that exudates can drive resolution, partly through SPMs, sets a new terrain to evaluate resolution physiology and pharmacology, in which SPMs are crucial as chemical signals for catabasis and host defence.

Note added in proof: A paper recently appeared online while the current Review was in press reporting the quantification of pro-resolving mediators using a new approach in human tissues and their functional relationship to known eicosanoids[121]. ■

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1. Flower, R. J. Prostaglandins, bioassay and inflammation. *Br. J. Pharmacol.* **147,** S182–S192 (2006).
2. Samuelsson, B. Role of basic science in the development of new medicines: examples from the eicosanoid field. *J. Biol. Chem.* **287,** 10070–10080 (2012).
3. Dinarello, C. A., Simon, A. & van der Meer, J. W. Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases. *Nature Rev. Drug Discov.* **11,** 633–652 (2012).
4. Serhan, C. N. & Savill, J. Resolution of inflammation: the beginning programs the end. *Nature Immunol.* **6,** 1191–1197 (2005).
5. Maderna, P. & Godson, C. Lipoxins: resolutionary road. *Br. J. Pharmacol*. **158,** 947–959 (2009).
6. Tabas, I. & Glass, C. K. Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science* **339,** 166–172 (2013).
7. Serhan, C. N. *et al*. 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.* **192,** 1197–1204 (2000).
8. Serhan, C. N. *et al*. 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*. **196,** 1025–1037 (2002).
9. Lands, W. E. M. *Fish, Omega-3 and Human Health* 2nd edn (AOCS Press, 2005).
10. Serhan, C. N. & Chiang, N. Resolution phase lipid mediators of inflammation: agonists of resolution. *Curr. Opin. Pharmacol.* **13,** 632–640 (2013).
11. Malawista, S. E., de Boisfleury Chevance, A., van Damme, J. & Serhan, C. N. Tonic inhibition of chemotaxis in human plasma. *Proc. Natl Acad. Sci. USA* **105,** 17949–17954 (2008).
12. Fullerton, J. N., O'Brien, A. J. & Gilroy, D. W. Lipid mediators in immune dysfunction after severe inflammation. *Trends Immunol*. **35,** 12–21 (2014).
13. Dalli, J. & Serhan, C. N. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. *Blood* **120,** e60–e72 (2012).
14. Serhan, C. N. The resolution of inflammation: the devil in the flask and in the details. *FASEB J.* **25,** 1441–1448 (2011).
15. Bailey, R. L., Gahche, J. J., Miller, P. E., Thomas, P. R. & Dwyer, J. T. Why US adults use dietary supplements. *JAMA Intern. Med*. **173,** 355–361 (2013).
16. Yates, C. M., Calder, P. C. & Rainger, G. E. Pharmacology and therapeutics of omega-3 polyunsaturated fatty acids in chronic inflammatory disease. *Pharmacol. Ther*. **141,** 272–282 (2014).
A thoughtful review of the use of omega-3 essential fatty acids in inflammatory diseases and their potential in supplementation, dietary manipulation and pharmacology.
17. Serhan, C. N. & Petasis, N. A. Resolvins and protectins in inflammation-resolution. *Chem. Rev*. **111,** 5922–5943 (2011).
18. Schwab, J. M., Chiang, N., Arita, M. & Serhan, C. N. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature* **447,** 869–874 (2007).
19. Miyahara, T. *et al*. D-series resolvins attenuate vascular smooth muscle cell activation and neointimal hyperplasia following vascular injury. *FASEB J*. **27,** 2220–2232 (2013).
20. Levy, B. D. & Serhan, C. N. Resolution of acute inflammation in the lung. *Annu. Rev. Physiol*. **76,** 467–492 (2014).
21. Spite, M., Claria, J. & Serhan, C. N. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. *Cell Metab*. **19,** 21–36 (2014).
22. Dalli, J. *et al*. Resolvin D3 and aspirin-triggered resolvin D3 are potent immunoresolvents. *Chem. Biol*. **20,** 188–201 (2013).
23. Serhan, C. N. *et al*. Macrophage pro-resolving mediator maresin 1 stimulates tissue regeneration and controls pain. *FASEB J*. **26,** 1755–1765 (2012).
24. Dalli, J. *et al*. The novel 13S,14S-epoxy-maresin is converted by human macrophages to maresin1 (MaR1), inhibits leukotriene A4 hydrolase (LTA4H), and shifts macrophage phenotype. *FASEB J.* **27,** 2573–2583 (2013).
25. Biswas, S. K. & Mantovani, A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nature Immunol*. **11,** 889–896 (2010).
26. Levy, B. D., Clish, C. B., Schmidt, B., Gronert, K. & Serhan, C. N. Lipid mediator class switching during acute inflammation: signals in resolution. *Nature Immunol.* **2,** 612–619 (2001).
27. Morris, T. *et al*. Effects of low-dose aspirin on acute inflammatory responses in humans. *J. Immunol*. **183,** 2089–2096 (2009).
28. Takano, T., Clish, C. B., Gronert, K., Petasis, N. & Serhan, C. N. Neutrophil-mediated changes in vascular permeability are inhibited by topical application of aspirin-triggered 15-epi-lipoxin A4 and novel lipoxin B4 stable analogues. *J. Clin. Invest*. **101,** 819–826 (1998).
29. Chan, M. M.-Y. & Moore, A. R. Resolution of inflammation in murine autoimmune arthritis is disrupted by cyclooxygenase-2 inhibition and restored by prostaglandin E2-mediated lipoxin A4 production. *J. Immunol.* **184,** 6418–6426 (2010).
30. Bannenberg, G. L. *et al*. Molecular circuits of resolution: Formation and actions of resolvins and protectins. *J. Immunol*. **174,** 4345–4355 (2005).
31. Lucas, C. D. *et al*. Downregulation of Mcl-1 has anti-inflammatory pro-resolution effects and enhances bacterial clearance from the lung. *Mucosal Immunol*. http://dx.doi.org/10.1038/mi.2013.102 (2013).
32. Navarro-Xavier, R. A. *et al*. A new strategy for the identification of novel molecules with targeted proresolution of inflammation properties. *J. Immunol*. **184,** 1516–1525 (2010).
33. Chiang, N. *et al*. Infection regulates pro-resolving mediators that lower antibiotic requirements. *Nature* **484,** 524–528 (2012).
34. Spite, M. *et al*. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. *Nature* **461,** 1287–1291 (2009).
35. Birnbaum, Y. *et al*. Augmentation of myocardial production of 15-epi-lipoxin-A4 by pioglitazone and atorvastatin in the rat. *Circulation* **114,** 929–935 (2006).
This is the first description of 15-epi-LXA$_4$ biosynthesis by statins *in vivo* in rats using a novel pathway and phosphorylated enzymes in the biosynthesis of 15-epi-LXA$_4$ that mimic the biosynthetic capacity of acetylated COX-2.
36. Cooray, S. N. *et al*. Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses. *Proc. Natl Acad. Sci. USA* **110,** 18232–18237 (2013).
This article reports exciting results indicating that heterodimers and homodimers of ALX/FPR2 signal different responses by activation of distinct intracellular signalling mechanisms.
37. Serhan, C. N. *et al*. Novel proresolving aspirin-triggered DHA pathway. *Chem. Biol.* **18,** 976–987 (2011).
38. Morris, T. *et al.* Dichotomy in duration and severity of acute inflammatory responses in humans arising from differentially expressed proresolution pathways. *Proc. Natl Acad. Sci. USA* **107,** 8842–8847 (2010).
39. Brancaleone, V. *et al*. A vasculo-protective circuit centered on lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 operative in murine microcirculation. *Blood* **122,** 608–617 (2013).
40. Norling, L. V. *et al*. Cutting edge: humanized nano-proresolving medicines mimic inflammation-resolution and enhance wound healing. *J. Immunol.* **186,** 5543–5547 (2011).
41. Stables, M. J. *et al*. Transcriptomic analyses of murine resolution-phase macrophages. *Blood* **118,** e192–e208 (2011).

42. Miki, Y. *et al*. Lymphoid tissue phospholipase A2 group IID resolves contact hypersensitivity by driving antiinflammatory lipid mediators. *J. Exp. Med.* **210,** 1217–1234 (2013).
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.
43. Levy, B. D. *et al*. Protectin D1 is generated in asthma and dampens airway inflammation and hyper-responsiveness. *J. Immunol.* **178,** 496–502 (2007).
44. Miyata, J. *et al*. Dysregulated synthesis of protectin D1 in eosinophils from patients with severe asthma. *J. Allergy Clin. Immunol*. **131,** 353–360 (2013).
45. Yamada, T. *et al*. Eosinophils promote resolution of acute periotonitis by producing proresolving mediators in mice. *FASEB J.* **25,** 561–568 (2011).
46. Isobe, Y. *et al*. Stereochemical assignment and anti-inflammatory properties of the omega-3 lipid mediator resolvin E3. *J. Biochem*. **153,** 355–360 (2013).
47. Arita, M. *et al*. Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* **201,** 713–722 (2005).
48. Ohira, T. *et al*. Resolvin E1 receptor activation signals phosphorylation and phagocytosis. *J. Biol. Chem*. **285,** 3451–3461 (2010).
49. El Kebir, D., Gjorstrup, P. & Filep, J. G. Resolvin E1 promotes phagocytosis-induced neutrophil apoptosis and accelerates resolution of pulmonary inflammation. *Proc. Natl Acad. Sci. USA* **109,** 14983–14988 (2012).
50. Krishnamoorthy, S., Recchiuti, A., Chiang, N., Fredman, G. & Serhan, C. N. Resolvin D1 receptor stereoselectivity and regulation of inflammation and pro-resolving microRNAs. *Am. J. Pathol.* **180,** 2018–2027 (2012).
51. Norling, L. V., Dalli, J., Flower, R. J., Serhan, C. N. & Perretti, M. Resolvin D1 limits polymorphonuclear leukocytes recruitment to inflammatory loci: receptor dependent actions. *Arterioscler. Thromb. Vasc. Biol.* **32,** 1970–1978 (2012).
52. Fredman, G., Li, Y., Dalli, J., Chiang, N. & Serhan, C. N. Self-limited versus delayed resolution of acute inflammation: temporal regulation of pro-resolving mediators and microRNA. *Sci. Rep.* **2,** 639 (2012).
53. Li, Y. *et al*. Plasticity of leukocytic exudates in resolving acute inflammation is regulated by microRNA and proresolving mediators. *Immunity* **39,** 885–898 (2013).
54. Li, D. *et al*. Resolvin D1 and aspirin-triggered resolvin D1 regulate histamine-stimulated conjunctival goblet cell secretion. *Mucosal Immunol*. http://dx.doi.org/10.1038/mi.2013.7 (2013).
55. Nelson, J. *et al*. The ALX/FPR2 receptor for RvD1 is expressed and functional in salivary glands. *Am. J. Physiol. Cell Physiol*. **306,** C178–C185 (2014).
56. Jones, C. N. *et al*. Microfluidic chambers for monitoring leukocyte trafficking and humanized nano-proresolving medicines interactions. *Proc. Natl Acad. Sci. USA* **109,** 20560–20565 (2012).
57. Kasuga, K. *et al*. Rapid appearance of resolvin precursors in inflammatory exudates: novel mechanisms in resolution. *J. Immunol*. **181,** 8677–8687 (2008).
58. Simiele, F. *et al*. Transcriptional regulation of the human FPR2/ALX gene: evidence of a heritable genetic variant that impairs promoter activity. *FASEB J.* **26,** 1323–1333 (2012).
59. Serhan, C. N. *et al*. Reduced inflammation and tissue damage in transgenic rabbits overexpressing 15-lipoxygenase and endogenous anti-inflammatory lipid mediators. *J. Immunol*. **171,** 6856–6865 (2003).
60. Hasturk, H. *et al*. Resolvin E1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis *in vivo*. *J. Immunol*. **179,** 7021–7029 (2007).
61. Oh, S. F., Pillai, P. S., Recchiuti, A., Yang, R. & Serhan, C. N. Pro-resolving actions and stereoselective biosynthesis of 18S E-series resolvins in human leukocytes and murine inflammation. *J. Clin. Invest.* **121,** 569–581 (2011).
62. Walker, J. *et al*. Lipoxin A4 increases survival by decreasing systemic inflammation and bacterial load in sepsis. *Shock* **36,** 410–416 (2011).
63. Prescott, D. & McKay, D. M. Aspirin-triggered lipoxin enhances macrophage phagocytosis of bacteria while inhibiting inflammatory cytokine production. *Am. J. Physiol. Gastrointest. Liver Physiol.* **301,** G487–G497 (2011).
64. Divangahi, M. *et al*. *Mycobacterium tuberculosis* evades macrophage defenses by inhibiting plasma membrane repair. *Nature Immunol*. **10,** 899–906 (2009).
65. Tobin, D. M. *et al*. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. *Cell* **148,** 434–446 (2012).
This systematic analysis of tuberculosis in infections in zebrafish identified the host gradation response to producing excess LTB_4 or excess LXA_4, each having different outcomes for the host and the clearance of tuberculosis infections.
66. Rajasagi, N. K., Reddy, P. B., Mulik, S., Gjorstrup, P. & Rouse, B. T. Neuroprotectin D1 reduces the severity of herpes simplex virus-induced corneal immunopathology. *Invest. Ophthalmol. Vis. Sci.* **54,** 6269–6279 (2013).
67. Rajasagi, N. K. *et al*. Controlling herpes simplex virus-induced ocular inflammatory lesions with the lipid-derived mediator resolvin E1. *J. Immunol*. **186,** 1735–1746 (2011).
This is an exciting first report demonstrating that resolvin E1 protects the eye from Herpes simplex virus infection and stimulates clearance of the virus.
68. Cilloniz, C. *et al*. Lethal dissemination of H5N1 influenza virus is associated with dysregulation of inflammation and lipoxin signaling in a mouse model of infection. *J. Virol.* **84,** 7613–7624 (2010).
69. Morita, M. *et al*. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. *Cell* **153,** 112–125 (2013).
70. Tam, V. C. *et al*. Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. *Cell* **154,** 213–227 (2013).
71. Haas-Stapleton, E. J. *et al*. *Candida albicans* modulates host defense by biosynthesizing the pro-resolving mediator resolvin E1. *PLoS ONE* **2,** e1316 (2007).
72. Baillie, J. K. & Digard, P. Influenza–time to target the host? *N. Engl. J. Med*. **369,** 191–193 (2013).
73. Pouliot, M., Clish, C. B., Petasis, N. A., Van Dyke, T. E. & Serhan, C. N. Lipoxin A4 analogues inhibit leukocyte recruitment to *Porphyromonas gingivalis*: a role for cyclooxygenase-2 and lipoxins in periodontal disease. *Biochemistry* **39,** 4761–4768 (2000).
74. Shen, J. *et al*. Macrophage-mediated 15-lipoxygenase expression protects against atherosclerosis development. *J. Clin. Invest.* **98,** 2201–2208 (1996).
75. Merched, A. J., Ko, K., Gotlinger, K. H., Serhan, C. N. & Chan, L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. *FASEB J.* **22,** 3595–3606 (2008).
76. Merched, A. J., Serhan, C. N. & Chan, L. Nutrigenetic disruption of inflammation-resolution homeostasis and atherogenesis. *J. Nutrigenet. Nutrigenomics* **4,** 12–24 (2011).
77. Hasturk, H. *et al*. RvE1 protects from local inflammation and osteoclast mediated bone destruction in periodontitis. *FASEB J.* **20,** 401–403 (2006).
78. Lima-Garcia, J. F. *et al.* The precursor of resolvin D series and aspirin-triggered resolvin D1 display anti-hyperalgesic properties in adjuvant-induced arthritis in rats. *Br. J. Pharmacol.* **164,** 278–293 (2011).
The authors of this article demonstrate that 17-HDHA and aspirin-triggered RvD1 have potent protective actions in adjuvant-induced arthritis and reduce pain in this model of arthritis.
79. Kowal-Bielecka, O., Kowal, K., Distler, O. & Gay, S. Mechanisms of disease: leukotrienes and lipoxins in scleroderma lung disease–insights and potential therapeutic implications. *Nature Clin. Pract. Rheumatol*. **3,** 43–51 (2007).
80. Martins, V. *et al*. ATLa, an aspirin-triggered lipoxin A4 synthetic analog, prevents the inflammatory and fibrotic effects of bleomycin-induced pulmonary fibrosis. *J. Immunol*. **182,** 5374–5381 (2009).
81. Börgeson, E. *et al*. Lipoxin A(4) and benzo-lipoxin A(4) attenuate experimental renal fibrosis. *FASEB J*. **25,** 2967–2979 (2011).
This article reported that expediting resolution and counter-regulation of pro-inflammatory mediators by administration of LXA_4 or its analogues can prevent organ fibrosis and renal fibrosis.
82. Qu, X. *et al.* Resolvins E1 and D1 inhibit interstitial fibrosis in the obstructed kidney via inhibition of local fibroblast proliferation. *J. Pathol*. **228,** 506–519 (2012).
83. Hsiao, H. M. *et al*. A novel anti-inflammatory and pro-resolving role for resolvin D1 in acute cigarette smoke-induced lung inflammation. *PLoS ONE* **8,** e58258 (2013).
84. Wang, S. B. *et al*. Estrogen negatively regulates epithelial wound healing and protective lipid mediator circuits in the cornea. *FASEB J.* **26,** 1506–1516 (2012).
85. Menon, R. *The Effect of Resolvins on Dermal Wound Healing*. MS thesis, Rutgers Univ. (2012).
86. Tang, Y. *et al.* Proresolution therapy for the treatment of delayed healing of diabetic wounds. *Diabetes* **62,** 618–627 (2013).
In this article, the authors demonstrate that pro-resolving mediators such as RvD1 can expedite healing of wounds encountered in diabetes.
87. Ramon, S., Gao, F., Serhan, C. N. & Phipps, R. P. Specialized proresolving mediators enhance human B cell differentiation to antibody-secreting cells. *J. Immunol*. **189,** 1036–1042 (2012).
88. Hong, S. *et al*. Resolvin D1, protectin D1, and related docosahexaenoic acid-derived products: analysis via electrospray/low energy tandem mass spectrometry based on spectra and fragmentation mechanisms. *J. Am. Soc. Mass Spectrom.* **18,** 128–144 (2007).
89. Ariel, A. *et al*. The docosatriene protectin D1 is produced by TH_2 skewing and promotes human T cell apoptosis via lipid raft clustering. *J. Biol. Chem.* **280,** 43079–43086 (2005).
90. Ariel, A. *et al*. Apoptotic neutrophils and T cells sequester chemokines during immune response resolution via modulation of CCR5 expression. *Nature Immunol.* **7,** 1209–1216 (2006).
91. Settimio, R., Clara, D. F., Franca, F., Francesca, S. & Michele, D. Resolvin D1 reduces the immunoinflammatory response of the rat eye following uveitis. *Mediators Inflamm*. **2012,** 318621 (2012).
92. Tian, H., Lu, Y., Sherwood, A. M., Hongqian, D. & Hong, S. Resolvins E1 and D1 in choroid-retinal endothelial cells and leukocytes: biosynthesis and mechanisms of anti-inflammatory actions. *Invest. Ophthalmol. Vis. Sci*. **50,** 3613–3620 (2009).
The authors of this report demonstrate the endogenous biosynthesis of RvE1 and RvD1 in the retina and the role of cell–cell interactions in this organ.
93. Vassiliou, E. K., Kesler, O. M., Tadros, J. H. & Ganea, D. Bone marrow-derived dendritic cells generated in the presence of resolvin E1 induce apoptosis of activated $CD4^+$ T cells. *J. Immunol*. **181,** 4534–4544 (2008).
94. Kim, T. H., Kim, G. D., Jin, Y. H., Park, Y. S. & Park, C. S. Omega-3 fatty acid-derived mediator, Resolvin E1, ameliorates 2,4-dinitrofluorobenzene-induced atopic dermatitis in NC/Nga mice. *Int. Immunopharmacol*. **14,** 384–391 (2012).
95. Hong, S., Gronert, K., Devchand, P., Moussignac, R.-L. & Serhan, C. N. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autacoids in anti-inflammation. *J. Biol. Chem*. **278,** 14677–14687 (2003).
96. Lukiw, W. J. *et al*. A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J. Clin. Invest.* **115,** 2774–2783 (2005).

97. Marcheselli, V. L. *et al*. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J. Biol. Chem.* **278,** 43807–43817 (2003).
This is an important first report of the production and actions of NPD1 in the mouse brain and its neural function by reducing inflammation.
98. Bazan, N. G., Calandria, J. M. & Serhan, C. N. Rescue and repair during photoreceptor cell renewal mediated by docosahexaenoic acid-derived neuroprotectin D1. *J. Lipid Res.* **51,** 2018–2031 (2010).
An authoritative, critical review of the bioactivity and actions of NPD1 in the eye and brain.
99. Sheets, K. G. *et al*. Microglial ramification and redistribution concomitant with the attenuation of choroidal neovascularization by neuroprotectin D1. *Mol. Vis.* **19,** 1747–1759 (2013).
The article is the first description of NPD1 action in regulating neovascularization by targeting microglia.
100. Bazan, N. G. *et al*. Novel aspirin-triggered neuroprotectin D1 attenuates cerebral ischemic injury after experimental stroke. *Exp. Neurol.* **236,** 122–130 (2012).
101. Wang, X. *et al.* Resolution of inflammation is altered in Alzheimer's disease. *Alzheimers Dement*. http://dx.doi.org/10.1016/j.jalz.2013.12.024 (2014).
102. Mizwicki, M. T. *et al*. 1α,25-Dihydroxyvitamin D3 and resolvin D1 retune the balance between amyloid-β phagocytosis and inflammation in Alzheimer's disease patients. *J. Alzheimers Dis.* **34,** 155–170 (2013).
103. Svensson, C. I., Zattoni, M. & Serhan, C. N. Lipoxins and aspirin-triggered lipoxin stop inflammatory pain processing. *J. Exp. Med.* **204,** 245–252 (2007).
104. Piomelli, D. & Sasso, O. Peripheral gating of pain signals by endogenous lipid mediators. *Nature Neurosci.* **17,** 164–174 (2014).
105. Xu, Z.-Z. *et al*. Resolvins RvE1 and RvD1 attenuate inflammatory pain via central and peripheral actions. *Nature Med.* **16,** 592–597 (2010).
106. Bang, S., Yoo, S., Yang, T. J., Cho, H. & Hwang, S. W. 17(R)-resolvin D1 specifically inhibits transient receptor potential ion channel vanilloid 3 leading to peripheral antinociception. *Br. J. Pharmacol.* **165,** 683–692 (2012).
107. Park, C. K. *et al*. Resolving TRPV1 and TNF-α-mediated spinal cord synaptic plasticity and inflammatory pain with neuroprotectin D1. *J. Neurosci.* **31,** 15072–15085 (2011).
108. Abdelmoaty, S. *et al*. Spinal actions of lipoxin A4 and 17(R)-resolvin D1 attenuate inflammation-induced mechanical hypersensitivity and spinal TNF release. *PLoS ONE* **8,** e75543 (2013).
109. Terrando, N. *et al*. Aspirin-triggered resolvin D1 prevents surgery-induced cognitive decline. *FASEB J.* **27,** 3564–3571 (2013).
This article reports an exciting discovery demonstrating that RvD1 counter-regulates pro-inflammatory mediators produced during surgery-induced cognitive decline.
110. Psychogios, N. *et al*. The human serum metabolome. *PLoS ONE* **6,** e16957 (2011).
111. Mas, E., Croft, K. D., Zahra, P., Barden, A. & Mori, T. A. Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation. *Clin. Chem.* **58,** 1476–1484 (2012).
112. Serhan, C. N. *et al*. Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J. Immunol.* **176,** 1848–1859 (2006).
113. Jones, M. L. *et al*. Maternal dietary omega-3 fatty acid intake increases resolvin and protectin levels in the rat placenta. *J. Lipid Res.* **54,** 2247–2254 (2013).
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.
114. Weiss, G. A. *et al*. High levels of anti-inflammatory and pro-resolving lipid mediators lipoxins and resolvins and declining docosahexaenoic acid levels in human milk during the first month of lactation. *Lipids Health Dis.* **12,** 89 (2013).
115. Markworth, J. F. *et al.* Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **305,** R1281–R1296 (2013).
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.
116. Wu, S. H., Chen, X. Q., Liu, B., Wu, H. J. & Dong, L. Efficacy and safety of 15(R/S)-methyl-lipoxin A_4 in topical treatment of infantile eczema. *Br. J. Dermatol.* **168,** 172–178 (2013).
This article reports a paediatric clinical trial that was the first to demonstrate that the topical addition of an aspirin-triggered LXA_4 stable analogue is safe and effective in reducing infantile eczema.
117. Raatz, S. K. *et al*. Baking reduces prostaglandin, resolvin, and hydroxy-fatty acid content of farm-raised Atlantic salmon (*Salmo salar*). *J. Agric. Food Chem.* **59,** 11278–11286 (2011).
118. Clària, J., Nguyen, B. T., Madenci, A., Ozaki, C. K. & Serhan, C. N. Diversity of lipid mediators in human adipose tissue depots. *Am. J. Physiol. Cell Physiol.* **304,** C1141–C1149 (2013).
119. Prüss, H. *et al*. Proresolution lipid mediators in multiple sclerosis - differential, disease severity-dependent synthesis — a clinical pilot trial. *PLoS ONE* **8,** e55859 (2013).
120. Giera, M. *et al*. Lipid and lipid mediator profiling of human synovial fluid in rheumatoid arthritis patients by means of LC-MS/MS. *Biochim. Biophys. Acta* **1821,** 1415–1424 (2012).
121. Colas, R., Shinohara, M., Dalli, J., Chiang, N. & Serhan, C.N. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. *Am. J. Physiol Cell Physiol*. http://dx.doi.org/10.1152/ajpcell.00024.2014 (2 April, 2014).

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