Published in final edited form as:

Microbiol Spectr. 2016 June ; 4(3): . doi:10.1128/microbiolspec.MCHD-0001-2014.

Macrophage Pro-Resolving Mediators—the When and Where

JESMOND DALLI¹ and CHARLES SERHAN¹

¹Center for Experimental Therapeutics and Reperfusion Injury, Harvard Institutes of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Abstract

Macrophages and neutrophils orchestrate acute inflammation and host defense as well as the resolution phase and return to homeostasis. In this article, we review the contribution of macrophages to local lipid mediator (LM) levels and the regulation of macrophage LM profiles by neutrophils and neutrophil-derived microparticles. We carried out LM metabololipidomics profiling distinct phagocytes: neutrophils (PMN), apoptotic PMN, and macrophages. Efferocytosis increased specialized proresolving mediator (SPM) biosynthesis, including Resolvin D1 (RvD1), RvD2, and RvE2, which were further elevated by PMN microparticles. Using deuterium-labeled precursors (d8-arachidonic acid, d5-eicosapentaenoic acid, and d5-docosahexaenoic acid), apoptotic PMN and microparticles contributed to SPM biosynthesis during efferocytosis. Assessment of macrophage LM profiles in M2 macrophages demonstrated higher SPM levels in this macrophage subset, including maresin 1 (MaR1), and lower amounts of leukotriene B4 and prostaglandins than M1. Apoptotic PMN uptake by both macrophage subtypes led to modulation of their LM profiles. Leukotriene B4 was down-regulated in M2 whereas SPM including lipoxin A4 were increased. Conversely, uptake of apoptotic PMN by M2 macrophages reduced (~ 25%) overall LM. MaR1 displays potent tissue regenerative and anti-nociceptive actions in addition to its pro-resolving and anti-inflammatory actions. In addition the MaR1 biosynthetic intermediate 13S,14S-epoxy-Maresin is also bioactive, inhibiting LTB4 biosynthesis and switching macrophage phenotypes from M1 to M2. Together, these results establish LM signature profiles of human phagocytes and related subpopulations. They demonstrate microparticle regulation of specific macrophage endogenous LM during defined stages of acute inflammation and their dynamic changes in human primary phagocytes.

Introduction

Inflammation is the organisms response to local injury in vascularized tissues programmed to traffic leukocytes and plasma delivery to an injured site or point of bacterial invasion (1), this protective response when uncontrolled in humans is associated with many widely occurring diseases. These include cardiovascular, metabolic and the classic inflammatory diseases, i.e. arthritis and periodontal disease along with cancers (reviewed in (2)). Non-resolving inflammation is now widely acknowledged as a major driver in most of these diseases (for review see ref (3)). The classical view of the resolution phase of the acute inflammatory response as thought and presented in pathology textbooks (1, 4) as well as in

medical dictionaries (5) was that local inflammatory chemical messengers and cells were *passively* diluted at the site (dilution of chemotactic gradient), hence halting further leukocyte recruitment resolving the exudate or battlefield of inflammation (6· 7). The historical perspective on the origins and concepts in the medical community regarding the resolution of inflammation trace back apparently as early as in the 11th Century in Europe, and interested readers can refer to a recent review (8).

Regarding the origins that led pathologists to consider the resolution of inflammation as a passive process, the reader is directed to ref. (9). In considering the outcomes of acute inflammation, pathologists considered four major paths: 1) complete resolution, 2) abscess formation, 3) healing by connective tissue replacement (fibrosis) and 4) progression of the tissue onto chronic inflammation. Specifically, in considering the complete resolution, it was noted that, in an ideal situation, all inflammatory responses, once they have succeeded in neutralizing injurious stimuli, must come to a termination or end with the restoration of the site of acute inflammation and its return to normality. This process, termed resolution, is the usual outcome when the injury is limited or short-lived or when there has been little tissue destruction and the damaged parenchymal cells can regenerate (9). Resolution thus involves “the neutralization or spontaneous decay of the local chemical mediators with the return of normal vascular permeability, cessation of leukocytic infiltration” (9) and cell death (largely by cellular apoptosis of neutrophils) and their removal as well as the removal of edema, foreign agents and necrotic debris from the inflammatory site. These events were held to take place primarily via phagocytic processes of phagocytes and the lymphatics (4· 9). Thus, while the events leading to resolution were thought to be seemingly chaotic, the process is highly organized as observable via microscopy of histological tissue sections, leading pathologists to the original ideas regarding resolution as a passive and spontaneous process.

The study of chemical mediators initially focused on those signals, local-acting autacoids, that play critical roles in the initiation process. Local mediators such as histamine, complement split products (C5a and C3b) and chemokines serve as chemoattractants to bring neutrophils, which are the first responders in many instances, via diapedesis from postcapillary venules into tissues to combat injury and invading organisms. In this process of initiation of inflammation intended to neutralize foreign invaders, arachidonic acid or n-6-derived mediators play a critical role. In this regard, prostaglandins are known to play pivotal roles in inflammation, and leukotrienes, in particular leukotriene B₄, plays a pivotal role as the key chemoattractant involved in the hierarchy of chemoattractants (10· 11). The gradient of chemoattractants such as LTB₄ is critical in enabling neutrophils to smell the chemical signal per se and move directedly along the gradient. Prostaglandin E₂ plays a critical role in the hemodynamic changes and endothelial cell responses, which permit neutrophils to migrate past them during diapedesis. All of these events are critical in the initiation process, and for the better part of the last century investigators focused their efforts on defining the mediators and mechanisms involved in initiating the acute inflammatory response, since this has led to therapeutic agents that can limit the dynamics and extent of the inflammatory response (12-14).

It became evident that there are lipid mediators that are also involved in endogenous anti-inflammation. In this regard, both PGE₂ and PGD₂ play special roles. There are multiple

receptors for these eicosanoids, and their cell-specific signaling is dictated by specific G protein coupling, which is cell type specific. PGE₂ has anti-inflammatory actions such as stopping neutrophil migration, as does PGD₂, but these mediators have a distinction in that they can induce the apparatus responsible for lipoxin production (15). The distinction between anti-inflammation and resolution is defined below, but at this juncture it is important to point out that anti-inflammation and resolution are distinct processes. Proresolving processes are active events stimulating the uptake, for example, of apoptotic neutrophils and/or bacteria and their debris, while anti-inflammation is generally an inhibitory process, stopping leukocytic infiltration for example. The distinction between these processes is now clear. One is an agonist, namely a proresolving mediator, while anti-inflammation can be initiated by a pharmacologic inhibitor or antagonist (16, 17).

Results from this laboratory demonstrated that resolution of self-limited inflammatory exudates is a biochemically active process that involves the local and temporal biosynthesis of a new genus of specialized pro-resolving mediators (SPM) with their novel functions mapped employing resolution indices (7, 18–20). SPM encompass several families of structurally and chemically distinct mediators. These chemical mediator families include lipoxins biosynthesized from arachidonic acid, E-series resolvins from EPA and docosahexaenoic acid (DHA) derived D-series resolvins, protectins and maresins. Each potent bioactive member of these families shares a defining action in resolving local inflammation. By definition, they each limit further neutrophil (PMN) recruitment to the site of injury and/or microbial invasion and enhance macrophage uptake of cellular debris and apoptotic PMN to bring about tissue homeostasis (2, 21). The placement of these mediators within the resolution of inflammation as well as the state-of-the art definitions are reviewed in (16, 21).

The process of macrophage clearance is a critical process conserved throughout evolution. Elie Metchnikoff first described it in the late 19th century in starfish larvae. Since then, the role of this process in mammalian systems has been extensively studied where it is appreciated to be critical in both the maintenance of homeostasis and clearance of cellular debris, bacteria (22, 23) and apoptotic cells, a process termed efferocytosis (24). Along with these defining properties, specific members of the SPM genus carry out more specialized tasks within programmed resolution (25) and hence the scope of their individual actions are non-overlapping and evoked *via* specific cell surface receptors that are GPCRs (2). The role for specific SPM in mediating the biological actions of SPM is established using both mice over expressing these receptors that give enhanced host protective actions as well as mice lacking the receptors that demonstrate excessive inflammation and loss of response to their specific SPM agonist(s). For example, in mice overexpressing the human lipoxin receptor (ALX), which also mediates the biological actions of RvD1, we found a reduction in the amplitude of the inflammatory response, accelerated resolution and enhanced responses to LXA₄ (26) and RvD1 (27). Similar results were also observed with transgenic mice overexpressing the receptor for RvE1, ChemR23/ERV (28), whereas in mice lacking the murine homologue of the ALX receptor there is excessive inflammation and decreased responses to these pro-resolving mediators (27, 29, 30). For a detailed recent review the reader is directed to the following review (31).

From the recent literature, it is clear that specific lipid mediators interact with GPCR receptors that can evoke cell type-specific responses. The specific responses of a given cell type could be mediated by different intracellular signals such as cyclic AMP in the case of resolvin D2 (32) or other intracellular signals such as phosphorylation and receptor cross-desensitization (33). Recent studies have also shown that proresolving actions of GPCR receptors as in the case of ALX receptor can differentially signal if they are in the heterodimer vs. homodimer state (34). These recent studies from Perretti and colleagues clearly demonstrate that heterodimer receptors with ALX and lipoxin A₄ as the ligand can differentially signal. Further research is needed along these lines to decode each of the SPM receptors and their target cell type and tissue responses and intracellular signal transduction.

A systems approach led to the identification of novel bioactive structures coined resolvins and protectins in murine inflammatory exudates and isolated human cells based on LC-MS-MS-based lipid mediator lipidomics and tandem assessment of their functions in anti-inflammation and pro-resolution (18, 35). The complete stereochemistry and total organic synthesis of several key resolvins, protectins as well as their aspirin-triggered forms are established (recently reviewed in (21, 36)). These include resolvin D1 (7*S*, 8*R*, 17*S*-trihydroxy-4*Z*, 9*E*, 11*E*, 13*Z*, 15*E*, 19*Z*-docosahexaenoic acid), resolvin D2 (7*S*, 16*R*, 17*S*-trihydroxy-4*Z*, 8*E*, 10*Z*, 12*E*, 14*E*, 19*Z*-docosahexaenoic acid), 17*R*-HDHA (17*R*-hydroxy-4*Z*, 7*Z*, 10*Z*, 13*Z*, 15*E*, 19*Z*-docosahexaenoic acid), neuroprotectin D1/NPD1 (10*R*, 17*S*-dihydroxy-4*Z*, 7*Z*, 11*E*, 13*E*, 15*Z*, 19*Z*-docosahexaenoic acid), resolvin E1 (5*S*, 12*R*, 18*R*-trihydroxy-6*Z*, 8*E*, 10*E*, 14*Z*, 16*E*-eicosapentaenoic acid) and most recently maresin 1 (7*R*, 14*S*-dihydroxy-4*Z*, 8*E*, 10*E*, 12*Z*, 16*Z*, 19*Z*-docosahexaenoic acid) (37). In addition to confirming the original structural assignments and potent anti-inflammatory and pro-resolving actions *in vivo* of resolvins, lipoxins and maresins (36), new studies from others demonstrate their potent actions in experimental colitis (38), arthritis (39), arthritic pain (40), ocular diseases (41), resolving obesity (42) and diabetes (43). Importantly, synthetic SPM permitted their identification in other biological sources including, for example, human inflammatory responses (44, 45), human serum (46), fish (47) and other marine organisms (48) and the invertebrate phyla (37). These mediators also counter-regulate the production of pro-inflammatory signals including lipid mediators (e.g. leukotrienes and prostaglandins) and cytokines (e.g. TNF- α , IL-6) (21) and upregulate levels of host protective and anti-inflammatory mediators including proteins such as Annexin A1 (reviewed in 49), gases including endogenous heme oxygenase-1 derived carbon monoxide (50) as well as endogenous hydrogen sulfide (51) and cytokines including TGF- β and IL-10 (reviewed in 52). For a detailed review of the role of cytokines in regulating steps during the resolution of inflammation the readers are directed to this recent review (52).

The discovery of potent bioactive resolvins and protectins in resolving exudates implicated that multiple cell types are involved in their biosynthesis given the dynamic process ongoing within evolving inflammatory exudates (7, 18). With authenticated SPM and pro-inflammatory LM, we can now carry out targeted lipid mediator (LM) metabololipidomics *via* profiling monitoring > 50 individual mediators and pathway markers from these autacoid pathways in initiation and resolution of inflammation (53).

Microbiol Spectr. Author manuscript; available in PMC 2016 June 27.

H₂O; 241=277-H₂O), RvD2, LXB₄ and 5,15-diHETE (Figure 1B and insets for diagnostic ions).

LM quantification is achieved via multiple reaction monitoring (MRM) of signature ion pairs Q1 (Parent ion) - Q3 (characteristic daughter ion) (Figure 1C). Macrophage efferocytosis of apoptotic PMN increased SPM biosynthesis, primarily RvD1 (18±10 vs. 127±14 pg/2.5×10⁵ cells), RvD2 (28±8 vs. 124±12 pg/2.5×10⁵ cells) and LXB₄ (19±2 vs. 44±6 pg/2.5×10⁵ cells). This was accompanied by increases in prostanoids including PGE₂ (7±1 vs. 44±12 pg/2.5×10⁵ cells; Figure 1C). Having found that microparticles stimulate efferocytosis, we assessed their actions on SPM biosynthesis during apoptotic PMN uptake. Microparticles stimulated macrophage biosynthesis of RvD2 (219±44 vs. 124±12 pg/2.5×10⁵ cells), LXB₄ (84±15 vs. 44±6 pg/2.5×10⁵ cells) and RvE2 (478±57 vs. 199±26 pg/2.5×10⁵ cells), to a greater extent than apoptotic PMN, whilst reducing PGF_{2α} (259±29 vs. 459 ± 54 pg/2.5×10⁵ cells) and TXB₂ (328±21 vs. 456±62 pg/2.5×10⁵ cells) (Figure 1C).

Temporal SPM biosynthesis contributes to regulating the onset of resolution programs (2). Therefore, we assessed the cumulative levels for each LM family identified (64). During human macrophages efferocytosis there was an increase in DHA (Figure 2A) and AA derived SPM (Figure 2B) biosynthesis, along with an augmentation in prostanoid production (Figure 2C). Microparticles further enhanced apoptotic PMN stimulated SPM biosynthesis (Figure 2A,B) whilst reducing prostanoid production (Figure 2C). To rule out the possibility that microparticles carried mediators in addition to their known ability to carry SPM precursors (63), we profiled the microparticles. Microparticles did not carry mature SPM. Hence, we profiled the amounts of LM associated with apoptotic PMN and microparticles in the absence of efferocytosis. Although specific SPM and LM were demonstrable, their values were baseline compared to levels produced via macrophages during efferocytosis (64).

Whether PMN microparticles are able to stimulate macrophage LM biosynthesis was also investigated. Incubation of PMN microparticles with macrophages led to the biosynthesis of lipoxygenase- and cyclooxygenase-derived LM (64). These included RvD5, PD1 and PGE₂ in accordance with criteria (53) that included matching retention times and at least 6 diagnostic ions as illustrated for MaR1, PD1, RvE2 and RvE1. These results are first to demonstrate each of these pro-resolving mediators in human macrophage incubations and their interrelationships.

In these experiments we observed, for example, that PMN microparticles led to an increase in MaR1 (13±4 vs. 21±2 pg/2.5×10⁵ macrophages), PD1 (7±1 vs. 28±5 pg/2.5×10⁵ macrophages), LXB₄ (78±4 vs. 248±26 pg/2.5×10⁵ macrophages) and RvE1 (14±8 vs. 72±9 pg/2.5×10⁵ macrophages) production. Also, we found that PMN microparticles stimulated macrophage prostanoids, in particular PGE₂ (18±2 vs. 40±6 pg/2.5×10⁵ macrophages) and TXB₂ (28±7 vs. 63±10 pg/2.5×10⁵ macrophages). Cumulative LM metabololipidomics for each LM family identified demonstrated that PMN microparticles stimulated biosynthesis of DHA- (Figure 2A), AA- (Figure 2B) and EPA- derived SPM along with prostanoids (Figure 2C). Of interest, incubation of macrophages with G-protein inhibitors (pertussis toxin and cholera toxin) reduced SPM biosynthesis without altering prostanoid levels. Together these

results demonstrate that microparticles selectively stimulate macrophage SPM production in a GPCR-dependent manner (64, 66).

Microparticles stimulate efferocytosis of apoptotic PMN

Since PMN microparticles exert anti-inflammatory and pro-resolving actions *in vivo* (63); we assessed their ability to regulate macrophage uptake of apoptotic cells (efferocytosis) (17). PMN-microparticles were obtained from activated PMN and incubated with macrophages prior to addition of fluorescent apoptotic PMN, dose dependently increased macrophage-associated fluorescence (Figure 2D). These results indicate that microparticles regulate efferocytosis of apoptotic cells and stimulate the biosynthesis of SPM (Figure 2E).

Apoptotic PMN possess a pro-resolving LM profile

Because apoptotic PMN stimulate macrophage SPM during efferocytosis (64), we investigated mediator profiles of apoptotic PMN in comparison to zymosan-stimulated PMN. Using targeted LM metabololipidomics, we identified a profile signature of LM that included RvD1, LXB₄, PGD₂ and PGE₂. Identification was achieved (*vide supra*) as illustrated for RvE2, PD1 (also known as NPD1/PD1 when produced in neural systems (19)), RvD2 and LTB₄. MRM LM quantification of signature ion pairs demonstrated that AA derived LM represented over ~85% of the apoptotic PMN AA, EPA and DHA identified LM metabolome. In these cells the DHA-derived mediators represented ~6% whilst EPA derived mediators amounted to ~11% of the targeted LM. On the other hand, with zymosan-stimulated PMN, AA-derived LM constituted ~81% of the targeted AA, EPA and DHA LM metabolomes.

Comparison of endogenous LM identified in apoptotic *versus* zymosan activated PMN revealed that RvD1 (42±12 vs. 5±2 pg/5×10⁶ cells) and LXB₄ (142±37 vs. 14±7 pg/5×10⁶ cells) biosynthesis was reduced in zymosan-stimulated cells. Similarly there was a down-regulation in a number of cyclooxygenase derived LM in zymosan stimulated PMN including PGD₂ (123±29 vs. 82±15 pg/5×10⁶ cells), PGE₂ (390±75 vs. 82±16 pg/5×10⁶ cells) and PGF_{2α} (127±40 vs. 52±18 pg/5×10⁶ cells). Production of the chemoattractant LTB₄ (including its 20-OH P450 metabolite) was elevated in zymosan stimulated cells as opposed to apoptotic PMN. For intact PMN without exposure to agonists, the levels for these mediators were close to or below limits. In order to gauge the effector functions endowed upon PMN by their individual LM profiles, we assessed the cumulative LM amounts for the distinct LM families identified using LM metabololipidomics. We found that apoptotic PMN produced significantly higher SPM and prostanoids than zymosan or intact PMN. Assessment of AA, EPA and DHA-derived monohydroxy products did not reveal significant differences between apoptotic, zymosan-stimulated or intact PMN (64).

In order to appreciate the sequence of events involved in LM production during human PMN apoptosis, we also identified LM present at distinct stages of PMN progression to apoptosis determined by Annexin V staining. Since, during inflammation, edema formation supplies the inflammatory response with extracellular substrate (69), we determined the time course of LM biosynthesis by apoptotic cells in the presence of extracellular substrate. MRM quantification of identified LM demonstrated a significant increase in SPM biosynthesis by

apoptotic cells, including D-and E-series resolvins that reached maximum within 30min (64). Prostanoids were also elevated, whilst LTB₄ (including its 20-OH metabolite) occurred during the initial phase and subsequently declined, both in the presence and absence of substrate. Although we were not able to rule out that a small proportion of potentially non-apoptotic PMN that might have been present could have contributed to LM profiles in incubations with apoptotic PMN, it is more than likely that at the 18h interval LM profiles reflect the capacity of apoptotic PMN to biosynthesize specific LM.

Apoptotic PMN and microparticles, a nidus for macrophage SPM biosynthesis

The potential contribution of transcellular biosynthesis was assessed, in particular for SPM, during macrophage efferocytosis (64). For this purpose we utilized deuterium- labeled (*d*) precursors, which allowed for the identification of mediators from substrate/precursors derived from either apoptotic PMN or microparticles. LM derived from *d*-substrate had a higher mass to charge ratio (*m/z*) than those biosynthesized from endogenous substrate. Specifically, LM produced from *d*₈-AA were 8 atomic mass units (amu) and those from *d*₅-EPA and *d*₅-DHA were 5 amu greater than LM biosynthesized from endogenous precursors. We first fortified microparticles and apoptotic PMN with *d*-labeled precursors and assessed levels of *d*-labeled LM, essential fatty acids (AA, EPA and DHA) and biosynthetic precursors (including 18-HEPE, 15-HEPE, 15-HETE, 17-HDHA and 14-HDHA). We identified LM precursors including *d*₅-DHA, *d*₅-17-HDHA, and *d*₈-AA along with a select number of mediators, including *d*₅-RvD5 and *d*₅-RvE2 in accordance with published criteria (53). MRM quantitation of the identified LM demonstrated that the levels of these *d*-labeled mediators within microparticles and apoptotic PMN were near limits of detection (<5pg LM/6×10⁵ microparticles and <1pg LM/1×10⁶ PMN). We also identified *d*-labeled biosynthetic precursors that included 16 ± 3 pg *d*₅-17-HDHA/5×10⁵ microparticles and 18 ± 1 pg *d*₅-17-HDHA/7.5×10⁵ apoptotic PMN and *d*-labeled essential fatty acids that included 423 ± 35 pg *d*₅-DHA/5×10⁵ microparticles and 459 ± 133 pg *d*₅-DHA/7.5×10⁵ apoptotic PMN. These results suggested that whilst there was a significant enrichment of *d*-labeled precursors in both microparticles and apoptotic PMN, only a relatively minor proportion was further converted to bioactive LM in the absence of macrophages.

In these studies, we added either *d*-labeled microparticles or *d*-labeled apoptotic PMN to macrophages assessing LM transcellular biosynthesis during macrophage efferocytosis (64). Apoptotic PMN uptake by macrophages led to the biosynthesis of *d*₅-RvD2 and *d*₅-RvD5 from the D-series resolvins, *d*₅-PD1 from the protectins, from the lipoxins *d*₈-LXB₄ and *d*₅-RvE2 from the E-series resolvins, whilst *d*₈-PGD₂ and *d*₈-PGF_{2α} were the only prostanoids identified and *d*₅-RvD5, *d*₅-RvE2 and *d*₈-LXB₄ where the atomic mass of the parent molecules was increased by either 5 or 8 amu. MRM quantification of *d*-LM biosynthesized during macrophage efferocytosis suggested that microparticles acted as a nexus for *d*₅-RvD2, *d*₅-RvD5, *d*₈-LXA₄, *d*₈-PGE₂ and *d*₈-PGF_{2α} production. *d*₅-MaR1 and *d*₈-LXB₄ biosynthesis was primarily sustained by precursors derived from within apoptotic PMN. *d*₅-PD1 and *d*₅-RvE2 biosynthesis were equally reliant on precursors from apoptotic cells and microparticles. These results demonstrate that transcellular biosynthesis contributes to LM production during macrophage efferocytosis were both apoptotic PMN and microparticles contribute to and sustain a nidus for LM biosynthesis.

Human macrophage subtypes yield distinct LM and SPM profiles

Following LM metabolomics of M1 and M2 macrophages, that were prepared from human peripheral blood monocytes, we identified 35 distinct LM that included RvD1, PD1, MaR1, LXA₅, LXB₅ and PGD₂, PGE₂. These mediators were identified using their diagnostic fragmentation patterns, matching a minimum of 6 characteristic fragments as shown for RvD2, PD1, RvE2 and LXA₅. LM metabololipidomics of M1 macrophage AA, EPA and DHA identified LM metabolome we found that AA derived LM amounted to ~48% of LM identified. On the other hand, LM metabololipidomics of M2 macrophages demonstrated that SPM represented ~50% of the identified AA, EPA and DHA metabolome, consisting of D-series resolvins, protectins and maresins (~11%), lipoxins (~15%) and E-series resolvins and lipoxins (~24%).

Using MRM, we compared the endogenous biosynthesis of individual LM between the two macrophage subtypes. In these experiments, we found that biosynthesis of distinct SPM was elevated in M2 in comparison to M1 macrophage, these included RvD5 (196±26 vs. 43±5 pg/2.5×10⁵ cells), MaR1 (299±8 vs. 45±8 pg/2.5×10⁵ cells), PD1 (3442±206 vs. 1339±206 pg/2.5×10⁵ cells), LXA₄ (977±173 vs. 675±167 pg/2.5×10⁵ cells), LXB₄ (11,750±724 vs. 7,560±659 pg/2.5×10⁵ cells), LXB₅ (4,370±397 vs. 2,551±859 pg/2.5×10⁵ cells) and RvE2 (20,680±4,910 vs. 10,910±4,232 pg/2.5×10⁵ cells). Conversely, production of cyclooxygenase derived LM was elevated in M1 macrophages, these included PGE₂ (2,285±435 vs. 1,573±490 pg/2.5×10⁵ cells), PGF_{2α} (11,325±428 vs. 2,108±256 pg/2.5×10⁵ cells), TXB₂ (550±135 vs. 222±73 pg/2.5×10⁵ cells) and the EPA derived prostanoids.

Because different human macrophage subtypes possess distinct LM profiles, we compared the cumulative amounts of different LM families identified in M1 and M2 macrophages in order to assess effector functions that these endow for the individual macrophage subtypes. SPM biosynthesis was higher in M2 compared to M1 macrophages, while production of prostanoids and leukotrienes LTB₄ and LTB₅ was elevated in M1 macrophages compared to M2 (Figure 3). Assessment of LM biosynthesis following apoptotic cell efferocytosis demonstrated that apoptotic PMN uptake gave SPM in M1 macrophages whilst reducing prostanoids and leukotrienes. Apoptotic PMN with M2 macrophages reduced overall LM production including SPM. These results indicate that human M1 and M2 macrophages, as defined (70), possess distinct endogenous LM signature profiles.

Maresin1 is pro-resolving and tissue regenerative

Self-resolving inflammatory exudates and lipid mediator metabolomics recently uncovered a new family of potent anti-inflammatory and pro-resolving mediators biosynthesized by macrophages, coined maresins (71). It was essential to establish the stereochemistry of this pathway. We determined that maresin 1 (MaR1) produced by human macrophages from endogenous docosahexaenoic acid (DHA) matched synthetic 7*R*,14*S*-dihydroxydocosa-4*Z*,8*E*,10*E*,12*Z*,16*Z*,19*Z*-hexaenoic acid. The MaR1 alcohol groups and Z/E geometry of conjugated double bonds were matched using isomers prepared by total organic synthesis. MaR1's potent defining actions were confirmed with synthetic MaR1, i.e. limiting neutrophil (PMN) infiltration in murine peritonitis (ng/mouse range) as well as enhancing human macrophage uptake of apoptotic PMNs. At 1 nM, MaR1 was slightly more potent than

Resolvin D1 (RvD1) in stimulating human macrophage efferocytosis, an action not shared by LTB₄.

MaR1 also accelerated surgical regeneration in planaria, increasing the rate of head reappearance (37). Upon injury of planaria, MaR1 was biosynthesized from deuterium-labeled (d₅)-DHA that was blocked with lipoxygenase inhibitor. Planaria are simple organisms capable of rapid regeneration, the process wherein mammalian tissue macrophages play distinct roles. Hence, we questioned whether MaR1 demonstrated properties in tissue regeneration. To this end, planaria were grown and their anterior portions were surgically removed and exposed to pro-resolving mediators (Figure 4). Planaria are increasingly recognized as a useful model system for tissue regeneration (72). Factors are known to be involved in planaria tissue regeneration (72). However, they remained to be identified. MaR1 and RvE1 at 100 nM each enhanced the rate of tissue regeneration with the appearance of the anterior portion, i.e. head regeneration, evident as early as three days post surgery (Figure 4). We examined MaR1 dose dependency (Figure 4). MaR1 at doses as low as 1, 10, and 100 nM enhanced tissue regeneration in the anterior head that was statistically significant by days 3 and 4. By days 6 and 7, the actions were less prominent. MaR1-enhanced tissue regeneration was concentration dependent (Figure 4) (37).

MaR1 also possess potent anti-nociceptive actions dose-dependently inhibiting TRPV1 currents in neurons, blocked capsaicin (CAP, 100 nM)-induced inward currents ($IC_{50} = 0.49 \pm 0.02$) and reduced both inflammatory and chemotherapy-induced neuropathic pain in mice. These results demonstrate the potent actions of MaR1 in regulating inflammation-resolution, tissue regeneration and resolving pain (8, 37, 67). These findings suggest that chemical signals are shared in resolute cellular trafficking key in tissue regeneration. Moreover, immunoresolvents of the innate immune response (8), such as MaR1, offer new opportunities for assessing macrophages and their local DHA metabolome in the return to tissue homeostasis.

The maresin biosynthetic pathway

In the biosynthesis of maresins, the 14-lipoxygenation of DHA by human macrophage 12-lipoxygenase (12-LOX) gave 14-hydro(peroxy)-docosahexaenoic acid (14-HpDHA) as well as several dihydroxy-docosahexaenoic acids implicating an epoxide intermediate formation by this enzyme. Using a stereocontrolled synthesis, enantiomerically pure 13*S*,14*S*-epoxy-docosa-4*Z*,7*Z*,9*E*,11*E*,16*Z*,19*Z*-hexaenoic acid (13*S*,14*S*-epoxy-DHA) was prepared and its stereochemistry was confirmed by NMR spectroscopy. When this 13*S*,14*S*-epoxide was incubated with human macrophages, it was converted to MaR1. The synthetic 13*S*,14*S*-epoxide inhibited leukotriene B₄ (LTB₄) formation by human leukotriene A₄ hydrolase (LTA₄H) ~40% ($p < 0.05$) to a similar extent as LTA₄ (~50%, $p < 0.05$) but was not converted to MaR1 by this enzyme. 13*S*,14*S*-epoxy-DHA also reduced (~60%; $p < 0.05$) arachidonic acid conversion by human 12-LOX. Incubation of 13*S*,14*S*-epoxy-DHA with M1 macrophage led to a change in macrophage phenotype towards an M2 profile (73). Macrophage phenotype was determined using flow cytometry where M1 macrophages were expressed higher CD80 and CD54 while M2 macrophages displayed higher CD163 and CD206 levels. Incubation of M1 macrophages with either 13*S*,14*S*-epoxy-DHA (10nM) or

MaR1 (10nM) led to significant reductions in CD54 and CD80 expression and a concomitant upregulation of CD163 and CD206 (Figure 5). For comparison, this regulation was also found with RvD1 [10 nM]. We also investigated the conversion of 13S,14S-epoxy-DHA to MaR1 by different human macrophage subtypes. Incubation of 13S,14S-epoxy-DHA (2 μ M) with M2 macrophages gave higher MaR1 levels than when the 13S,14S-epoxy-DHA was incubated with M1 macrophages as determined by lipid mediator metabololipidomics.

These results establish the biosynthesis of the 13S,14S-epoxide, its absolute stereochemistry, precursor role in MaR1 biosynthesis and its own intrinsic bioactivity (73). Given its actions and role in MaR1 biosynthesis, this epoxide is now termed 13,14-epoxy-maresin (13,14-eMaR) and exhibits new mechanisms in resolution of inflammation in its ability to inhibit pro-inflammatory mediator production by LTA₄ hydrolase and to block arachidonate conversion by human 12-LOX rather than merely terminating phagocyte involvement. Thus, these findings identify novel regulatory actions of DHA-derived mediators within the eicosanoid cascade and in macrophage phenotypes (see Figure 6) that can play a role in vascular integrity (74) as well as in aging (66), tissue regeneration (37, 71), pain (67) and host defense mechanism (75), all critical life processes that are regulated by neutrophils and ultimately by specific macrophage subtypes where resolution phase signals such as the pro-resolving mediators (resolvins, protectins and maresins) actively turn on homeostatic mechanisms.

Therefore SPM emerge as potent regulators of macrophage responses of interest during the resolution phase of acute inflammatory responses. Since the means and methodologies to identify these mediators have only recently become widely available a number of aspects of their biology in humans have only just started to be explored such as the production of these mediators in human tissues (76-78). In addition, a number of questions still remain to be addressed such as the when and where these mediators are produced in human tissues in health and disease and the relevance of resolution processes that may fail giving to human disease. For those interested in the future directions and questions to be addressed, we direct readers to this recent review (21).

Looking Ahead—In the near future, given the ability now to profile both pro-inflammatory and anti-inflammatory-proresolving mediators such as the SPM in human tissues (78), and in other human organs (79-81) including human placenta (82), plasma (78, 81) and milk (83), it is possible that lipid mediator and SPM signature profiles will be very useful in both precision and personalized medicine. In this regard, the profile of SPMs can serve as a barometer of diagnostic value for ranking the nutritional status of an individual and whether or not they can be timely or slow resolvers of a local acute inflammatory challenge (44). For example, a randomized controlled trial in human chronic kidney disease showed that n-3 fatty acid supplementation increases SPM, which may help improve inflammation in this disease (84). Most importantly, we will be able to establish whether specific SPMs (resolvins, protectins or maresins) act locally to evoke their biological responses or if they have a distal site of action. In this regard, we recently identified resolvins, protectins and maresins in human milk, which suggests that they could have a local site of action but also could be active in the newborn (85). Most importantly, such

methods of profiling initiators of inflammation and proresolving mediators in human tissues will help to assess the nutritional status of an individual. For example, what are the optimal levels of omega-3 essential fatty acids such as EPA and DHA as well as n-6 arachidonic acid needed at a local tissue site to insure timely neutralization of invading foreign injury as well as timely resolution and homeostasis? There are clearly age- and gender-related components in the resolution of inflammation, as we have found that the ability to resolve acute inflammatory responses in a timely fashion diminishes with age in mouse models (66). Perhaps it will be possible to personalize our nutrition to impact resolution in both an age- and gender-dependent manner to improve health and reduce disease burden by enhancing local resolution of inflammation that could diminish chronic inflammation and the impact of chronic inflammatory diseases on organs throughout the body. In addition to tailoring designer medical foods to personalized nutrition, it is also possible to use each of the proresolving mediators as templates for analogs that can be used as therapeutics to stimulate resolution of inflammation as a new approach to resolution pharmacology (86). This has already been extended to ongoing human clinical trials in ocular indications (87) and for periodontal disease (88).

Given that resolution of inflammation is a fundamental process in all human tissues and that phagocytes and specifically macrophages play a central role in orchestrating this response as well as tissue repair and regeneration, immunoresolvents (such as resolvins, protectins and maresins) may provide a novel therapeutic approach for diseases characterized by uncontrolled inflammation and failed resolution.

Acknowledgments

The authors thank Mary Halm Small for expert assistance in manuscript preparation. Studies in the authors' lab were supported by the National Institutes of Health (grant numbers R01GM38765 and P01GM095467).

References

1. Majno, G.; Joris, I. Cells, tissues, and disease : principles of general pathology. 2nd ed.. Oxford University Press; New York ; Oxford: 2004.
2. Serhan CN. Novel resolution mechanisms in acute inflammation: to resolve or not? *Am J Pathol.* 2010; 177:1576–1591. [PubMed: 20813960]
3. Nathan C, Ding A. Nonresolving inflammation. *Cell.* 2010; 140:871–882. [PubMed: 20303877]
4. Robbins, SL.; Cotran, R. Pathologic Basis of Disease. 2nd ed.. W.B. Saunders Co.; Philadelphia: 1979. Chapters 2 and 3
5. Taber, CW. Taber's Cyclopedic Medical Dictionary. 11th ed.. F. A. Davis Company; Philadelphia: 1970.
6. Willoughby DA, Moore AR, Colville-Nash PR, Gilroy D. Resolution of inflammation. *Int J Immunopharmacol.* 2000; 22:1131–1135. [PubMed: 11137620]
7. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med.* 2000; 192:1197–1204. [PubMed: 11034610]
8. Serhan CN. The resolution of inflammation: the devil in the flask and in the details. *FASEB J.* 2011; 25:1441–1448. [PubMed: 21532053]
9. Cotran, RS.; Kumar, V.; Collins, T. Robbins Pathologic Basis of Disease. 6th ed.. W.B. Saunders Co.; Philadelphia: 1999. p. 78Chap. 3

10. Lammermann T, Afonso PV, Angermann BR, Wang JM, Kastenmuller W, Parent CA, Germain RN. Neutrophil swarms require LTB₄ and integrins at sites of cell death in vivo. *Nature*. 2013; 498:371–375. [PubMed: 23708969]
11. Malawista SE, de Boisleury Chevance A, van Damme J, Serhan CN. Tonic inhibition of chemotaxis in human plasma. *Proc Natl Acad Sci U S A*. 2008; 105:17949–17954. [PubMed: 18997012]
12. Samuelsson B, Dahlén SE, Lindgren JÅ, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science*. 1987; 237:1171–1176. [PubMed: 2820055]
13. Samuelsson B. Role of basic science in the development of new medicines: examples from the eicosanoid field. *J Biol Chem*. 2012; 287:10070–10080. [PubMed: 22318727]
14. Vane, JR. Les Prix Nobel: Nobel Prizes, Presentations, Biographies and Lectures. Almqvist & Wiksell; Stockholm: 1982. Adventures and excursions in bioassay: the stepping stones to prostacyclin; p. 181-206.
15. Levy BD, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol*. 2001; 2:612–619. [PubMed: 11429545]
16. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LAJ, Perretti M, Rossi AG, Wallace JL. Resolution of inflammation: state of the art, definitions and terms. *FASEB J*. 2007; 21:325–332. [PubMed: 17267386]
17. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol*. 2005; 6:1191–1197. [PubMed: 16369558]
18. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac R L. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter pro-inflammation signals. *J Exp Med*. 2002; 196:1025–1037. [PubMed: 12391014]
19. Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN. Molecular circuits of resolution: Formation and actions of resolvins and protectins. *J Immunol*. 2005; 174:4345–4355. [PubMed: 15778399]
20. Schwab JM, Chiang N, Arita M, Serhan CN. Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature*. 2007; 447:869–874. [PubMed: 17568749]
21. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature*. 2014; 510:92–101. [PubMed: 24899309]
22. Tauber, AI.; Chernyak, L. Metchnikoff and the Origins of Immunology: From Metaphor to Theory. Oxford University Press; New York: 1991.
23. Gordon S. The macrophage: past, present and future. *Eur J Immunol*. 2007; 37(Suppl 1):S9–17. [PubMed: 17972350]
24. Savill JS, Wyllie AH, Henson JE, Walport MJ, Henson PM, Haslett C. Macrophage phagocytosis of aging neutrophils in inflammation. Programmed cell death in the neutrophil leads to its recognition by macrophages. *J Clin Invest*. 1989; 83:865–875. [PubMed: 2921324]
25. Buckley CD, Gilroy DW, Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity*. 2014; 40:315–327. [PubMed: 24656045]
26. Devchand PR, Arita M, Hong S, Bannenberg G, Moussignac R-L, Gronert K, Serhan CN. Human ALX receptor regulates neutrophil recruitment in transgenic mice: Roles in inflammation and host-defense. *FASEB J*. 2003; 17:652–659. [PubMed: 12665478]
27. Krishnamoorthy S, Recchiuti A, Chiang N, Fredman G, Serhan CN. Resolvin D1 receptor stereoselectivity and regulation of inflammation and pro-resolving microRNAs. *Am J Pathol*. 2012; 180:2018–2027. [PubMed: 22449948]
28. Gao L, Faibish D, Fredman G, Herrera BS, Chiang N, Serhan CN, Van Dyke TE, Gyurko R. Resolvin E1 and Chemokine-like Receptor 1 Mediate Bone Preservation. *J Immunol*. 2013; 190:689–694. [PubMed: 23241890]
29. Norling LV, Dalli J, Flower RJ, Serhan CN, Perretti M. Resolvin D1 limits polymorphonuclear leukocytes recruitment to inflammatory loci: receptor dependent actions. *Arterioscler Thromb Vasc Biol*. 2012; 32:1970–1978. [PubMed: 22499990]

30. Brancaleone V, Gobbetti T, Cenac N, le Faouder P, Colom B, Flower RJ, Vergnolle N, Nourshargh S, Perretti M. A vasculo-protective circuit centered on lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 operative in murine microcirculation. *Blood*. 2013; 122:608–617. [PubMed: 23733341]
31. Serhan CN, Chiang N. Resolution phase lipid mediators of inflammation: agonists of resolution. *Curr Opin Pharmacol*. 2013; 13:632–640. [PubMed: 23747022]
32. Chiang N, Dalli J, Colas RA, Serhan CN. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J Exp Med*. 2015; 212:1203–1217. [PubMed: 26195725]
33. Li D, Hodges RR, Jiao J, Carozza RB, Shatos MA, Chiang N, Serhan CN, Dartt DA. Resolvin D1 and aspirin-triggered resolvin D1 regulate histamine-stimulated conjunctival goblet cell secretion. *Mucosal Immunol*. 2013; 6:1119–1130. [PubMed: 23462912]
34. Cooray SN, Gobbetti T, Montero-Melendez T, McArthur S, Thompson D, Clark AJ, Flower RJ, Perretti M. Ligand-specific conformational change of the G-protein-coupled receptor ALX/FPR2 determines proresolving functional responses. *Proc Natl Acad Sci U S A*. 2013; 110:18232–18237. [PubMed: 24108355]
35. Lu Y, Hong S, Tjonahen E, Serhan CN. Mediator-lipidomics: databases and search algorithms for PUFA-derived mediators. *J Lipid Res*. 2005; 46:790–802. [PubMed: 15722568]
36. Serhan CN, Petasis NA. Resolvins and protectins in inflammation-resolution. *Chem Rev*. 2011; 111:5922–5943. [PubMed: 21766791]
37. Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA. Macrophage pro-resolving mediator maresin 1 stimulates tissue regeneration and controls pain. *FASEB J*. 2012; 26:1755–1765. [PubMed: 22253477]
38. Bento AF, Claudino RF, Dutra RC, Marcon R, Calixto JB. Omega-3 fatty acid-derived mediators 17(R)-hydroxy docosahexaenoic acid, aspirin-triggered resolvin D1 and resolvin D2 prevent experimental colitis in mice. *J Immunol*. 2011; 187:1957–1969. [PubMed: 21724996]
39. Chan MM, Moore AR. Resolution of inflammation in murine autoimmune arthritis is disrupted by cyclooxygenase-2 inhibition and restored by prostaglandin E2-mediated lipoxin A4 production. *J Immunol*. 2010; 184:6418–6426. [PubMed: 20435922]
40. Bang S, Yoo S, Yang TJ, Cho H, Hwang SW. 17(R)-resolvin D1 specifically inhibits transient receptor potential ion channel vanilloid 3 leading to peripheral antinociception. *Br J Pharmacol*. 2012; 165:683–692. [PubMed: 21718307]
41. Rajasagi NK, Reddy PB, Suryawanshi A, Mulik S, Gjorstrup P, Rouse BT. Controlling herpes simplex virus-induced ocular inflammatory lesions with the lipid-derived mediator resolvin E1. *J Immunol*. 2011; 186:1735–1746. [PubMed: 21187448]
42. Titos E, Rius B, Gonzalez-Periz A, Lopez-Vicario C, Moran-Salvador E, Martinez-Clemente M, Arroyo V, Claria J. Resolvin D1 and its precursor docosahexaenoic acid promote resolution of adipose tissue inflammation by eliciting macrophage polarization toward an M2-like phenotype. *J Immunol*. 2011; 187:5408–5418. [PubMed: 22013115]
43. White PJ, Arita M, Taguchi R, Kang JX, Marette A. Transgenic restoration of long-chain n-3 fatty acids in insulin target tissues improves resolution capacity and alleviates obesity-linked inflammation and insulin resistance in high-fat-fed mice. *Diabetes*. 2010; 59:3066–3073. [PubMed: 20841610]
44. Morris T, Stables M, Colville-Nash P, Newson J, Bellingan G, de Souza PM, Gilroy DW. Dichotomy in duration and severity of acute inflammatory responses in humans arising from differentially expressed proresolution pathways. *Proc Natl Acad Sci U S A*. 2010; 107:8842–8847. [PubMed: 20421472]
45. Morris T, Stables M, Hobbs A, de Souza P, Colville-Nash P, Warner T, Newson J, Bellingan G, Gilroy DW. Effects of low-dose aspirin on acute inflammatory responses in humans. *J Immunol*. 2009; 183:2089–2096. [PubMed: 19597002]
46. Psychogios N, Hau DD, Peng J, Guo AC, Mandal R, Bouatra S, Sinelnikov I, Krishnamurthy R, Eisner R, Gautam B, Young N, Xia J, Knox C, Dong E, Huang P, Hollander Z, Pedersen TL, Smith SR, Bamforth F, Greiner R, McManus B, Newman JW, Goodfriend T, Wishart DS. The human serum metabolome. *PLoS One*. 2011; 6:e16957. [PubMed: 21359215]

47. Raatz SK, Golovko MY, Brose SA, Rosenberger TA, Burr GS, Wolters WR, Picklo MJ Sr. Baking reduces prostaglandin, resolvin, and hydroxy-fatty acid content of farm-raised Atlantic salmon (*Salmo salar*). *J Agric Food Chem*. 2011; 59:11278–11286. [PubMed: 21919483]
48. Tobin DM, Roca FJ, Oh SF, McFarland R, Vickery TW, Ray JP, Ko DC, Zou Y, Bang ND, Chau TT, Vary JC, Hawn TR, Dunstan SJ, Farrar JJ, Thwaites GE, King MC, Serhan CN, Ramakrishnan L. Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. *Cell*. 2012; 148:434–446. [PubMed: 22304914]
49. Perretti M, Dalli J. Exploiting the Annexin A1 pathway for the development of novel anti-inflammatory therapeutics. *Br J Pharmacol*. 2009; 158:936–946. [PubMed: 19845684]
50. Chiang N, Shinohara M, Dalli J, Mirakaj V, Kibi M, Choi AMK, Serhan CN. Inhaled carbon monoxide accelerates resolution of inflammation via unique pro-resolving mediator–heme oxygenase-1 circuits. *J Immunol*. 2013; 190:6378–6388. [PubMed: 23650615]
51. Flannigan KL, Agbor TA, Blackler RW, Kim JJ, Khan WI, Verdu EF, Ferraz JG, Wallace JL. Impaired hydrogen sulfide synthesis and IL-10 signaling underlie hyperhomocysteinemia-associated exacerbation of colitis. *Proc Natl Acad Sci U S A*. 2014; 111:13559–13564. [PubMed: 25187563]
52. Dinarello CA. Anti-inflammatory agents: present and future. *Cell*. 2010; 140:935–950. [PubMed: 20303881]
53. Yang R, Chiang N, Oh SF, Serhan CN. Metabolomics-lipidomics of eicosanoids and docosanoids generated by phagocytes. *Curr Protoc Immunol*. 2011; (Suppl 95):14.26.11–14.26.26. [PubMed: 21462168]
54. Boilard E, Nigrovic PA, Larabee K, Watts GF, Coblyn JS, Weinblatt ME, Massarotti EM, Remold-O'Donnell E, Farndale RW, Ware J, Lee DM. Platelets amplify inflammation in arthritis via collagen-dependent microparticle production. *Science*. 2010; 327:580–583. [PubMed: 20110505]
55. Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol*. 1967; 13:269–288. [PubMed: 6025241]
56. Norling LV, Dalli J. Microparticles are novel effectors of immunity. *Curr Opin Pharmacol*. 2013; 13:570–575. [PubMed: 23742926]
57. Hunter MP, Ismail N, Zhang X, Aguda BD, Lee EJ, Yu L, Xiao T, Schafer J, Lee ML, Schmittgen TD, Nana-Sinkam SP, Jarjoura D, Marsh CB. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One*. 2008; 3:e3694. [PubMed: 19002258]
58. Cantaluppi V, Gatti S, Medica D, Figliolini F, Bruno S, Deregibus MC, Sordi A, Biancone L, Tetta C, Camussi G. Microvesicles derived from endothelial progenitor cells protect the kidney from ischemia-reperfusion injury by microRNA-dependent reprogramming of resident renal cells. *Kidney Int*. 2012; 82:412–427. [PubMed: 22495296]
59. Brown GT, McIntyre TM. Lipopolysaccharide signaling without a nucleus: kinase cascades stimulate platelet shedding of proinflammatory IL-1 β -rich microparticles. *J Immunol*. 2011; 186:5489–5496. [PubMed: 21430222]
60. Agouni A, Mostefai HA, Porro C, Carusio N, Favre J, Richard V, Henrion D, Martinez MC, Andriantsitohaina R. Sonic hedgehog carried by microparticles corrects endothelial injury through nitric oxide release. *FASEB J*. 2007; 21:2735–2741. [PubMed: 17428963]
61. Mack M, Kleinschmidt A, Bruhl H, Klier C, Nelson PJ, Cihak J, Plachy J, Stangassinger M, Erfle V, Schlondorff D. Transfer of the chemokine receptor CCR5 between cells by membrane-derived microparticles: a mechanism for cellular human immunodeficiency virus 1 infection. *Nat Med*. 2000; 6:769–775. [PubMed: 10888925]
62. Dalli J, Norling LV, Renshaw D, Cooper D, Leung K-Y, Perretti M. Annexin 1 mediates the rapid anti-inflammatory effects of neutrophil-derived microparticles. *Blood*. 2008; 112:2512–2519. [PubMed: 18594025]
63. Norling LV, Spite M, Yang R, Flower RJ, Perretti M, Serhan CN. Cutting edge: Humanized nanoproresolving medicines mimic inflammation-resolution and enhance wound healing. *J Immunol*. 2011; 186:5543–5547. [PubMed: 21460209]
64. Dalli J, Serhan CN. Specific lipid mediator signatures of human phagocytes: microparticles stimulate macrophage efferocytosis and pro-resolving mediators. *Blood*. 2012; 120:e60–72. [PubMed: 22904297]

65. Dalli J, Norling LV, Montero-Melendez T, Federici Canova D, Lashin H, Pavlov AM, Sukhorukov GB, Hinds CJ, Perretti M. Microparticle alpha-2-macroglobulin enhances pro-resolving responses and promotes survival in sepsis. *EMBO Mol Med*. 2014; 6:27–42. [PubMed: 24357647]
66. Arnardottir HH, Dalli J, Colas RA, Shinohara M, Serhan CN. Aging delays resolution of acute inflammation in mice: reprogramming the host response with novel nano-proresolving medicines. *J Immunol*. 2014; 193:4235–4244. [PubMed: 25217168]
67. Ji RR, Xu ZZ, Gao YJ. Emerging targets in neuroinflammation-driven chronic pain. *Nat Rev Drug Discov*. 2014; 13:533–548. [PubMed: 24948120]
68. Mirakaj V, Dalli J, Granja T, Rosenberger P, Serhan CN. Vagus nerve controls resolution and pro-resolving mediators of inflammation. *J Exp Med*. 2014; 211:1037–1048. [PubMed: 24863066]
69. Kasuga K, Yang R, Porter TF, Agrawal N, Petasis NA, Irimia D, Toner M, Serhan CN. Rapid appearance of resolvins precursors in inflammatory exudates: novel mechanisms in resolution. *J Immunol*. 2008; 181:8677–8687. [PubMed: 19050288]
70. Bellora F, Castriconi R, Dondero A, Reggiardo G, Moretta L, Mantovani A, Moretta A, Bottino C. The interaction of human natural killer cells with either unpolarized or polarized macrophages results in different functional outcomes. *Proc Natl Acad Sci U S A*. 2010; 107:21659–21664. [PubMed: 21118979]
71. Serhan CN, Yang R, Martinod K, Kasuga K, Pillai PS, Porter TF, Oh SF, Spite M. Maresins: novel macrophage mediators with potent anti-inflammatory and pro-resolving actions. *J Exp Med*. 2009; 206:15–23. [PubMed: 19103881]
72. Pellettieri J, Fitzgerald P, Watanabe S, Mancuso J, Green DR, Alvarado AS. Cell death and tissue remodeling in planarian regeneration. *Dev Biol*. 2010; 338:76–85. [PubMed: 19766622]
73. Dalli J, Zhu M, Vlasenko NA, Deng B, Haeggstrom JZ, Petasis NA, Serhan CN. 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*. 2013; 27:2573–2583. [PubMed: 23504711]
74. Libby P, Tabas I, Fredman G, Fisher EA. Inflammation and its resolution as determinants of acute coronary syndromes. *Circ Res*. 2014; 114:1867–1879. [PubMed: 24902971]
75. Legg K. Infectious disease: Pro-resolving lipids offer a helping hand to antibiotics. *Nat Rev Drug Discov*. 2012; 11:441. [PubMed: 22596252]
76. Markworth JF, Vella LD, Lingard BS, Tull DL, Rupasinghe TW, Sinclair AJ, Maddipati KR, Cameron-Smith D. Human inflammatory and resolving lipid mediator responses to resistance exercise and ibuprofen treatment. *Am J Physiol Regul Integr Comp Physiol*. 2013; 305:R1281–R1296. [PubMed: 24089379]
77. Sasaki A, Fukuda H, Shiida N, Tanaka N, Furugen A, Ogura J, Shuto S, Mano N, Yamaguchi H. Determination of omega-6 and omega-3 PUFA metabolites in human urine samples using UPLC/MS/MS. *Anal Bioanal Chem*. 2015; 407:1625–1639. [PubMed: 25577352]
78. Colas RA, Shinohara M, Dalli J, Chiang N, Serhan CN. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. *Am J Physiol Cell Physiol*. 2014; 307:C39–54. [PubMed: 24696140]
79. Barden A, Mas E, Croft KD, Phillips M, Mori TA. Short-Term n-3 Fatty Acid Supplementation but not Aspirin Increases Plasma Proresolving Mediators of Inflammation. *J Lipid Res*. 2014; 55:2401–2407. [PubMed: 25187667]
80. Jones ML, Mark PJ, Keelan JA, Barden A, Mas E, Mori TA, Waddell BJ. Maternal dietary omega-3 fatty acid intake increases resolvins and protectins levels in the rat placenta. *J Lipid Res*. 2013; 54:2247–2254. [PubMed: 23723388]
81. Mas E, Croft KD, Zahra P, Barden A, Mori TA. Resolvins D1, D2, and other mediators of self-limited resolution of inflammation in human blood following n-3 fatty acid supplementation. *Clin Chem*. 2012; 58:1476–1484. [PubMed: 22912397]
82. Keelan JA, Mas E, D'Vaz N, Dunstan JA, Li S, Barden AE, Mark PJ, Waddell BJ, Prescott SL, Mori TA. Effects of maternal n-3 fatty acid supplementation on placental cytokines, pro-resolving lipid mediators and their precursors. *Reproduction*. 2015; 149:171–178. [PubMed: 25504868]
83. Weiss GA, Troxler H, Klinker G, Rogler D, Braegger C, Hersberger M. 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.* 2013; 12:89. [PubMed: 23767972]
84. Mas E, Barden A, Burke V, Beilin LJ, Watts GF, Huang RC, Puddey IB, Irish AB, Mori TA. A randomized controlled trial of the effects of n-3 fatty acids on resolvins in chronic kidney disease. *Clin Nutr.* 2015 doi: 10.1016/j.clnu.2015.1004.1004.
85. Arnardottir H, Orr SK, Dalli J, Serhan CN. Human milk proresolving mediators stimulate resolution of acute inflammation. *Mucosal Immunol.* 2015 in press.
86. Arita M, Oh S, Chonan T, Hong S, Elangovan S, Sun Y-P, Uddin J, Petasis NA, Serhan CN. Metabolic inactivation of resolvin E1 and stabilization of its anti-inflammatory actions. *J Biol Chem.* 2006; 281:22847–22854. [PubMed: 16757471]
87. Aven Therapeutics. RX 10045 -- ocular inflammation. 2013. <http://www.auventx.com/aven/products/rx10045.php>
88. Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, Petasis NA, Levy BD, Serhan CN, Van Dyke TE. RvE1 protects from local inflammation and osteoclast mediated bone destruction in periodontitis. *FASEB J.* 2006; 20:401–403. [PubMed: 16373400]
89. Deng B, Wang CW, Arnardottir HH, Li Y, Cheng CY, Dalli J, Serhan CN. Maresin biosynthesis and identification of maresin 2, a new anti-inflammatory and pro-resolving mediator from human macrophages. *PLoS One.* 2014; 9:e102362. [PubMed: 25036362]

Figure 1. SPM identification in human macrophage: regulation during efferocytosis and by neutrophil microparticles

Macrophages were incubated with PMN microparticles (MP) before addition of apoptotic (Apop) PMN. (A) Representative MRM traces for identified LMs. (B) Accompanying MS/MS spectra used for SPM identification. (C) Specific bioactive lipid mediator and precursor/pathway markers where: Q1, M-H (parent ion); and Q3, diagnostic ion in the MS-MS (daughter ion) along with mean $\pm$ SEM values for each of the mediators identified. Quantification and values obtained after PMN (3×10^5 PMN) and MP (2×10^5 MP) incubations. The detection limit was ~ 1 pg. *Below limits. Results are expressed as mean $\pm$ SEM; n = 4 distinct cell preparations. For additional details, see ref. (64) and text for further details.

Figure 2. Upregulation of SPM in macrophages by apoptotic PMN and PMN microparticles
Incubations were conducted as in Figure 1, lipid mediators were identified and quantified by LC-MS-MS. (A) D-series resolvins, protectins, and maresins. (B) Lipoxins. (C) Prostaglandins and thromboxanes. Results are expressed as mean $\pm$ SEM; $n = 4$ distinct cell preparations. * $P < .05$ vs macrophage group. ** $P < .01$ vs macrophage group. # $P < .05$ vs macrophage plus Apo PMN group. (D) Uptake of the CFDA-labeled Apo PMN was monitored after incubation with the indicated MP concentrations. Results are expressed as mean $\pm$ SEM ($n = 4$ distinct cell preparations). * $P < .05$ vs macrophage plus PMN group. ** $P < .01$ vs macrophage plus Apo PMN group. (E) Schematic highlighting the contribution of apoptotic PMN and microparticles to SPM biosynthesis in macrophages. For additional details, see ref. (64) and text for further details.

Figure 3. Distinct macrophage subtypes display characteristic LM profiles

Human M1 and M2 macrophages were obtained by incubating peripheral blood monocytes with GM-CSF, IFN- γ and LPS or M-CSF and IL4 respectively and LM profiles assessed by LC-MS-MS. Results are representative on n=6-8 separate cell preparations. Results are representative of 6-8 separate cell suspensions. The original results were obtained in ref. (64) and replotted here in Fig. 3.

Figure 4. The macrophage derived pro-resolving mediator MaR1 stimulates tissue regeneration (A-C) Anterior portions of brown planaria were surgically removed, planaria were exposed to vehicle or MaR1 and regeneration assessed 4 days after surgery using fluorescently conjugated *Erythrina cristagalli* lectin to visualize secretory cells. (D) After surgery planaria were kept in water containing vehicle, or the indicated concentrations of MaR1. We direct interested readers to the following publication for detailed experimental conditions (37). Results are means $\pm$ SEM (n=5 planaria/group). *P < 0.05, **P < 0.01, ***P < 0.001 vs. vehicle alone.

Figure 5. Maresins and Resolvin D1 promote macrophage phenotype switch

M1 macrophages were incubated with vehicle, 13S,14S-epoxy-MaR, MaR1, or RvD1 for 6 h. Expression of M1 (CD54, CD80) and M2 (CD163, CD206) was assessed by flow cytometry using fluorescently conjugated antibodies. Results are representative of $n = 3$ for each incubation conditions. For additional details, see the text and ref. (64) for original data set.

Figure 6. Maresin biosynthesis and actions

DHA is converted by human 12-lipoxygenase through a lipoxygenase reaction involving the abstraction of hydrogen and the antarafacial addition of molecular oxygen at carbon 14 to produce 14*S*-HpDHA, which is also converted to the 13,14-epoxide by the same enzyme (89) denoted 13*S*,14*S*-epoxide-maresin, which is further converted to the bioactive Maresin 1, which promotes resolution, reduces pain signaling, and promotes tissue regeneration. See text for further details and refs. (37, 71, 73). The epoxide 13,14*S*-epoxy-maresin is also bioactive and inhibits LTA₄ hydrolase, limiting the production of LTB₄ as well as the conversion of arachidonic acid to 12*S*-HpETE. These actions of the maresin epoxide indicate that activation of the pathway leads to down regulation of pro-inflammatory mediators during resolution.