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LIPID MEDIATORS IN IMMUNE REGULATION AND RESOLUTION

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ABSTRACT

We are all too familiar with the events that follow a bee sting – heat, redness, swelling and pain. These are Celsus' four cardinal signs of inflammation that are driven by very well defined signals and hormones; in fact targeting the factors that drive this onset phase is the basis upon which most current anti-inflammatory therapies were developed. We are also very well aware that within a few hours these cardinal signs normally disappear. In other words, inflammation resolves. When it does not, inflammation persists resulting in damaging chronic conditions. While inflammatory onset is actively driven so also is its resolution – years of research has identified novel internal counter-regulatory signals that work together to switch off inflammation. Among these signals, lipids are potent signaling molecules that regulate an array of immune responses including vascular hyper reactivity and pain as well as leukocyte trafficking and clearance, so-called resolution. Here, we collate bioactive lipid research to date and summarise the major pathways involved in their biosynthesis and their role in inflammation as well as resolution.

ABBREVIATIONS

Arachidonic acid (AA)

Cyclooxygenase (COX)

Thromboxane A synthase (TXAS)

Prostaglandin D synthase (PGDS)

Prostaglandin E synthase (PGES-1, -2 and -3)

Prostaglandin F synthase (PGFS)

Resolvins (Rvs)

Protectins (PDs)

IκB kinase (IKK),

Cytochrome P450 (CYP450)

Soluble (sEH) and microsomal (mEH) epoxide hydrolase's

Dihydroxy-eicosatrienoic acids (DHETs).

Intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1)

Cysteinyl leukotriene receptor 1 and 2 (cys-LT1 and cys-LT2).

Lipoxins (LXs)

15 epimeric-LX (15-epi-LXs) or aspirin-trigged LXs (ATL)

18*R*-hydroxyeicosapentanoic acid (18*R*-HEPE)

Eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA)

Omega-3 polyunsaturated fatty acids (ω3-PUFA),

Resolvin D1 (RvD1)

Protectin D1 (PD1)

Neuroprotectin D1 (NPD1).

14*S*-hydroperoxydocosahexaenoic acid (14*S*-HPDHA; maresin, MaR1)

Maresins (MaR)

List of Hyperlinks for Crosschecking

naproxen

Prednisone

Infliximab

Anakinra

Cyclooxygenase

arachidonic acid

PGG_2

PGH_2

PGD_2

PGE_2

$PGF_{2\alpha}$

PGI_2

TXA_2

prostanoid receptors

PGJ_2, $\Delta 12,14\text{-}PGJ_2$

15-deoxy-$\Delta 12,14\text{-}PGJ_2$ [

PPAR-γ

Cytochrome P450s

CYP2J2

linoleic acid

docosahexaenoic acid

eicosapentaenoic acid

lipoxygenase

5,6--EET

20-hydroxyeicosatetraenoic

TRP

GRP40

LTC_4

LTD_4

LTE_4

LTB_4

LT receptors

lipoxins

LXB_4

ALX

resolvins

ChemR23

INFLAMMATION AND ITS RESOLUTION

Inflammation is a protective response against infection and/or injury. However, when it becomes dysregulated as a consequence of genetic abnormalities, the ageing process or environmental factors, our immune system has the capacity to cause extensive damage. Arthritis, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, atherosclerosis and even cancer, while aetiologically disparate, are diseases unified by a dysregulated immune component. The current strategy of treating such diseases is based, largely, upon inhibiting the factors that drive acute inflammation such as nonsteroidal anti-inflammatory drugs (NSAIDS - naproxen, diclofenac, etc), steroids (Prednisone) and 'biologic' drugs such as Infliximab (anti-TNF) and Anakinra (anti-IL-1). Although these medicines ameliorate disease symptoms they do not bring about a 'cure' and are ineffective in a significant subset of patients. Furthermore, side effects can hamper endogenous homeostatic systems, predisposing to infection. Thus, there is a need to develop more efficient and effective therapeutics; with one approach being to harness the bodies own healing process for therapeutic gain.

Consequently, attention has turned to the other end of the inflammatory spectrum, resolution, in order to understand the endogenous processes that switch off inflammation. Our objective has been to identify novel internal counter-regulatory systems that terminate inflammation in order to provide new targets that can be harnessed pharmacologically to push on-going inflammation down a pro-resolution pathway. Consequently, resolution is now been studied in great detail with clear evidence suggesting that resolution is an active process with quantifiable indices and

specific requirements. Along these lines, lipid mediators have emerged as internal regulatory signals that activate many aspect of the inflammation/resolution cascade including terminating leukocyte trafficking into tissue once the inflammatory signal has been removed, scavenging pro-inflammatory signals as well as clearing dead cells from the resolves site. Hence, in this review the role of lipids in the resolution cascade will be discussed.

CYCLOOXYGENASE AND PROSTANOIDS

Cyclooxygenase (COX) converts arachidonic acid (AA) to form PGG_2 (Pagels, Sachs, Marnett, Dewitt, Day & Smith, 1983) with the peroxidase element of the enzyme further reducing PGG_2 to PGH_2 (Hamberg & Samuelsson, 1973), which serves as a precursor for all major prostanoid mediators. There are two principle isoforms involved in the conversion of AA to prostanoids, namely COX-1 and COX-2. Unlike COX-1, which is constitutively expressed in most cells and tissues and is broadly involved in house-keeping functions, COX-2 is induced in response to inflammatory (Dubois et al., 1998) being expressed at sites of infection and injury with the exception of parts of the brain and kidney (Harris, McKanna, Akai, Jacobson, Dubois & Breyer, 1994). Formation of prostanoids from PGH_2 occurs through the actions of downstream synthases that are expressed in a tissue and cell type-selective fashion including prostaglandin D synthase (PGDS) (Shimizu, Yamamoto & Hayaishi, 1982) prostaglandin E synthase (PGES-1, -2 and -3) (Tanaka, Ward & Smith, 1987), prostaglandin F synthase (PGFS) (Hayashi, Fujii, Watanabe, Urade & Hayaishi, 1989), prostaglandin I synthase (PGIS), and thromboxane A synthase (TXAS) (Ullrich & Haurand, 1983), which form PGD_2, PGE_2,

$PGF_{2\alpha}$, PGI_2 (also known as prostacyclin) and TXA_2, respectively. The differential expression of these downstream enzymes within cells determines the profile and levels of prostanoid production generated under resting and inflammatory conditions.

Presently there are nine known prostanoid receptors in mice and man. These include the PGD receptors DP1 and DP2, the PGE_2 receptors, EP1, EP2, EP3 and EP4; the PGF receptor, FP; the PGI receptor, IP; and the TXA receptor, TP. In addition, there are splice variants of the EP3, FP and TP receptors differentiated only in their C-terminal tails. All of these receptors belong to the G-protein coupled receptor (GPCR) superfamily of proteins, with the exception of DP2 (also known as CRTH2), which is a member of the chemoattractant receptor family (Hirai et al., 2001). The IP, DP1, EP2 and EP4 receptors signal through G_s resulting in an increased intracellular cAMP, whereas the EP3 receptor couples to G_i to reduce cAMP, while EP1, FP and TP receptors signal through G_q to induce calcium mobilization.

The more common prostanoids, PGE_2 and PGI_2, both enhance vasodilation (Kaley, Hintze, Panzenbeck & Messina, 1985), oedema formation and vascular permeability particularly in the presence of histamine, bradykinin and 5-HT (Hata & Breyer, 2004). Mice that are genetically depleted for their respective receptors (IP, EP2 and EP3) show reduced pleural exudation following treatment with inflammogens including carrageenan and zymosan (Yuhki et al., 2004).

Robust evidence from EP-deficient mice has shown that the febrile response to PGE_2 arises from the actions of PGE_2 on its EP3 receptor, which is present on sensory neurons in the periphery and brain (Dantzer, Konsman, Bluthe & Kelley,

2000). Equally, PGE_2 is a potent pyretic agents known with elevated concentrations found in cerebrospinal fluid taken from patients with bacterial or viral infections (Saxena, Beg, Singhal & Ahmad, 1979). While none of the prostanoids cause pain directly, PGI_2 and PGE_2 reduce the threshold of nociceptor sensory neurons to stimulation when bound to IP, EP1, EP3 and EP4 receptors, respectively (Ahmadi, Lippross, Neuhuber & Zeilhofer, 2002).

Prostanoids also play an important role in protecting against oxidative injury in cardiac tissue and in maintaining cardiovascular (CV) homeostasis. Indeed, their protective effect has been demonstrated in clinical studies, which found an increase risk of myocardial infarction (MI), stroke, systemic and pulmonary hypertension, thrombosis and sudden cardiac death following the use of COX-2 specific inhibitors (Garcia Rodriguez, Tacconelli & Patrignani, 2008). Furthermore, deleting specific prostanoid synthases and receptors result in an augmentation of ischemia/reperfusion injury (Xiao et al., 2001) as well as contributing to the decline in cardiac function following MI. CV health is regulated by vasodilatory PGI_2 and pro-thrombotic TXA_2 (Bunting, Moncada & Vane, 1983), where PGI_2 counterbalance the actions of TXA_2 (Grosser, Fries & FitzGerald, 2006). Indeed, endothelial PGI_2 along with NO prevent TXA_2-induced platelet aggregation and thrombosis. TXA_2 is derived from platelet COX-1 causing platelet aggregation and vascular smooth muscle contraction (Ellis et al., 1976). Clinical CV diseases including unstable angina, MI and stroke can arise from overproduction of TXA_4. Importantly, the cardio-protective properties of aspirin can be attributed to the covalent inhibition of COX-1 (Rocca et al., 2002).

As well as being pro-inflammatory, many prostanoids upregulate intracellular cAMP triggering immuno-suppressive effects. For example, PGE_2 and PGI_2 reduce the ability of inflammatory leukocytes to phagocytose and kill microorganisms (Aronoff, Canetti & Peters-Golden, 2004), as well as inhibit the production of downstream pro-inflammatory mediators (Aronoff et al., 2007) while, in contrast, triggering the synthesis of IL-10 and IL-6 (Harizi, Juzan, Pitard, Moreau & Gualde, 2002). Indeed, in a number of conditions associated with increased susceptibility to infection, including cancer (Starczewski, Voigtmann, Peskar & Peskar, 1984), aging (Hayek et al., 1997) and cystic fibrosis (Medjane, Raymond, Wu & Touqui, 2005) overexpression of PGE_2 has been reported. Interestingly, during the very early phase of acute inflammation, PGE_2 indirectly exerts pro-resolution effects by switching on the transcription of enzymes necessary for the generation of LXs (Levy, Clish, Schmidt, Gronert & Serhan, 2001), resolvins (Rvs) and protectins (PDs) (Hong, Gronert, Devchand, Moussignac & Serhan, 2003); these represent other classes of lipids mediators with pro-resolution properties.

While PGD_2 can elevate cAMP via its DP1, PGD2 may also act independently of its DP1 and DP2 receptors when it non-enzymatically dehydrates into prostaglandins of the J2 series (e.g. PGJ_2, $\Delta 12,14\text{-}PGJ_2$ and 15-deoxy-$\Delta 12,14\text{-}PGJ_2$ [15d-PGJ_2]) (Clark, Bishop-Bailey, Estrada-Hernandez, Hla, Puddington & Padula, 2000). These cyclopentenone PGs form covalent attachments with reactive sulphydryl groups on intracellular regulatory proteins, which enables modulation of their function. For instance, 15d-PGJ_2 upon ligation to the nuclear receptor PPAR-γ (Khan, 1995), decreases pro-inflammatory cytokine release and modifies gene expression (Jiang, Ting & Seed, 1998) as well as directly inhibiting the actions of IκB kinase (IKK),

which is responsible for the activation of NF-κB (Cernuda-Morollon, Pineda-Molina, Canada & Perez-Sala, 2001). 15d-PGJ_2, identified in rodent peritonitis resolution exudates (Rajakariar et al., 2007), independently of PPAR-γ, can preferentially inhibit monocyte rather than neutrophil trafficking through differential regulation of cell-adhesion molecule and chemokine expression (Gilroy, Colville-Nash, McMaster, Sawatzky, Willoughby & Lawrence, 2003); regulate macrophage activation and pro-inflammatory gene expression (Lawrence, 2002); and induce leukocyte apoptosis through a caspase-dependent mechanism (Bishop-Bailey & Hla, 1999). Moreover, it has been shown that PGD_2-derived compounds function as endogenous breaking signals for lymphocytes to stimulate resolution (Trivedi et al., 2006). See Table 1 for prostanoids their bio-actions and concentrations at sites of inflammation.

PROSTANOIDS AND POST-RESOLUTION BIOLOGY

Recently, we demonstrated that classical resolution may not be the end of the local immune response to infection/injury, but rather that a third phase subsequent to these exists: post-resolution (Motwani et al., 2017). Traditionally, resolution processes were deemed successful if acute inflammation, as described by leukocyte clearance and cytokine catabolism, was terminated; however they may have a hitherto unappreciated role in controlling adaptive immune responses and maintaining tolerance. Specifically, we found that murine innate immune-mediated responses to low-dose yeast cell wall extract (zymosan, administaered intra perioneally [i.p.]) or bacteria (*S. pneumoniae*$^{ovalbumin\text{-}labelled}$*, i.p.*) resolved. Interestingly, these low-dose stimuli elicited a previously overlooked second wave of leukocyte influx into tissues that persisted for weeks. These cells comprised three separate populations of $Ly6c^{hi}$ monocyte-derived macrophages (MDMs) including

$CD11B^+/CD49d^+/CD115^+/MHC\text{-}II^+$ myeloid-derived suppressor cells (MDSCs), $F4\text{-}80^{lo}/MHC\text{-}II^+/CD11c^+$ dendritic cells (DCs) and $F4\text{-}80^{int}/CD11B^{hi}/CD11c^-$ macrophages. In addition, tissue-resident (embryonic-derived) macrophages, which disappear during the acute inflammatory response, re-appear. These diverse populations of macrophages were observed alongside lymph node expansion and increased numbers of peripheral blood and tissue memory T and B lymphocytes. polymorphonuclear (PMNs) were not present during this phase. One of the key events in this process is the sustained synthesis of PGE_2, which is derived from macrophage COX-1/mPGES and that is triggered by IFNγ. It transpires that this post-resolution phase of prostanoid biosynthesis creates a window of susceptibility to repeat infections on the one hand, while also controlling local adaptive immune processes on the other (Newson et al., 2017). The nature of these prostanoid/adaptive immune interactions is being investigated.

CYTOCHROME P450

Cytochrome P450s (CYP450s) are a family of membrane-bound, haem-containing enzymes found in the liver, Kidneys, brain, heart, CV system and lung and are best characterized for the catalysis of NADPH-dependent oxidation of drugs, chemicals and carcinogens and hormones (Nelson et al., 1996). The CYP450 family contains 57 genes in humans, and although approximately one quarter of these have been shown capable of metabolizing PUFAs, the CYP2J2 and CYP2C family members (CYP2C8, 2C9) are thought to be the major enzymes responsible for lipid mediator production (Bishop-Bailey, Thomson, Askari, Faulkner & Wheeler-Jones, 2014). In addition to metabolizing AA **(Figure 1)**, CYP450s also readily metabolise the related ω6 PUFA linoleic acid (LA) **Figure 2**, and ω3 PUFAs (see below also)

docosahexaenoic acid (DHA, **Figure 3**) and eicosapentaenoic acid (EPA, **Figure 4**) in to series of related biologically active mediators (Smilowitz et al., 2013). CYP450 are capable or metabolizing PUFA substrates by epoxygenase, lipoxygenase and ω-hydroxylase type activities (Zeldin, 2001). The epoxygenase activity inserts a single molecular oxygen in to one of the double bonds of each PUFA e.g. for AA to form one of 4 regioisomers of epoxyeicosatrienoic acid (5,6-, 8,9-, 11,12- or 14,15-EET; the numbers indicating the double bond in AA subject to epoxygenation (Zeldin, 2001). Each EET can be formed as either an *R/S* or *S/R* stereoisomer, with ratios of production depending on the generating CYP450. Stereoisomers of EETs may have different biological activities, but little research exists to understand the extent if these differences. CYP450s can also have lipoxygenase activity producing mid-chain (12[*R*]-), and ω-hydroxylase activity producing terminal (19[*S*>*R*]-, and 20-) hydroxyeicosatetraenoic acids (HETEs) (Roman, 2002). Once formed, epoxygenase products in particular are quickly metabolized by epoxide hydrolases (EH) or reincorporated in to membranes (Zeldin, 2001). Soluble (sEH) and microsomal (mEH) epoxide hydrolase's (EH; encoded by the *ephx2 and ephx1 respectively*) combine to metabolize virtually all epoxygenase products *in vivo* (Edin et al., 2018). e.g. EETs get converted to dihydroxy-eicosatrienoic acids (DHETs). Importantly, a number of sEH-inhibitors (sEH-I) have been developed that inhibit the breakdown of epoxygenase products to potentiate there signalling (Hwang, Wecksler, Wagner & Hammock, 2013).

AA and related PUFA are metabolised by CYP epoxygenase and epoxide hydolases in the vascular endothelium (Zhang, Oltman, Lu, Lee, Dellsperger & VanRollins, 2001) (Roman, 2002), and vascular smooth muscle. In vascular smooth muscle, AA

is also catalysed by CYP hydroxylases to 20-HETE (Wang, Guan, Nguyen, Zand, Nasjletti & Laniado-Schwartzman, 1999). Indeed, CYP4F3A in myeloid tissue catalyzes the ω-hydroxylation of leukotriene B_4 to 20-hydroxy leukotriene B_4, an inactivation process that is critical for the regulation of the inflammatory response (Johnson, Edson, Totah & Rettie, 2015). However, it is unknown whether CYP4F3 is the source of 20-HETE produced by PMNs (Bednar et al., 2000). These metabolites play a large and complex role in maintaining cardiac, renal and pulmonary homeostasis by regulating vascular tone and reactivity, ion transport, renal and pulmonary functions as well as growth responses (Fleming, 2007). Moreover, they have been shown to exert striking anti-inflammatory actions (Inceoglu et al., 2008), see below.

CYTOCHROME P450 AND INFLAMMATION

EETs catalysed by CYPs 2C8, 2C9 and 2J2 inhibit the activation of the transcription factor NF-κB via the inhibitor of κB kinase (IKK) (Node et al., 1999). Consequently, EETs may therefore have the propensity to down-regulate various cytokine-induced pro-inflammatory signalling pathways downstream of NF-κB activation. This may explain how EETs prevent the adhesion of PMNs to the vascular wall by suppressing the expression of cell adhesion molecules, including intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin on the surface of endothelial cells in response to cytokines (TNF-α and IL-1α), and LPS (Fleming, 2007), **Figure 5**. We recently published that epoxygenases are anti-inflammatory in human primary monocytes and macrophages (Bystrom et al., 2011), regulate M1 and M2 phenotype (Bystrom et al., 2011) and promote bacterial and lipid phagocytosis (Bystrom et al., 2013). In a mouse model of inflammatory

resolution we took this further using a CYP450 epoxygenase inhibitor SKF525A and sEH knockout mice (Gilroy et al., 2016). We reported how CYP450 epoxygenase-derived mediators play a crucial role in controlling the infiltration of monocytes into sites of inflammation and are essential for the pro-resolution phenotype of cells of the monocyte lineage (Gilroy et al., 2016) driving macrophage efferocytosis. Additionally, it was recently reported that EETs display analgesic bioactions during experimental inflammatory pain (Inceoglu et al., 2008). In general CYP450-derived epoxygenase products are anti-atherosclerotic, vasodilatory and anti-inflammatory (Chaudhary et al.), with the notable exception of linoleic acid-derived / epoxide hydrolase product DiHOMEs. DiHOMEs have recently been shown to mediate thermal hyperalgesia (Zimmer et al., 2018), and at high levels are toxic to PMNs, and were originally termed ‘leukotoxins’ (Moghaddam, Grant, Cheek, Greene, Williamson & Hammock, 1997).

The use of sEH-Is and sEH knockout mice has been invaluable to understanding the *in vivo* roles or epoxygenase products. Inhibiting sEH has revealed protective roles for epoxygenase products in injury-induced vascular neointima formation (Revermann et al.), atherosclerosis and aneurysm formation (Zhang et al., 2009), and inflammatory cell recruitment (Gilroy et al., 2016). sEH inhibition or overexpression of producing enzymes such as CYP2J2 are also protective in various acute inflammatory lung injury models (Revermann et al., 2009). EETs released from platelets exert anti-thrombotic properties by inhibiting platelet aggregation induced by AA and vascular injury (Briggs, Xiao, Parkin, Shen & Goldman, 2000). EETs can also increase the expression of tissue plasminogen activator in a cAMP-dependent

mechanism, thus suggesting potentially important roles in controlling the fibrinolytic balance at sites of inflammation (Node et al., 2001).

The identification of epoxygenase-product receptors has almost exclusively focused on arachidonic acid derived- EETs and HETEs, with very little research so far on other n3 and n6-PUFA products. Putative receptor targets include transient receptor potential (TRP) channels, peroxisome-proliferator-activator receptors, and GRP40 (Bishop-Bailey, Thomson, Askari, Faulkner & Wheeler-Jones, 2014). EETs can directly activate PPAR-γ in endothelial cells (Liu et al., 2005) and PPAR-α in monocytes with EET-mediated anti-inflammatory effects blocked by PPAR-γ (Liu et al., 2005) or PPAR-α antagonists, respectively. PPAR activation does not however account for all the anti-inflammatory effects of EETs. It has been suggested that the anti-inflammatory properties of EETs occurred through its ligation to a cell surface receptor. It was reported that EETs bind with high-affinity to an 'EET-receptor' on the surface of a monocytic cell line, belonging to a specific class of GPCRs (Behm, Ogbonna, Wu, Burns-Kurtis & Douglas, 2009). GRP40 can be activated by 14,15-EET (Ma, Wang, Chen, Zhang, Harris & Chen, 2015). However, it must be noted that GRP40 activation only occurred above 10μM (Ma, Wang, Chen, Zhang, Harris & Chen, 2015); whereas most biological effects occur in the nM range. These receptors are not present in all cells and their known actions don't always correlate with the vascular and anti-inflammatory activities of epoxygenase products. The identity of this receptor and its role, if any, in initiating the immuno-modulatory actions of EETs is yet to be determined. By contrast, intracellular signalling pathways are more established. Depending on the model system used, epoxygenases or its

products can reduce cellular activation by inhibiting NFκB, inhibiting ERK activation, elevate cAMP, and/ or induce cellular hyperpolarization (Thomson, Askari & Bishop-Bailey, 2012). Recently it has also been proposed that inhibiting inflammatory endoplasmic reticulum stress may be critical for the beneficial effects of epoxygenase products, in particular neuropathic pain.

As stated above CYP hydroxylases metabolites also possess anti-inflammatory properties. For instance, 16-HETE can block the adhesion of leukocytes to the microvascular endothelium (Bednar et al., 2000) while also suppressing the synthesis of LTs as well as inhibiting rises in cerebrospinal fluid pressure, which represents index of tissue damage and swelling, in thrombo-embolic model of stroke in rabbits (Bednar et al., 2000). Moreover, PMN-derived 20-HETE and 16-HETE also counteract TX-induced platelet aggregation (Hill, Fitzpatrick & Murphy, 1992). Therefore, it can surmised that not only do metabolites of CYPs maintain CV and renal, but they also regulate other diverse signalling pathways pertinent to fibrinolysis, platelet aggregation, inflammation and cellular injury.

LEUKOTRIENE AND LIPOXINS – BIOSYNTHESIS

Lipoxygenase (LOX) enzymes include 5-, 12-, or 15-LOX and are expressed in leukocytes, platelets and endothelial cells, respectively. 5-LOX, for instance, metabolise AA to the slow-reacting substances of anaphylaxis (LTC_4, LTD_4 and LTE_4: potent mediators of the allergic response) (Lewis, Austen, Drazen, Clark, Marfat & Corey, 1980) as well as LTB_4, a powerful PMN and eosinophil chemoattractant (Borgeat & Samuelsson, 1979).

To date, four subtypes of LT receptors have been described including B leukotriene receptor 1 and 2 (BLT1 and BLT2), and cysteinyl leukotriene receptor 1 and 2 (cys-LT1 and cys-LT2). Once bound, LTs a signal via a G-protein in the cytoplasm to increase intracellular calcium and block formation of cAMP, which then modulates diverse cellular activities ranging from motility to transcriptional activation. While Cys-LT1 mediate mucus secretion, oedema accumulation and broncho-constriction in airways (Lynch et al., 1999), Cys-LT2 drives inflammatory responses, tissue fibrosis in the lung as well as vascular permeability (Beller, Friend, Maekawa, Lam, Austen & Kanaoka, 2004). Not surprisingly, Cys-LT1 is overexpressed in patients with chronic rhinosinusitis or asthma who have aspirin sensitivity (Sousa, Parikh, Scadding, Corrigan & Lee, 2002). By comparison, BLT1 is a high-affinity receptor for LTB_4, and is responsible for its chemo-attractant and pro-inflammatory properties (Tager & Luster, 2003). Although BLT2 acts in a similar fashion to BLT1, LTB_4 affinity towards BLT1 is much higher.

In contrast, lipoxins (LXs) are a series of trihydroxytetraene-containing bioactive eicosanoids that were first isolated from human leukocytes in the mid 1980's (Serhan, Hamberg & Samuelsson, 1984). However, in contrast to LTs, which are manufactured by intracellular biosynthesis, LXs are generated through cell-cell interactions by a process known as transcellular biosynthesis. In different human cell types, during the first biosynthetic step of LX biosynthesis, LOX inserts molecular oxygen into AA. This can be achieved by two major routes - the first pathway occurs in eosinophils, monocytes, or epithelial cells and involves the oxygenation of AA at C-15 by 15-LOX yielding 15*S*-HPETE. Secreted 15*S*-HPETE is then taken up by monocytes or PMNs and converted to 5,6-epoxytetraene by 5-LOX, which is then

hydrolysed within these cells by either LXA_4 or LXB_4 hydrolase to LXA_4 or LXB_4. Activation of this pathway concomitantly reduces LT synthesis, which requires 5-LOX to convert AA into LTA_4 (Claria & Serhan, 1995). The second major route of LX biosynthesis occurs in a LTA_4-dependent manner and involves platelet-leukocyte interactions. 5-LOX within leukocytes converts AA into LTA_4, which when secreted is taken up by platelets adhering on the surface of the leukocyte and is subsequently transformed to LXA_4 and LXB_4. This occurs *via* the LX synthase activity of human 12-LOX (Romano & Serhan, 1992). A third pathway of LX generation was discovered following aspirin ingestion, which irreversibly acetylates COX-2 in endothelial cells and other activated cell types; this is a property specific to aspirin and not shared with other NSAIDs. Consequently, instead of COX-2 converting AA into PGG_2, aspirin acetylation reprograms the enzyme resulting in the transformation of AA into 15*R*-HETE (C-15 alcohol carried in the *R*-configuration). This is then metabolised in a transcellular manner by adherent leukocyte, vascular endothelial or epithelial 5-LOX to form 15 epimeric-LX (15-epi-LX) or aspirin-trigged LXs (ATL) that carry their C-15 alcohol in the R configuration rather than 15S native LX. Although initially thought to be only aspirin triggered, a pathway of endogenous 15-epi-LX generation has recently been described, where neuronal sphingosine kinase 1 mediates this COX-2 acetylation(Lee et al., 2018). ATL's share many of the immune regulatory characteristics of native LXs.

LIPOXINS – RECEPTORS AND BIO-ACTIONS

The biological actions of LXA_4 and 15-epi-LXs are mediated through ALX receptor, which is a specific G-protein-coupled receptor (GPCR) isolated and cloned in mouse, human and rat tissues (Chiang, Takano, Arita, Watanabe & Serhan, 2003); ALX is

also known as the FPRL1 receptor. Human ALX was identified and cloned in various leukocytes populations including T cells (Ariel, Chiang, Arita, Petasis & Serhan, 2003), monocytes (Maddox, Hachicha, Takano, Petasis, Fokin & Serhan, 1997) as well as tissue-resident macrophages, synovial fibroblasts (Sodin-Semrl, Taddeo, Tseng, Varga & Fiore, 2000) and intestinal epithelial cells (Gronert, Gewirtz, Madara & Serhan, 1998). LXA_4 and 15-epi-lipoxin A_4 (not for LXB_4, LTB_4, LTD_4 or PGE_2) show high affinity towards ALX (Kd = 1.7nM) [231]. ALX also has the ability to interact with other small peptides/proteins such as Ac2-26 and glucocorticoid-derived annexin-1, which carry out similar anti-inflammatory effects as LXs and 15-epi-LXs. Studies in transgenic mice over-expressing human ALX showed that the protective and immune modulatory effects of LXs and 15-epi-LXs were ligand- and receptor-dependent (Devchand et al., 2003). In a peritonitis model of zymosan-induced acute inflammation, infiltration of neutrophils was substantially diminished in ALX transgenic mice compared to their wild-type equivalents (Devchand et al., 2003) with the site of lipoxin action identified as being the leukocyte/endothelial interface mediated by the generation of nitric oxide's anti-adhesive properties (Paul-Clark, Van Cao, Moradi-Bidhendi, Cooper & Gilroy, 2004).

15-epi-LX analogues also regulate an ALX-dependent p38/MAPK cascade, known to promote chemotaxis by inhibiting leukocyte-specific AP-1 phosphorylation and activation (Ohira et al., 2004). In addition to ALX, LXs also function as partial agonists to a subclass of rhodopsin receptors (CysLT1) more commonly activated by LTs, mediating bioactions in several tissues and cell types other than leukocytes (Badr, DeBoer, Schwartzberg & Serhan, 1989). At nanomolar concentrations LXA_4 has been shown to compete for binding with LTD_4 on mesangial cells (Badr, DeBoer,

Schwartzberg & Serhan, 1989) and human umbilical vein endothelial cells (HUVECs) (Fiore, Romano, Reardon & Serhan, 1993) as well as opposing the pro-inflammatory effects of LTD_4. There is also evidence that another intracellular receptor; the Ah receptor (AhR) mediates the bioactions of LXs; AhR is a ligand activated transcription factor that can trigger such anti-inflammatory events as the expression of suppressor of cytokine signalling 2 (SOCS-2) (Aliberti, Serhan & Sher, 2002).

Lipoxins are anti-inflammatory at nanomolar concentrations controlling both granulocyte and myeloid cell entry into sites of inflammation. Indeed, the ability of LXs to diminish neutrophil trafficking was corroborated when an analogue of 15-epi-LX was intravenously administered to BLT1 knockout mice that have dramatically elevated neutrophils in the lungs after high limb ischemia-reperfusion (Chiang, Gronert, Clish, O'Brien, Freeman & Serhan, 1999). Furthermore, research in our laboratory has uncovered in humans that 15-epi-LXs regulates PMN influx in forearm blisters, accounting for low-dose aspirin's anti-inflammatory properties (Morris et al., 2009). Our additional work on resolving inflammation has revealed that humans fall into two categories, those who resolved their acute inflammatory responses in an immediate manner and those that show a more delayed or prolonged healing process, with the severity and duration controlled by endogenous epi-lipoxins/ALX expression(Morris et al., 2010). Paradoxically, while they inhibit neutrophil and eosinophil transmigration (Maddox, Colgan, Clish, Petasis, Fokin & Serhan, 1998), lipoxins promote monocyte infiltration into sites of inflammation, which, when differentiated into macrophages bring about some of they key aspects of resolution and wound healing (Maddox & Serhan, 1996) without inducing neutrophil

degranulation or release of other reactive oxygen species (Jozsef, Zouki, Petasis, Serhan & Filep, 2002)..

Once at the site of inflammation and resolution, monocyte-derived macrophages are stimulated by lipoxins to ingest and clear apoptotic neutrophils (Godson, Mitchell, Harvey, Petasis, Hogg & Brady, 2000), which maybe facilitated by changes in the actin cytoskeleton (Maderna, Cottell, Berlasconi, Petasis, Brady & Godson, 2002). Moreover, lipoxins increase levels of the anti-inflammatory cytokine TGF-β1, which, in turn, dampen a range of pro-inflammatory pathways (Bannenberg et al., 2005). LXs also anti-fibrotic thereby improve tissue remodelling by reducing the proliferation of fibroblasts and mesanglial cells induced by a numbers of factors, including connective-tissue growth factor, platelet-derived growth factor, TNF-α, LTD_4 and TGF-β (Leonard et al., 2002). 15-epi-LXs exert the same biological effects as endogenously produced LXs, but with additional properties including causing increased vasorelaxation (Serhan, 1994) and endothelial cell production of anti-inflammatory nitric oxide synthesis (Paul-Clark, Van Cao, Moradi-Bidhendi, Cooper & Gilroy, 2004). In addition, 15-epi-lipoxin A_4 inhibits TNF-α-induced IL-1β in periodontitis *in vivo* (Hachicha, Pouliot, Petasis & Serhan, 1999), down-regulates SOCS-2 signalling (Machado et al., 2006) and dampens TNF-α-induced IL-8 biosynthesis (Gronert, Gewirtz, Madara & Serhan, 1998). Expectedly, LXs and 15-epi-LXs exert beneficial effects in a range of experimental models of inflammation and human diseases including cystic fibrosis (Karp, Flick, Yang, Uddin & Petasis, 2005), glomerulonephritis (Munger et al., 1999), periodontitis (Pouliot, Clish, Petasis, Van Dyke & Serhan, 2000), ischemia/reperfusion injury (Chiang, Gronert, Clish, O'Brien, Freeman & Serhan, 1999), various cutaneous inflammation models

(Schottelius et al., 2002), pleuritis (Paul-Clark, Van Cao, Moradi-Bidhendi, Cooper & Gilroy, 2004), asthma (Levy, Bonnans, Silverman, Palmer, Marigowda & Israel, 2005), wound healing processes in the eye (Gronert, Maheshwari, Khan, Hassan, Dunn & Laniado Schwartzman, 2005), colitis, inflammation-induced hyperalgesia in rats, as well as microbial infection in mice (Aliberti, Hieny, Reis e Sousa, Serhan & Sher, 2002). See Table 1 for SPMs their bio-actions and concentrations at sites of inflammation.

SPECIALISED PRO-RESOLVING LIPID MEDIATORS (SPMs) - BIOSYNTHESIS

Omega-3 polyunsaturated fatty acids (ω3-PUFA), including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) are known to maintain organ function and health but also in reducing severity of inflammatory reactions and incidences of infection (Arita et al., 2005b). Although, also now known to be metabolized by COX, LOX and CYP450 pathways into distinct lipid mediators, a novel series of ω-3 PUFA products were identified in the resolving exudate of a mouse dorsal air pouch or peritonitis model using lipidomic and bio-informatic analysis (Lu, Hong, Tjonahen & Serhan, 2005). These endogenous mediators are called resolvins (Rvs), protectins (PDs) and maresins.

EPA or DHA generate the Rvs and are categorised as members of the E-series (from EPA) or D-series (from DHA). Both series Rvs were initially isolated from murine dorsal air pouches treated with EPA or DHA as well as aspirin. Transcellular formation of E-series Rvs occurs with the conversion of EPA to 18*R*-hydroxyeicosapentanoic acid (18*R*-HEPE) by COX-2 expressed within endothelial cells treated with aspirin. Similar to 15*R*-HETE in 15-epi-LX formation, 18R-HEPE is

released from endothelial cells to neighboring leukocytes for its conversion by 5-LOX to either RvE1 or RvE2, via a 5(6) epoxide-containing intermediate (Arita, Clish & Serhan, 2005). This interaction is blocked by selective COX-2 inhibition but not by indomethacin or paracetamol (Serhan, Clish, Brannon, Colgan, Chiang & Gronert, 2000). Although this transcellular route was proposed as the synthetic pathway for Rvs, intracellular production of resolvins and maresins have been observed in macrophages without the need for transcellular interactions. RvE1 is spontaneously produced in healthy subjects with levels increasing after treatment with either aspirin or EPA (Arita et al., 2005a). D-series Rvs, aspirin-triggered RvD1 (AT-RvD1) and RvD1 are synthesised via a pathway involving sequential oxygenations, initiated by 15-LOX or aspirin-acetylated COX-2 in the microvascular, respectively, followed by 5-LOX in human neutrophils with an epoxide containing intermediate. For AT-RvD1s, DHA is initially converted to epimeric 17*R*-hydroxydocosahexaenoic acid (17*R*-HDHA). In the absence of aspirin, however, DHA is enzymatically converted to 17*S*-HDHA (Hong, Gronert, Devchand, Moussignac & Serhan, 2003). Interestingly, generation of E-series Rvs can also be mediated by microbial and mammalian cytochrome P450 enzymes, which convert EPA into 18-HEPE. 18-HEPE can then be transformed by human neutrophils into either RvE1 or RvE2 (Serhan, Clish, Brannon, Colgan, Chiang & Gronert, 2000). Hence, it is possible that microbes at sites of infection may contribute to the production of Rvs in a similar pathway.

DHA is also a precursor for the generation of PDs being enzymatically converted by 15-LOX to a 17*S*-hydroperoxide-containing intermediate. This intermediate is then converted by leukocytes into a 16(17)-epoxide that is subsequently converted in these cells to a 10,17-dihydroxy-containing compound (Hong, Gronert, Devchand,

Moussignac & Serhan, 2003). PDs are distinguished by the presence of a conjugated triene double bond and by their potent bioactivity. One specific DHA-derived lipid mediator, 10,17*S*-docosatriene was termed protectin D1 (PD1), which when generated in neural tissue is called neuroprotectin D1 (NPD1). Moreover, PD1 exhibits tissue-specific bioactivity as in humans this lipid is synthesised by peripheral blood mononuclear cells and Th2 CD4+ T-cells, while in mice it has been isolated from exudates and brain cells, human microglial cells (Serhan et al., 2002) and in peripheral blood (Hong, Gronert, Devchand, Moussignac & Serhan, 2003).

SPMs IN INFLAMMATION AND RESOLUTION

One of the broader immunomodulatory properties of RvE1 is its ability to inhibit the accumulation neutrophil and dendritic cells at sites of inflammation. This occurs by blocking trans-endothelial migration of these cells across the microvascular endothelium as well as enhancing their clearance from inflammatory sites (Arita et al., 2005a). Other actions of RvE1 includes inhibition of reactive oxygen intermediate production from neutrophil in response to bacterial peptide, fMLP and TNFα (Gronert et al., 2004); inhibition of LTB_4-BLT1 signalling via NF-κB and hence the biosynthesis of pro-inflammatory chemokine and cytokines (Arita, Ohira, Sun, Elangovan, Chiang & Serhan, 2007); enhancement of macrophage efferocytosis of apoptotic bodies (Schwab, Chiang, Arita & Serhan, 2007); upregulation of the CC-chemokine receptor 5 (CCR5) on late apoptotic neutrophils (Ariel et al., 2006), which, in turn, abrogates chemokine signaling. RvE1 has also been shown to regulate leukocyte pro-inflammatory cell surface markers including L-selectin, whilst selectively disrupting TX-mediated platelet aggregation (Dona et al., 2008), adding further insight into its anti-inflammatory/pro-resolution properties. In disease states,

RvE1 suppresses *Porphyromonas gingivalis*-induced oral inflammation and bone loss during periodontitis (Hasturk et al., 2006), is protective in trinitrobenzene-sulphonic acid-induced colitis in rodents (Arita et al., 2005b) as well as mediating re-epithelisation of mouse cornea after thermal-injury (Gronert, Maheshwari, Khan, Hassan, Dunn & Laniado Schwartzman, 2005). Taken together, RvE1 triggers various aspects of the pro-resolution cascade ranging from the timely inhibition of granulocyte accumulation at sites of inflammation to the efferocytosis or clearance of inflammatory debris, reviewed in (Serhan, 2008).

RvE1 binds to ChemR23 with high affinity (K_d = 48.3nm) resulting in the down-regulation of NF-κB activity and consequently pro-inflammatory cytokine synthesis such as TNF-α as well as modulating pathways involved in mitogen-activated protein kinase (MAPK) signalling (Arita et al., 2005a). Although it has been found in the kidney, gastro-intestinal system, brain as well as CV tissue and cells of the myeloid lineage, the percentage of ChemR23 expression is highly variable. For example, ChemR23 is significantly increased on human monocytes but comparatively less so on neutrophils in response to anti-inflammatory mediators such as TGF-β. As with ALX, ChemR23 is also receptor for peptide ligands including chemerin, which also exerts anti-inflammatory actions (Cash et al., 2008). RvE1 also interacts with the LTB_4 receptor, BLT1 and is a partial antagonist preventing neutrophil activation (Arita, Ohira, Sun, Elangovan, Chiang & Serhan, 2007). Therefore, RvE1 couples to two distinct receptors to suppress pro-inflammatory mechanisms while enhancing pro-resolution pathways.

While structurally distinct from RvE1, RvE2 is a second member of the EPA-derived family of E-series resolvins. In PMNs from human, it is generated at higher concentrations than RvE1, but is equipotent when given intravenously and additive when administered alongside RvE1 (Tjonahen et al., 2006). RvE2 also suppresses PMN migration into the peritoneum after zymosan (Tjonahen et al., 2006) and it is still unclear what receptor RvE2 couples to it is reported to mediate resolution by activating the chemerin receptor ChemR23 and antagonising the LTB_4 receptor BLT1.

There are four members of the D-series Rvs including RvD1, RvD2, RvD3 and RvD4 (Hong, Gronert, Devchand, Moussignac & Serhan, 2003). As with RvE1, RvD1/D2 exerts both anti-inflammatory and pro-resolution properties by blocking neutrophil infiltration, while also enhancing macrophage efferocytosis of apoptotic bodies (Krishnamoorthy et al.). The latter occurs via the binding of RvD1 to either ALX or GPR32, which are present on the surface of monocytes and PMNs, the expression of which is upregulated by inflammatory stimuli including granulocyte-macrophage-colony-stimulating factor (GM-CSF) and zymosan (Krishnamoorthy et al.). Importantly, in a model of cecal ligation and puncture (CLP) RvD2, whose receptor is GPR18(Chiang, Dalli, Colas & Serhan, 2015), in addition to blocking peritoneal PMN accumulation markedly reduced bacteria numbers and pro-inflammatory cytokines leading to increased animal survival (Spite et al., 2009).

As already mentioned, in addition to D-series Rvs, DHA also acts as a precursor for the synthesis of PDs. PD1, for instance, is synthesised in the human brain, microglial (Serhan et al., 2002) and peripheral blood mononuclear cells (Hong, Gronert,

Devchand, Moussignac & Serhan, 2003). As with Rvs, PD1 may also inhibit PMN migration as well as toll-like receptor-mediated activation (Duffield et al., 2006) while suppressing Th2 inflammatory cytokines and pro-inflammatory lipid mediator synthesis (Levy et al., 2007). PD1 also blocks T-cell migration *in vivo* and promotes T-cell apoptosis (Ariel et al., 2005). PD1 is protective in experimental models of oxidative stress (Mukherjee, Marcheselli, Barreiro, Hu, Bok & Bazan, 2007), ischemic stroke (Marcheselli et al., 2003), ischemia-reperfusion renal injury (Duffield et al., 2006), asthma (Levy et al., 2007) and Alzheimer's (Lukiw et al., 2005). Indeed, peripheral blood mononuclear cells from Alzheimer's patients given a DHA-rich dietary supplement show dampened biosynthesis of IL-1β, IL-6 and granulocyte-colony-stimulating factor (G-CSF) (Vedin et al., 2008). As with RvE2, a receptor is yet to be identified. However, it is possible that it couples to a distinct receptor to RvE1 as its anti-inflammatory effects are additive with those of RvE1 *in vivo*.

Maresins (MaR1 and MaR2) are produced in tissues by macrophages *via* the actions of 12-LOX, through a 13,14-epoxide intermediate (Serhan et al., 2009). MaR1 can also be generated at sites of vascular inflammation during human platelet–neutrophil interactions *via* platelet 12-LOX conversion of DHA to 13S,14S-epoxy-maresin, followed by neutrophil conversion to MaR1 (Abdulnour et al., 2014). The receptors for maresins have yet to be identified. Though maresins have only been recently it has been reported that, as with Rvs and PD1, MaR1 block the infiltration of PMNs, whilst stimulating macrophage phagocytosis of apoptotic PMNs/zymosan (Serhan et al., 2009).

SUMMARY

Inflammation is a good thing; it kills bacteria and helps to heal wounds while imparting long term memory against the inciting antigen. Lipids play a key role in these events and come in many forms, including those that drive the cardinal signs of inflammation and those that help to restrain it and bring the response to a timely end. In fact, studying lipids and their inhibitors, NSAIDs, has given us a great deal of insight into homeostasis, immune responses to infection/injury and the wound healing process. Indeed, inflammatory onset has been an historical point of interest for the development of anti-inflammatory drug therapies. Research on the other end of the inflammatory spectrum, resolution, has provided the opportunity to harness internal mediators and their receptors to help drive on-going inflammation down a pro-resolution pathway. Moreover, this is achievable without compromising host defence. Such complex manipulation of the immune system provides new opportunities to develop further pro-resolution therapies based upon what we have learned from studying lipids in this setting.

FOOTNOTE

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017).

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Figure 1. Cytochrome p450 metabolism of arachidonic acid to EETs and their subsequent conversion by she to DHETs.

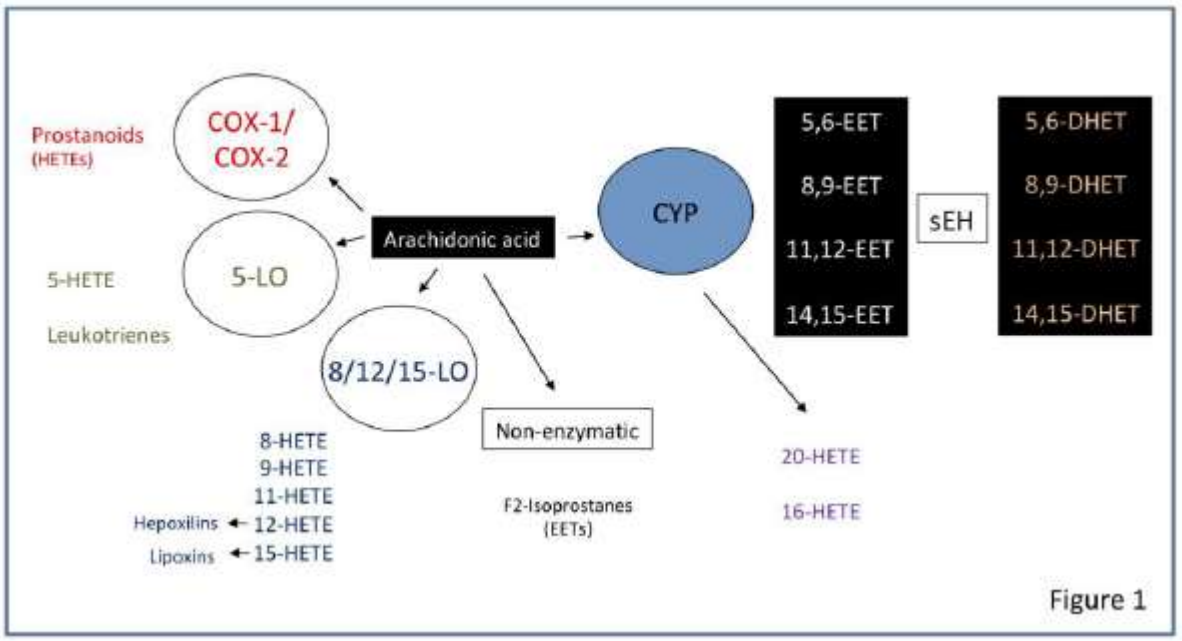

Figure 2. Cytochrome p450 metabolism of linoleic acid acid to EPOMEs and DHOMEs

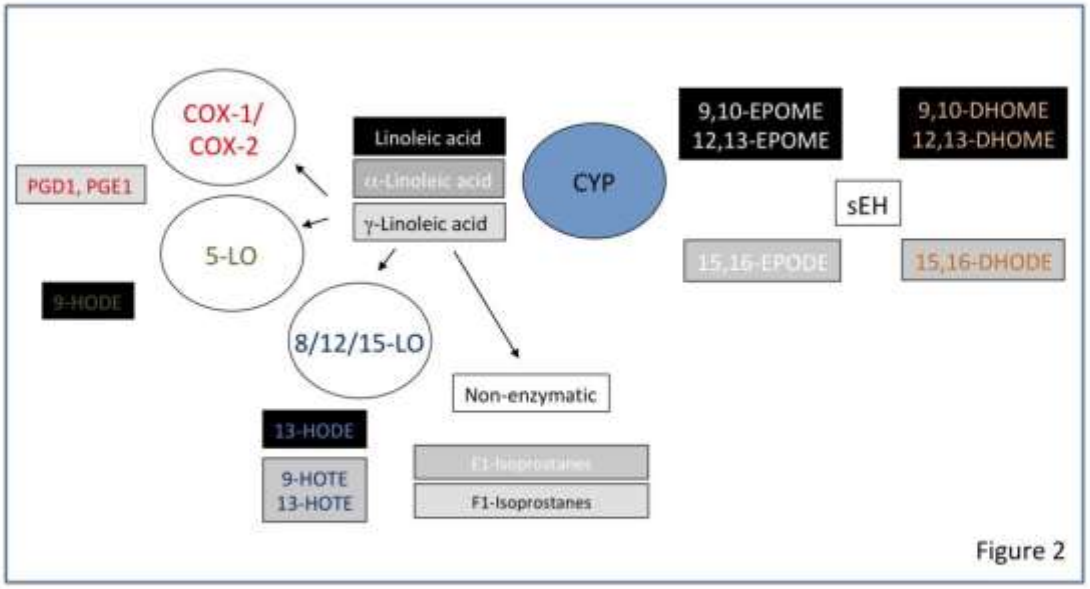

Figure 3. Cytochrome p450 metabolism of DHA to Epoxide docosapentaenoic acids.

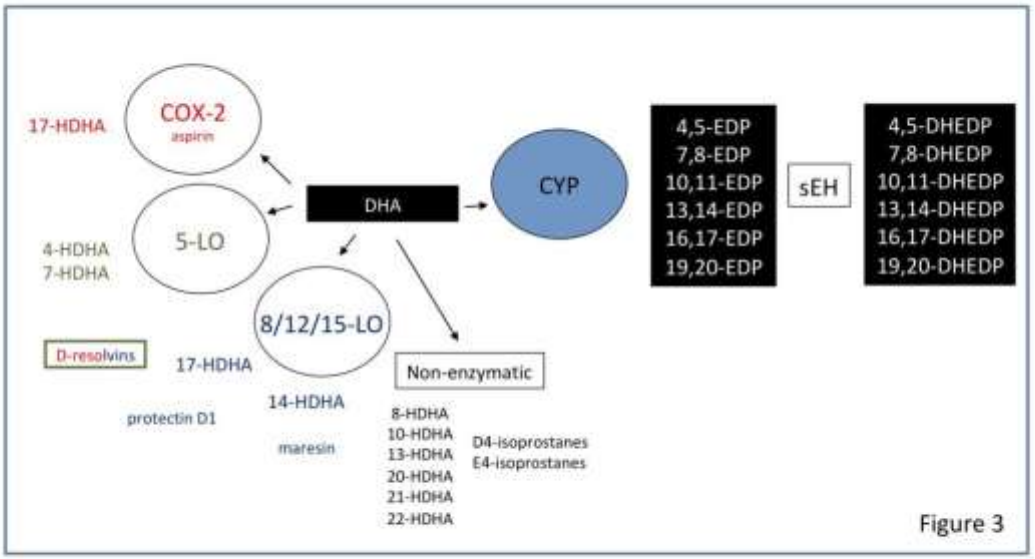

Figure 4. Cytochrome p450 metabolism of DHA to Epoxyeicosatetraenoic acids

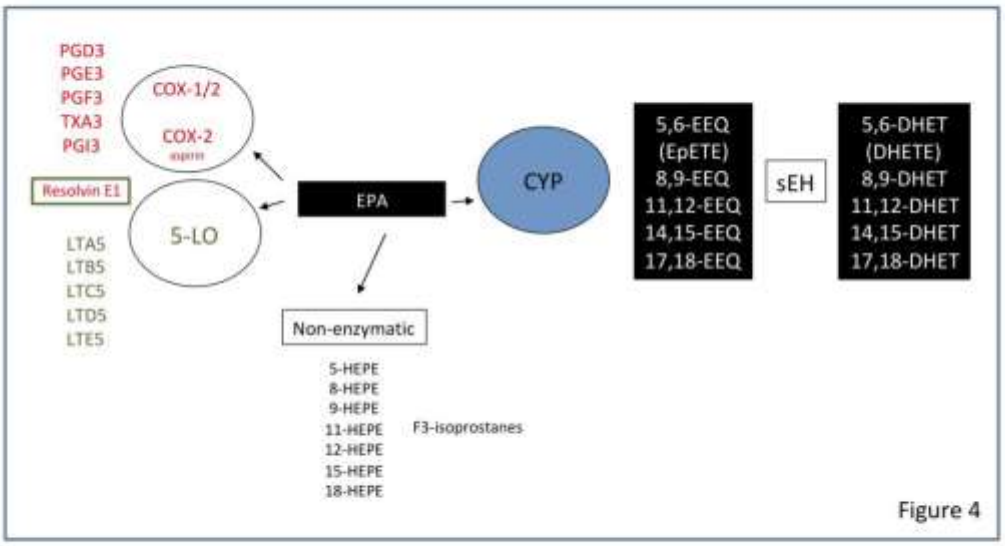

Figure 5. Biological properties of cytochrome p450 metabolites

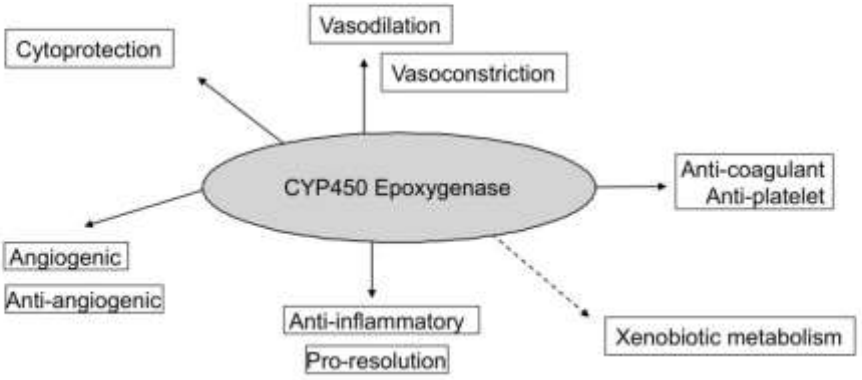

Table 1. Biological actions of lipid mediators and their relative concentrations at sites of inflammation

Lipid	Biological actions						Concentrations at sites of inflammation (*Motwani et al, 2018, Clin Pharm Ther; Motwani et al, 2018 JCI Insight.*)
	Inhibits granulocyte trafficking	Cytokine scavenging	Dampens pro-inflammatory signaling	Apoptosis	Efferocytosis	M1-M2 conversion	
Lipoxins A_4 and B_4	✓	✓	–	–	✓	✓	3.5-10.0pg/ml (Human *E. coli*-driven blister fluid)
Resolvin E1	✓	–	✓	–	✓	✓	– 2.0pg/ml (Human *E. coli*-driven blister fluid)
Resolvin D1	✓	–	✓	–	✓	✓	0.5-3.5pg/ml (Human *E. coli*-driven blister fluid)
Resolvin D2	✓	–	–	–	✓	–	10-15.0pg/ml (Human *E. coli*-driven blister fluid)
Maresins	✓	–	–	–	✓	–	–3.5pg/ml (Human *E. coli*-driven blister fluid)
Protectin D1	✓	–	–	✓	–	–	–1.0pg/ml (Human *E. coli*-driven blister fluid)
PGD_2	✓	–	–	–	–	–	–35.0pg/ml (Human *E. coli*-driven blister fluid)
15-deoxyΔ12–14-PGJ2	✓	–	✓	–	–	–	1,500pg/ml (mouse peritonitis) *Rajakariar et al PNAS, 2007*
PGE_2	Along with PGI_2 (–5,000pg/ml in *E. coli*-driven blister fluid) PGE_2 enhances vasodilation, edema formation and vascular permeability. PGE_2 is also a potent pyrogen and dampens innate-immune mediated responses to bacteria. PGE_2 also suppresses tumor immunity and has contrasting roles in the adaptive immune response dampening T cell proliferation in some instances and driving TH-17 responses in others.						–700pg/ml (Human *E. coli*-driven blister fluid)
PGF2α	PGF2α has potent smooth muscle stimulator, vaso- and broncho-constrictor, is also involved in acute, and chronic inflammatory diseases and sub-chronic inflammation in various cardiovascular dysfunctions.						75-1000gp/ml (Human *E. coli*-driven blister fluid)
TxB_2	TxB_2 is a potent vasoconstrictor and modulates T cell proliferative responses and the spread of bacterial infections						50-350pg/ml (Human *E. coli*-driven blister fluid)