Eicosanoid storm in infection and inflammation

Edward A. Dennis and Paul C. Norris

Abstract | Controlled immune responses to infection and injury involve complex molecular signalling networks with coordinated and often opposing actions. Eicosanoids and related bioactive lipid mediators derived from polyunsaturated fatty acids constitute a major bioactive lipid network that is among the most complex and challenging pathways to map in a physiological context. Eicosanoid signalling, similar to cytokine signalling and inflammasome formation, has primarily been viewed as a pro-inflammatory component of the innate immune response; however, recent advances in lipidomics have helped to elucidate unique eicosanoids and related docosanoids with anti-inflammatory and pro-resolution functions. This has advanced our overall understanding of the inflammatory response and its therapeutic implications. The induction of a pro-inflammatory and anti-inflammatory eicosanoid storm through the activation of inflammatory receptors by infectious agents is reviewed here.

Eicosanoids
Bioactive oxygenated polyunsaturated fatty acids containing 20 carbons.

Non-steroidal anti-inflammatory drugs
(NSAIDs). Drugs, such as aspirin and naproxen, that are used to ablate the inflammatory response. They work by inhibiting cyclooxygenase 1 (COX1) and COX2, thereby blocking the biosynthesis of prostaglandins including thromboxane.

Docosanoids
Bioactive oxygenated polyunsaturated fatty acids containing 22 carbons.

Department of Chemistry and Biochemistry and Department of Pharmacology, School of Medicine, University of California at San Diego, La Jolla, California 92093, USA.
Correspondence to E.A.D.
e-mail: edennis@ucsd.edu
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Eicosanoids are locally acting bioactive signalling lipids derived from arachidonic acid and related polyunsaturated fatty acids (PUFAs) that regulate a diverse set of homeostatic and inflammatory processes[1,2] linked to numerous diseases. Inhibiting the formation or receptor-mediated actions of classical eicosanoids (that is, prostaglandins and leukotrienes) by aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), by the leukotriene inhibitor zileuton and by leukotriene receptor antagonists during inflammation remains a prevailing strategy to alleviate pain, swelling, fever and asthmatic conditions. However, pleiotropic effects are becoming increasingly appreciated for most eicosanoids and their related docosanoids.

Hundreds of structurally and stereochemically distinct eicosanoid species can be made from arachidonic acid and other ω-6-derived PUFAs such as dihomo-γ-linolenic acid (DGLA) — the origin of which is the 18-carbon essential fatty acid linoleic acid — as well as from ω-3-derived PUFAs from α-linolenic acid (ALA) such as eicosapentaenoic acid (EPA), which can be further elongated to docosapentaenoic acid (DPA) and further desaturated to docosahexaenoic acid (DHA). Although the physiological roles of only a few of the eicosanoid and related docosanoid species are well understood, some of the agonists and receptors that activate inflammasome formation and the cytokine storm that accompanies infection[3,4] seem to also initiate the release of arachidonic acid and related PUFAs, resulting in an eicosanoid storm[5].

Mass spectrometry-based lipidomic profiling[6] is now being used to identify, monitor and quantify hundreds of distinct eicosanoids and related PUFA species[7] that seem to be involved in infection and inflammation, as well as in the resolution of inflammation[8]. These lipidomics approaches, sometimes referred to as 'eicosadomics', facilitate the identification of the 'eicosadome' and can be used to accurately determine global changes in cellular lipid levels during specific physiological processes. Lipidomics is currently being used to screen more effectively for potential disease biomarkers[9] and to provide scientists with a mechanistic understanding of eicosanoid biosynthesis and signalling at the cellular and multicellular tissue level. Integrating the latest genomics (transcriptomics), proteomics and lipidomics of the production of pro-inflammatory and pro-resolution eicosanoids and related PUFAs should lead to new insights into their roles during infection and inflammation.

In this Review, we summarize and discuss our current understanding of cellular eicosanoid metabolism[2,10–12] as well as eicosanoid signalling and its physiological consequences, which include homeostatic, pro-inflammatory and resolving functions. We also consider the importance of subcellular enzyme compartmentalization and stimulatory contexts involved in the regulation of eicosanoid metabolism[5,13–16]. Although the production of cytokines is considered a hallmark of infectious disease, we highlight the importance of the accompanying

Inflammasome
A molecular complex of several proteins that, upon assembly, cleaves pro-interleukin-1β (pro-IL-1β) and pro-IL-18, thereby producing the active forms of these pro-inflammatory cytokines.

Toll-like receptor
(TLR). A pattern recognition receptor that recognizes conserved molecules from pathogens, such as lipopolysaccharide, and initiates innate immune responses.

temporal production of both pro-inflammatory and anti-inflammatory (pro-resolution) eicosanoids and related docosanoids during the initiation and resolution of infection. The recent progress in lipidomic monitoring of the metabolism of arachidonic acid and related PUFAs provides us with the comprehensive perspective needed to tackle the challenges of therapeutic targeting of eicosanoid pathways, which range from traditional enzyme inhibition[17,18] with NSAIDs and prostanoid mimetics to 'natural' fish oil ω-3 fatty acid supplementation[19,20].

Eicosanoid biosynthesis and function

Eicosanoids arise from the oxidation of arachidonic acid and related PUFAs by cyclooxygenase[21] (COX), lipoxygenase[22] (LOX) and cytochrome P450 (CYP) enzymes, or via non-enzymatic free radical mechanisms (FIG. 1; also see Supplementary information S1–S3 (figures)). Although eicosanoids are most frequently associated with inflammation, they also have homeostatic functions (BOX 1).

The vast collection of lipids produced by these eicosanoid and related biosynthetic pathways is the starting point for *in vivo* eicosanoid signalling networks in cells through their cognate receptors (FIG. 1; TABLE 1). Cells are highly selective as to the specific eicosanoids that they synthesize, but the quantities produced are altered by the activation state and the physiological conditions of the specific tissues in which they reside. For example, macrophages produce an array of arachidonic acid-derived prostaglandins in response to Toll-like receptor (TLR) stimuli, but the specific prostaglandins and quantities produced after stimulation differ markedly between macrophages depending on their tissue origin and the isolation method used: there are differences in prostaglandin production between thioglycollate-elicited peritoneal macrophages and bone marrow-derived macrophages, as well as between primary macrophages and a transformed macrophage cell line[23]. Furthermore, the expression of receptors for specific eicosanoids is also cell and tissue specific.

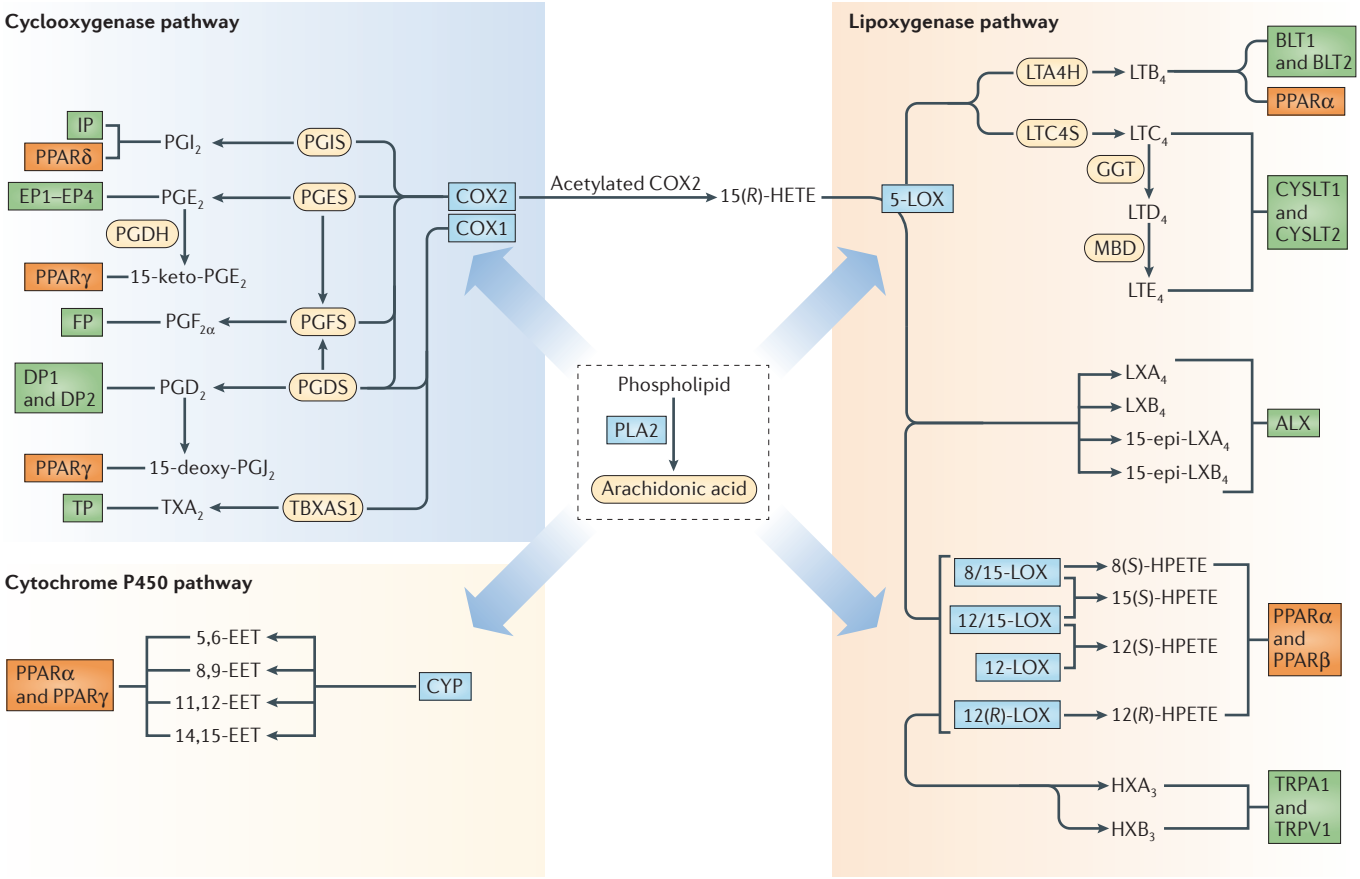

Figure 1 | **Eicosanoid biosynthesis and receptor signalling.** A lipidomic view of phospholipase A2 (PLA2), cyclooxygenase 1 (COX1), COX2, 5-lipoxygenase (5-LOX), 8-LOX, 12-LOX, 15-LOX and cytochrome P450 (CYP) epoxyhydrolase pathways of eicosanoid biosynthesis from arachidonic acid. Downstream enzymes are shown as yellow ovals; eicosanoid species and their receptors are shown as green boxes. The peroxisome-proliferator activated receptors (PPARs) that are potentially activated by the eicosanoids are also shown (orange boxes). The CYP ω-hydroxylase pathway and lipid species derived from other fatty acids are not shown. CYSLT1, cysteinyl leukotriene receptor 1; DP, PGD_2 receptor; EET, epoxyeicosatrienoic acid; EP, PGE receptor; FP, PGF receptor; GGT, γ-glutamyl transferase; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HX, hepoxilin; IP, PGI receptor; LT, leukotriene; LTA4H, LTA_4 hydrolase; LTC4S, LTC_4 synthase; LX, lipoxin; MBD, membrane-bound dipeptidase; PG, prostaglandin; PGDH, PG dehydrogenase; PGDS, PGD synthase; TBXAS1, TXA synthase; TP, thromboxane receptor; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; TX, thromboxane.

Peroxisome proliferator-activated receptor-α (PPARα). Member of a family of nuclear receptors that participate in the regulation of cellular metabolism and differentiation. PPARs have anti-inflammatory properties as they regulate the availability of limited cofactors or block promoters of pro-inflammatory genes.

Specialized pro-resolving mediators (SPMs). Eicosanoids and docosanoids that promote efferocytosis and also inhibit neutrophil diapedesis and pro-inflammatory cytokine expression. SPMs include lipoxins, resolvins, protectins, maresins and the newly discovered maresin conjugates in tissue regeneration (MCTR) sulfido-conjugate series.

Studies of eicosanoids in inflammation have mainly focused on the signalling pathways activated by lipids that are produced by the COX enzymes, as they collectively elicit the cardinal signs of inflammation, including heat, swelling, redness, pain and loss of function[24]. Understanding the physiological roles of these lipids is complicated by the differing effects that they have in different tissues: for example, the binding of prostaglandin E_2 (PGE_2) to its cognate G protein-coupled receptors (GPCRs), the EP receptor family, in neurons causes pain associated with inflammation, whereas autocrine EP signalling by PGE_2 in macrophages (and possibly in other leukocytes) can downregulate the production of tumour necrosis factor (TNF) and upregulate that of interleukin-10 (IL-10)[25], leading to a net reduction in inflammatory signalling.

Although eicosanoids of the COX pathway control a wide range of processes, the 5-LOX pathway (FIG. 1) is more specifically operative during inflammation to promote bronchoconstriction[26] and leukocyte recruitment to sites of tissue damage[27]. Whereas the functions of 5-LOX-derived leukotrienes in asthma and allergy are well understood, definitive biological functions for the intermediate metabolites 8-hydroperoxyeicosatetraenoic acid (8-HPETE), 12-HPETE and 15-HPETE, as well as their hydroxyeicosatetraenoic acid (HETE) products, have not been identified, but some may be ligands for peroxisome proliferator-activated receptor-α (PPARα)[28] and PPARγ[29], which induce anti-inflammatory effects and modulate liver X receptor (LXR; which regulates cholesterol homeostasis). Thus, metabolites of specific LOX enzymes may be anti-inflammatory, but the interconnections between LOX and COX enzymes during inflammation confound simple strategies to inhibit individual pathways for therapeutic gain. Despite a direct role for many of the LOX-derived monohydroxylated fatty acids produced by a single enzyme, numerous families of more complex di- and trihydroxylated fatty acids — specialized pro-resolving mediators (SPMs) — are produced by LOX and COX enzymes functioning in combination; these SPMs have remarkably potent and specific binding to GPCRs that accelerate bacterial clearance and neutrophil clearance, and turn on anti-inflammatory cytokine programmes.

The CYP pathway (FIG. 1) comprises a large number of enzymes that contain a haem iron. Many CYPs are expressed in the liver, but they are also expressed in other tissues where they inactivate and eliminate toxins and metabolites. The most upstream CYPs in the eicosanoid pathway convert arachidonic acid into epoxyeicosatrienoic acids (EETs) or ω-HETEs[2], which are thought to be anti-inflammatory, whereas the downstream diHETEs (which can be formed by soluble epoxide hydrolase (sEH)) are thought to be pro-inflammatory or inactive. Bioactive functions for the ω-HETEs are not well understood, as no cognate receptor or second messenger has been identified to date[2,30]. It is also important to clarify that epoxidation is essential for the formation of all SPMs and thus, in addition to LOX enzymes, some CYPs and sEH may be partially responsible for the synthesis of SPMs. Clearly, additional work is needed to identify the physiological roles of all of the eicosanoids and related PUFAs, as well as their receptors, and in particular their pro-inflammatory and anti-inflammatory functions. The systematic characterization of prostaglandin and leukotriene structures, biosynthetic pathways, natural receptors and biological functions has resulted in the production of new drug classes in the form of analogues, as well as receptor and enzyme inhibitors. These therapies that target eicosanoids have led to substantial improvements in treating inflammatory symptoms including swelling and pain, although more chronic diseases, such as arthritis and atherosclerosis, and more life-threatening pathogenic diseases are largely unaffected by the inhibition of

Box 1 | **Homeostatic functions of eicosanoids**

Classical eicosanoids have important homeostatic roles ranging from regulating vascular leakage and barrier formation to protecting mucosal integrity in the stomach and regulating platelet aggregation. For example, the cyclooxygenase 1 (COX1)-derived metabolite thromboxane A_2 (TXA_2) is produced by platelets and some other cell types and has a homeostatic role in platelet aggregation, as well as a role in the platelet response to injury. By contrast, COX2-derived prostaglandin I_2 (PGI_2) produced by endothelial cells inhibits platelet aggregation and promotes vasodilation.

Unique therapeutic side effects have been attributed to blocking COX1 or COX2 isoforms: COX1-specific inhibitors can cause stomach toxicity and delays in blood clotting, whereas COX2-specific inhibitors (coxibs) largely avoid stomach toxicity and promote platelet aggregation. Although coxibs potently inhibit COX2, which is upregulated during inflammation, the resulting vascular imbalance of PGI_2 and TXA_2 has been associated with an increase in the frequency of myocardial infarction and stroke events in coxib-treated patients. The most potent and specific COX2 inhibitor, rofecoxib, was removed from world markets a decade ago owing to cardiovascular risks.

The significance and complexity of eicosanoid signalling in crucial physiological processes have recently been evaluated in detail[73,74]. As a result, low doses of aspirin are now commonly prescribed as cardioprotective agents, and they limit TXA_2 formation by COX1 in platelets without inhibiting COX2-mediated PGI_2 formation in endothelial cells. One approach that could potentially circumvent this would be to inhibit the major inflammatory PGE_2 synthase (microsomal PGE_2 synthase 1 (mPGES1)), which is coupled to (and upregulated with) COX2 in many tissues during inflammation. However, this approach is questionable in light of the several anti-inflammatory effects that have now been attributed to PGE_2.

Fish oil-derived ω-3 fatty acid supplementation is also commonly prescribed for the treatment of various inflammatory ailments and for cardioprotection. The rationale for this is based on the ability of the ω-3 fatty acids eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) to inhibit arachidonic acid metabolism by COX1 (but less so by COX2)[20], similar to low-dose aspirin. However, the cardioprotective effects of ω-3 fatty acids may be obscured by the widespread use of statins, which seem to have anti-inflammatory properties.

Table 1 | **Eicosanoid pathways, mediators and receptors, and their physiological roles**

Major pathway	Mediator	Receptor	Physiological responses and biochemical effects	Refs
COX	PGE_2	EP1, EP2, EP3 and EP4	Vasodilation and vascular leakage; hyperalgesia; fever; ↑IL-10 levels; ↓TNF levels; neutrophil eicosanoid class switching	25,44,79–86
	15-keto- PGE_2	PPARγ	↑Adipogenesis; ↓mucus retention; ↑bicarbonate secretion	51,67
	PGD_2	DP1	Mast cell maturation; vasodilation; neuroprotection	87–89
		DP2	↑Eosinophil recruitment and allergic response	90,91
	15-deoxy-PGJ_2	PPARγ	↑Adipogenesis	92,93
	$PGF_{2\alpha}$	FP	Uterine, vascular and respiratory smooth muscle contraction; ↓intraocular pressure	94,95
	PGI_2	IP	↓Platelet aggregation; hyperalgesia; vasodilation; ↑IL-10 levels; ↓TNF levels	25,83,96–98
		PPARδ	Embryo implantation	99,100
	TXA_2	TP	↑Platelet aggregation; vasoconstriction; ↓T cell activation	101–104
5-LOX	LTB_4	BLT1	Neutrophil recruitment; vascular leakage	26,27,80
		BLT2	Enhanced epithelial barrier function	105
		PPARα	Negative feedback of LTB_4 biosynthesis	106,107
	LTC_4, LTD_4 and LTE_4	CYSLT1 and CYSLT2	Bronchoconstriction; vascular leakage; neutrophil extravasation	26,108
8-LOX, 12-LOX and 15-LOX	HPETEs, HETEs and diHETEs	TRPV1	Hyperalgesia	109
		PPARα and PPARγ	↑Expression of fatty acid translocase (also known as CD36)	28,29
CYP	EETs	PPARα and PPARγ	Vasodilation; antihyperalgesia; ↓COX2 expression	110–112
LOX–LOX and COX–LOX	HXA_3 and HXB_3	TRPA1 and TRPV1	Hyperalgesia	113
		Unknown	Mucosal epithelium-directed neutrophil recruitment	114–116
	LXA_4, 15-epi-LXA_4, LXB_4 and 15-epi-LXB_4	ALX	↓Neutrophil recruitment; ↑efferocytosis	8
Membrane-esterified eicosanoids	PL–HETE	Unknown	Modulation of signal transduction; lipoxin precursor storage	117,118
	PL–PG	Unknown	↓TLR response	119
Non-enzymatic	IsoPs	Unknown	Function not established	
	Arachidonic acid–NO_2	Unknown	COX1 inhibition	120

COX, cyclooxygenase; CYP, cytochrome P450; CYSLT, cysteinyl leukotriene receptor; DP, PGD receptor; EET, epoxyeicosatrienoic acid; EP, PGE receptor; FP, PGF receptor; HETE, hydroxyeicosatetraenoic acid; HPETE, hydroperoxyeicosatetraenoic acid; HX, hepoxilin; IL-10, interleukin-10; IP, PGI receptor; IsoPs, isoprostanes; LOX, lipoxygenase; LT, leukotriene; LX, lipoxin; NO_2, nitrogen dioxide; PG, prostaglandin; PL, phospholipid; PPAR, peroxisome-proliferator activated receptor; TLR, Toll-like receptor; TNF, tumour necrosis factor; TP, thromboxane receptor; TRPA1, transient receptor potential ankyrin 1; TRPV1, transient receptor potential vanilloid 1; TXA_2, thromboxane A_2.

eicosanoids. As many additional mediators derived from PUFAs during infection and inflammation have continued to be identified and characterized, there is now a greater focus on determining which lipid mediators enhance specific aspects of host protection and which mediate a return to homeostasis. As well as the regulation of anti-inflammatory responses, specific eicosanoid mediators have been associated with enhancing pathogen clearance, neutrophil clearance and antibody-mediated immune responses.

Prostaglandins and leukotrienes are produced rapidly after the initiation of inflammation and promote the early induction of oedema from postcapillary venules. Although this occurs naturally to mediate complement and leukocyte recruitment to a site of acute injury, the eicosanoid-mediated response can be overwhelming and life-threatening to the host during septic or toxic shock. For example, prostaglandins produced via COX1 (also known as PTGS1) during inflammasome activation have recently been shown to contribute to excessive vascular leakage and to lethality in mice, and they constitute part of a major pro-inflammatory eicosanoid response to infection[31]. Also, dual inhibition of COX2 (also known as PTGS2) and 5-LOX with flavocoxid has been shown to improve survival in mice after caecal ligation and puncture[32], with a reduction in the levels of circulating PGE_2 and leukotriene B_4 (LTB_4), an increase in lipoxin A_4 (LXA_4) expression, and a reduction in lung and liver myeloperoxidase (MPO) levels.

Caecal ligation and puncture
An experimental model of peritonitis in rodents in which the caecum is ligated and then punctured, thereby forming a small hole. This leads to leakage of intestinal bacteria into the peritoneal cavity and subsequent peritoneal infection.

Cellular control of eicosanoid biosynthesis

Phospholipase A2. The majority of eicosanoid metabolism requires free arachidonic acid, but it is primarily stored in an esterified form. Phospholipase A2 (PLA2) enzymes are crucial for increasing the levels of free arachidonic acid for metabolism and eicosanoid biosynthesis under most physiological conditions but particularly following inflammatory cell activation. Three members of the PLA2 superfamily have been implicated most strongly in cellular eicosanoid production: cytosolic calcium-dependent PLA2 (cPLA2), cytosolic calcium-independent PLA2 (iPLA2) and secreted PLA2 (sPLA2). iPLA2 is thought to be the primary PLA2 involved in most homeostatic cellular functions, particularly membrane homeostasis and remodelling[16,33] and constitutively generating a low level of free fatty acids with relatively minimal specificity for the particular esterified fatty acid; however, iPLA2 activity may include the de-esterification of arachidonic acid. cPLA2 is largely inactive during homeostatic conditions, but when activated by TLRs, purinergic receptors and other receptors that initiate signalling during an infection or inflammatory response, it translocates to the perinuclear and endoplasmic reticulum (ER) membranes. There, it hydrolyses arachidonic acid-containing phospholipids, leading to the production of pro-inflammatory eicosanoids. cPLA2 may also liberate phospholipid-esterified ω-3 PUFAs EPA, DPA and DHA, which are precursors for anti-inflammatory resolvins[20]. sPLA2 is an inducible enzyme that augments cPLA2 function to control the magnitude and duration of elevated free fatty acid levels including arachidonic acid[34].

As the most upstream regulators of eicosanoid biosynthesis, PLA2 enzymes regulate the eicosanoid response during different phases of an inflammatory response and probably do so downstream of various receptor-mediated cues (see below). Indeed, the activation of cPLA2 was shown to be responsible for the storm of pro-inflammatory eicosanoids that accompanies inflammasome formation induced by systemic flagellin or anthrax lethal toxin[31]. This study highlights the fact that a specific branch of the eicosanoid pathway — namely, prostaglandins produced by COX1 — is life-threatening when functioning systemically rather than acutely. The specialized delivery of autocoids to an infectious site can therefore break down during severe infections and supports the use of COX1-targeting NSAIDs during severe traumas and infections, whereas chronic diseases require more refined therapies.

Functional enzyme coupling. As free fatty acids and oxygenated eicosanoids and related PUFAs can rapidly diffuse out of the cell[1,35] or become reincorporated into membrane lipids by reacylation, and as activated PLA2 enzymes are concentrated at specific organelle interfaces, concentrations of free arachidonic acid and other fatty acids exist as gradients that restrict most downstream eicosanoid metabolism only to sites that are in very close proximity to PLA2 activity. For this reason, the colocalization of downstream enzymes in relation to the immediate substrate pool is crucial for effective bioactivity and resultant signalling by eicosanoids. Similarly, the close proximity of different cell types to one another is required to create intercellular communication pathways for distinct metabolons and eicosanoid profiles at the site of inflammation.

The COX pathway has received the most attention regarding the tight colocalization of PLA2 enzymes with downstream enzymes within a cell, defined as functional coupling[14] (FIG. 2). As cPLA2 predominantly translocates to the perinuclear membrane and ER, downstream enzymes that are also expressed at, or that can migrate to, these sites can preferentially participate in the metabolism of arachidonic acid. Functional coupling of thromboxane A synthase 1 (TBXAS1) and PGD synthase (PGDS) with COX1, which all partially colocalize to the ER, has been shown to preferentially produce the eicosanoids thromboxane A_2 (TXA_2) and PGD_2 during short-term stimulation of rat peritoneal macrophages[36]. Furthermore, microsomal PGE synthase 1 (mPGES1; also known as PTGES) and PGI_2 synthase (PGIS) are coupled with COX2 at the perinuclear membrane, and they preferentially produce PGE_2 and PGI_2 during the later phase of the lipopolysaccharide (LPS)-induced response (3–24 hours of stimulation)[36,37].

Another example is the 5-LOX pathway, in which 5-LOX-activating protein (FLAP; which is constitutively present and facilitates the transfer of arachidonic acid substrate to the 5-LOX active site) is responsible for the coupling of cPLA2 to 5-LOX at the perinuclear membrane. Note that both cPLA2 and 5-LOX are calcium dependent. Furthermore, the activation-dependent formation of LTC_4 and LTB_4 is dependent on distinct complexes of LTC_4 synthase (LTC4S) with 5-LOX at the outer nuclear membrane and of LTA_4 hydrolase (LTA4H) with 5-LOX at the inner nuclear membrane, respectively[38], giving rise to two potent but distinct inflammatory mediators.

The challenge now is to better understand functional coupling in the context of eicosanoid biosynthesis and function during inflammation, the study of which is complicated by the dynamic changes in the expression of eicosanoid biosynthetic enzymes and in their subcellular trafficking. A number of these dynamic changes have been recently documented by combining experimental and computational approaches: in one study[39], essentially complete lipidomic monitoring of eicosanoids was used with kinetic modelling to accurately predict the fluxes and temporal changes in the quantities and composition of COX and LOX metabolites (on the basis of enzyme transcript expression and functional coupling) in macrophages during stimulation of TLR4 and/or the P2X7 purinergic receptor, as well as in the presence of COX1- and COX2-specific inhibitors[39]. This study demonstrated differential coupling of COX1 and COX2 with downstream synthases and showed that fluxomics modelling can predict levels of inflammatory mediators in macrophages as a function of time, even with synergistic activation by TLR4 (priming) followed by a P2X7 agonist at a later time. Thus, the systems view of eicosanoids provided by lipidomics is improving our ability to predict the outcomes of specific enzyme inhibition at the cellular level and potentially at a physiological level as a computational tool to predict outcomes of drug candidates.

Myeloperoxidase
(MPO). An enzyme that is most highly expressed by neutrophils, where it is stored in azurophilic granules. MPO produces hypochlorous acid from hydrogen peroxide and chloride ions during the respiratory burst in neutrophils.

Purinergic receptors
A family of plasma membrane-bound molecules that are involved in several known cellular functions, such as vascular reactivity, apoptosis and cytokine secretion.

Resolvins
Lipid mediators that are induced in the resolution phase following acute inflammation. They are synthesized from the essential ω-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid.

Metabolons
In the context of this Review refers to complexes of multiple enzymes (bound or in close proximity) that coordinately synthesize eicosanoids and docosanoids, which is often dependent on receptor activation and cell–cell transfer of intermediates.

Fluxomics
The study of the flux or the change in the concentration of products and/or metabolites in a biosynthetic pathway in a cell as a function of time.

Transcellular eicosanoid metabolism. The biosynthesis of eicosanoids and related lipid species is markedly increased when cells are exposed to inflammatory stimuli that activate PLA2 and certain downstream enzymes. Some pathway enzymes can be robustly upregulated to enhance fatty acid metabolism and eicosanoid biosynthesis; the clearest examples are COX2 and mPGES1. Most cell types produce negligible amounts of eicosanoids in the steady state and when activated produce only a small number of distinct eicosanoids; this is determined by the specific enzymes that are expressed in that cell type[23]. This physiological compartmentalization of the expression of specific eicosanoid biosynthetic enzymes to specific cell types (for example, macrophages) greatly limits the production of eicosanoids and related lipids. During an immune response,

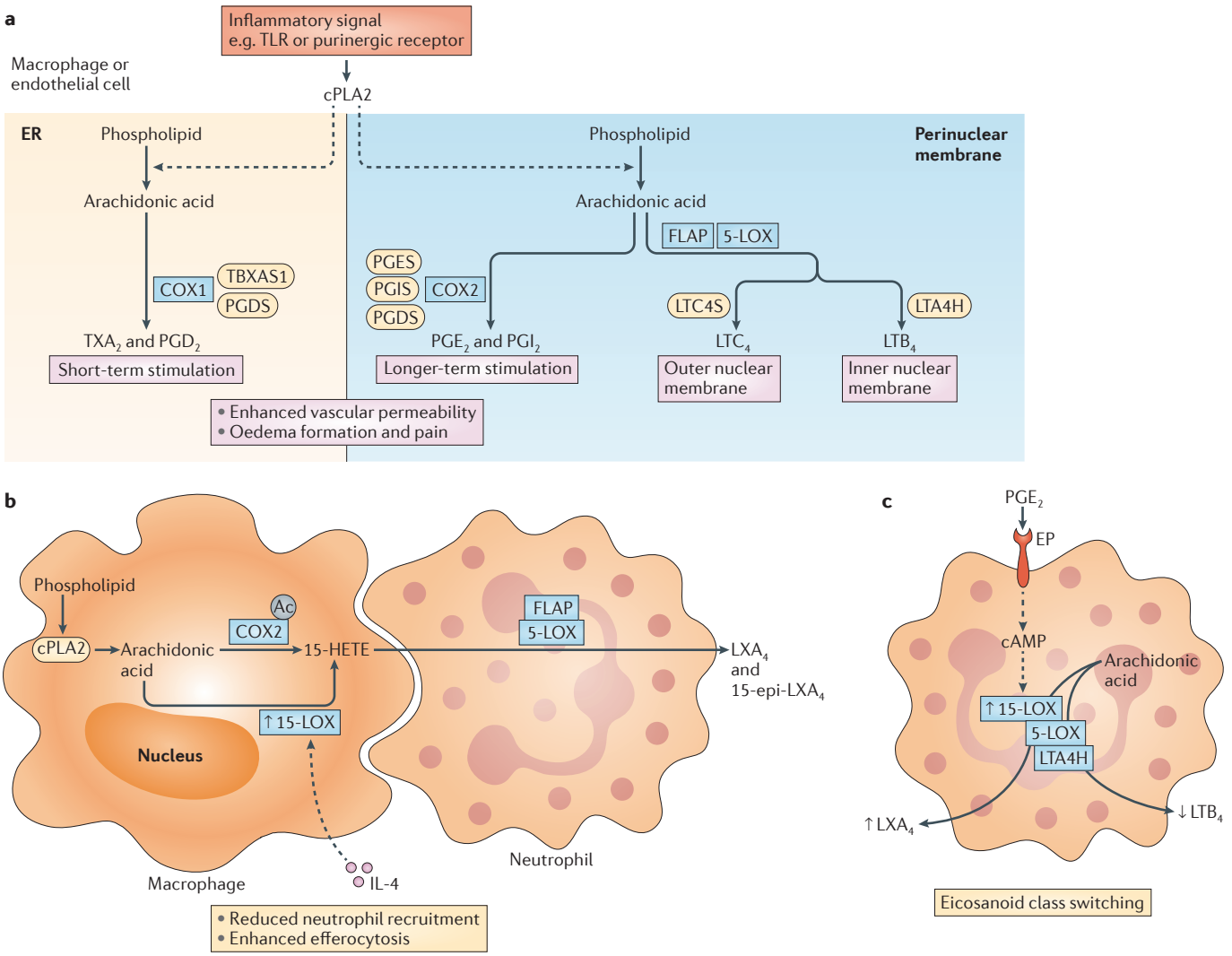

Figure 2 | **Enzyme functional coupling, transcellular biosynthesis and eicosanoid class switching. a** | Functional coupling of calcium-dependent phospholipase A2 (cPLA2) with metabolons comprised of cyclooxygenase 1 (COX1) or COX2 and different prostaglandin (PG) or thromboxane (TX) synthases. 5-lipoxygenase (5-LOX) is coupled to leukotriene A_4 (LTA_4) hydrolase (LTA4H) or LTC_4 synthase (LTC4S). PGES represents microsomal PGE synthase 1 (mPGES1) and PGDS represents haematopoietic PGD synthase; other isoforms are not shown. Coupling schemes are not completely isolated and thus products may derive from alternative routes depending on the cell type and the degree of cell activation. The functions of these products collectively describe the cardinal signs of inflammation. **b** | Transcellular biosynthesis of lipoxins (LXs) from arachidonic acid involving a cell expressing COX2 acetylated by aspirin (indicated by 'Ac') and/or 15-LOX that is upregulated by interleukin-4 (IL-4), both of which produce the same intermediate 15-hydroxyeicosatetraenoic acid (15-HETE). 15-HETE diffuses or is transported to an adjacent cell where it becomes incorporated into and subsequently released from membrane phospholipids by cPLA2 (not shown). This 15-HETE is converted by 5-LOX (with its functionally coupled 5-LOX-activating protein (FLAP)) to LXA_4 and/or the epimer 15-epi-LXA_4, thereby initiating eicosanoid class switching. Coordinated macrophage–neutrophil LX biosynthesis and the production of related specialized pro-resolving mediators (SPMs) provides putative mechanisms to enhance efferocytosis and redirection of neutrophils to the vasculature. **c** | Eicosanoid class switching involves receptor-mediated reprogramming of biosynthetic enzyme expression or activation. A neutrophil is depicted being activated by PGE_2 binding to an EP receptor that signals through cyclic AMP (cAMP) to increase 15-LOX expression, which shunts arachidonic acid synthesis from LTB_4 production by 5-LOX and LTA4H to LXA_4 production by 5-LOX and 15-LOX. Dashed arrows indicate that intermediate signal transduction steps are not shown; solid arrows indicate biosynthetic routes. ER, endoplasmic reticulum; TBXAS1, TXA synthase; TLR, Toll-like receptor.

this compartmentalization of specific enzymes can be overcome by the convergence of different cell types at an inflammatory site. Such conditions allow for the synthesis of more complex molecules by cell–cell transfer of intermediate eicosanoid metabolites and/or the metabolism of intermediate substrates, a process known as transcellular biosynthesis[40]. Transcellular biosynthesis has a role in the generation of several COX and 5-LOX metabolites and is particularly important in the formation of 'complex' eicosanoids synthesized by multiple LOX enzymes.

In isolation, a number of cell types express primarily one or two eicosanoid-producing enzymes (for example, 12-LOX in platelets, 5-LOX in neutrophils, and 15-LOX–COX2 in epithelial and endothelial cells); however, combinations of these cells cooperatively produce lipoxins that are relevant in inflammatory contexts[41], whereas other SPMs are produced through the same or similar mechanisms. The production of epimers of LXA_4 and LXB_4 (aspirin-triggered lipoxins) that are less prone to degradation by prostaglandin dehydrogenase (PGDH) was first demonstrated using co-cultures of neutrophils with endothelial cells, in which the upstream metabolite 5(*S*)-HPETE produced by 5-LOX in neutrophils was converted to lipoxins by acetylated COX2 that upregulated the production of 15(*R*)-HPETE, 15-epi-LXA_4 and 15(*R*)-LXB_4 in the endothelial cells following treatment with aspirin[42]. The conversion occurs because acetylation of the active site of COX2 fills its pocket to inhibit the normal prostaglandin-producing COX activity, thereby promoting the synthesis of 15(*R*)-HPETE rather than that of 15(*S*)-HPETE; thus, aspirin provides a novel activity that is distinct from that of other NSAIDs (which bind but do not acetylate COX). Lipoxins, the synthesis of which is increased by low-dose aspirin, promote the resolution of acute inflammation in humans[43]. Furthermore, the resolution of inflammation in mouse models of arthritis is mediated by the upregulation of 15-LOX through a COX2- and PGE_2-mediated mechanism (REFS 44,45).

Recently, it was found that activation of two or more receptors, such as TLRs and purinergic receptors, that are known to induce inflammasome formation also triggers lipoxin synthesis in macrophages[5]. In addition, cPLA2 regulates eicosanoid class switching — that is, switching the class of eicosanoids produced by a cell from pro-inflammatory prostaglandins to anti-inflammatory lipoxins — in a process that occurs in parallel with inflammasome formation and caspase activation[5] (FIG. 3). These data suggest that the same receptor-mediated events that lead to inflammasome formation might also trigger an eicosanoid storm that, in addition to pro-inflammatory mediators, can include mediators that trigger the initiation of resolution.

This receptor-mediated single-cell formation of lipoxins probably provides a means for cells including macrophages to efficiently enhance production without the need for multiple cell types to be involved via transcellular routes, largely through colocalization of numerous enzymes, including COX2, on particular membranes such as those of the ER and nuclear envelope[5]. Thus, aspirin not only enhances formation of the lipoxin precursor (15(*R*)-HETE) but also enables concentrated positioning of 15(*R*)-HETE in close proximity to activated 5-LOX for maximal conversion in a receptor-activated cell.

Further exploration of the specific association of these enzymes is likely to provide answers to other related and important questions: for instance, how are precursors of SPMs efficiently transferred via microparticles to other cells for processing? Microparticles shed from neutrophils have been shown to inhibit the pro-inflammatory actions of macrophages activated by zymosan or LPS[46]. More recently, these microparticles were found to contain the DHA-derived SPM precursors 14-hydroxydocosahexaenoic acid (14-HDHA) and 17-HDHA, and they could enhance efferocytosis of apoptotic neutrophils[47]. If SPM production involves PLA2-mediated mechanisms — for example, a role for cPLA2 in releasing arachidonic acid for lipoxin synthesis during inflammasome activation or releasing DHA for SPM synthesis — the effectiveness of nanomedicines that mimic microparticles could be enhanced. Of note, a 'resolving sPLA2' has been identified as having a role in the production of SPMs and in the resolution of skin inflammation[48], and it provides a potential candidate molecule that could be included in microparticles for the efficient delivery of SPM precursors to final lipoxygenation pathways.

Lipidomic perspective on the inflammatory process. The cell-specific compartmentalization of eicosanoid pathway enzymes in macrophages, neutrophils and other immune cells can be exploited using lipidomic strategies to assess the homeostatic and inflammatory environment. An elegant example of this approach comes from a study[49] that demonstrates how 12/15-LOX in resident peritoneal macrophages regulates the phenotype of leukocytes entering and exiting the peritoneal cavity during sterile inflammation. In this study, the disappearance and reappearance of resident macrophages, as well as the appearance and clearance of infiltrating macrophages, corresponded to the steady fall and eventual rise in the levels of 12-HETE and other LOX products[49]. Thus, by using temporal lipidomic or fluxomic[39] monitoring of specific eicosanoids, one can follow the progression of leukocyte trafficking as the infection progresses.

Although the presence of specific leukocytes is commonly used as an indicator of disease severity, lipidomic profiles can potentially be used in addition to cell-specific protein markers as a more complete read-out of cell phenotype and disease severity but with the added benefit of assessing dietary, metabolic and biosynthetic factors (the quantities of eicosanoids and related molecules derived from EPA, DHA and other fatty acids). A recent study using a macrophage-specific knockout of NCOR, which is a co-repressor associated with nuclear factor-κB (NF-κB)-specific genes, showed robust insulin sensitization and decreased inflammatory markers in obese mice compared with in control mice[50]. Using quantitative profiling, this study showed that the expression of ω-3 fatty acids — which are the precursors of many anti-inflammatory and pro-resolution mediators — was increased, a phenotype that protects against the

Lipoxins
A class of eicosanoids, comprising LXA_4 and LXB_4, that are produced by lipoxygenase-mediated metabolism of arachidonic acid. They are trihydroxytetraene-containing structures with potent biological activities in the resolution of inflammation.

Eicosanoid class switching
A process by which pro-inflammatory eicosanoid synthesis changes to anti-inflammatory or pro-resolution eicosanoid and docosanoid synthesis.

Efferocytosis
The phagocytic clearance of apoptotic cells (from the Latin word *effero*, meaning to take to the grave or bury) before they undergo secondary necrosis. The process usually triggers an anti-inflammatory response.

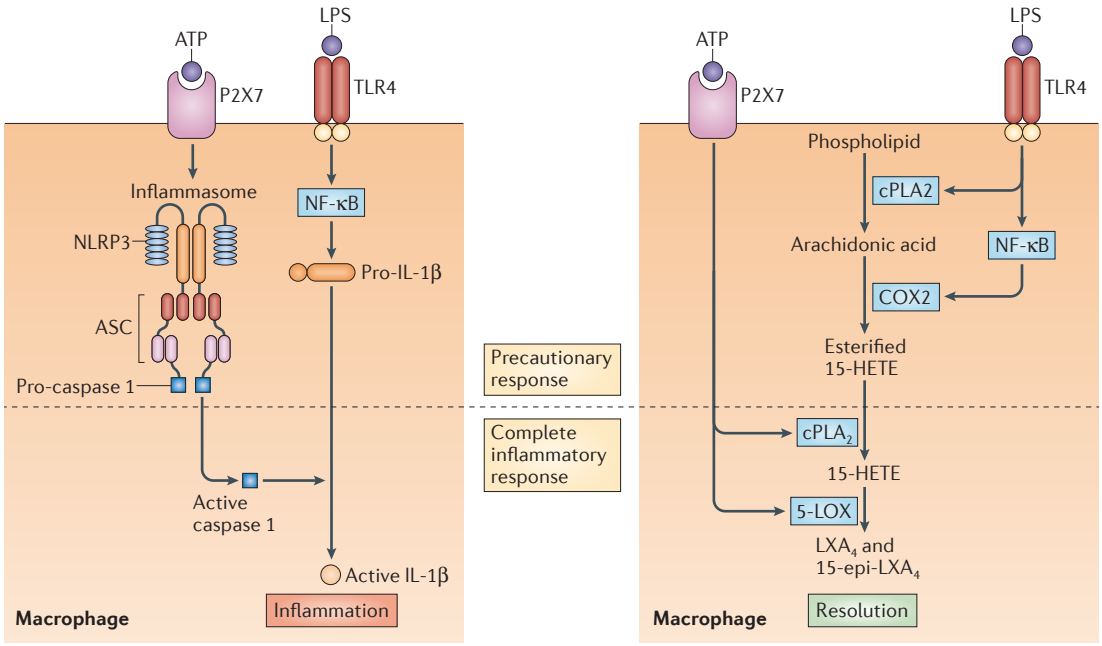

Figure 3 | **Inflammasome formation and caspase activation parallel lipoxin formation for a complete inflammatory response.** Toll-like receptor 4 (TLR4)-mediated priming of macrophages by lipopolysaccharide (LPS) induces the production of pro-interleukin-1β (pro-IL-1β) via activation of nuclear factor-κB (NF-κB). Subsequent P2X7 purinergic receptor engagement by ATP initiates the process of caspase 1 activation through inflammasome formation, which converts pro-IL-1β to active IL-1β (left panel), but a parallel pathway in macrophages exists to initiate the resolution of inflammation (right panel). TLR4-mediated priming also activates calcium-dependent phospholipase A2 (cPLA2) and induces the production of cyclooxygenase 2 (COX2) via NF-κB; this results in the release of arachidonic acid and its conversion into 15- hydroxyeicosatetraenoic acid (15-HETE), which becomes esterified into phospholipids. Subsequent P2X7 purinergic receptor engagement activates cPLA2 and 5-lipoxygenase (5-LOX) through calcium-dependent processes, leading to the release of 15-HETE from membrane phospholipids and the conversion of the 15-HETE to lipoxin A_4 (LXA_4) and the epimer 15-epi-LXA_4. The first step in this process is enhanced by aspirin, which acetylates an active site serine in COX2; this inhibits COX activity, but spares the LOX activity that converts arachidonic acid to 15-HETE, which causes complete eicosanoid class switching from pro-inflammatory prostaglandins to anti-inflammatory lipoxins. NLRP3, NOD-, LRR- and pyrin domain-containing 3.

Necrotic cell death
A form of cell death that frequently results from toxic injury, hypoxia or stress. Necrosis involves the loss of cell integrity and release of cell contents into the interstitium. This form of cell death usually occurs together with inflammation. Depending on the context, the self-antigens that are released by necrosis could become immunogenic.

Lyme disease
A disease caused by the bacterium *Borrelia burgdorferi* or other *Borrelia* spp. that are transmitted to humans via the bites of infected black-legged ticks. Symptoms can include skin rash, fever, fatigue, headache, muscle pain, stiff neck, and swelling of the knee and other large joints. Most cases can be successfully treated with antibiotics.

effects of high-fat diets. This demonstrates the use of quantitative lipidomics to monitor disease phenotypes and severity.

The complexity of eicosanoid biosynthesis is attributable to the dynamic expression and intracellular compartmentalization of the biosynthetic machinery, which are dependent on time, conditions and cell type. Generally, the upregulation of COX2 and mPGES1 expression begins early during inflammatory programmes in cells such as macrophages, endothelial cells and dendritic cells to form PGE_2; by contrast, IL-4- and PGE_2-mediated increases in 12/15-LOX expression begin at later stages in the activation of macrophages, neutrophils and several other cell types, and this results in the increased formation of SPMs that stimulate resolution. The 'inactivation' of prostaglandins by downstream enzymes may also function to produce anti-inflammatory PPAR agonists[51], and similar downstream mechanisms may also exist for other lipid mediators that are less well understood. This model of progression is tightly regulated and will require further dissection in specific disease states to identify specific intervention strategies.

Eicosanoids in infection and inflammation

A recurring theme in the case of *Mycobacterium tuberculosis* infections is that PGE_2 inhibits necrotic cell death in macrophages, which promotes pathogen resistance and host protection, whereas a specific level of TNF — determined by a delicate balance between LTB_4 and LXA_4 — is crucial for maximal control of infection[52,53]. Although the protective effect of PGE_2 in *M. tuberculosis* infection has been linked to the inhibition of the type I interferon response[54], PGE_2 has been shown to compromise immunity to influenza A virus by inhibiting macrophage antigen presentation and T cell immunity[55]. Although different eicosanoid species are emerging as enhancers of various aspects of inflammation and innate immunity in general, skewing the profile of these mediators to effectively target specific pathogens is a potential therapeutic approach that will require multiple strategies in line with personalized medicine.

Genetic variability also has a role in determining how the host is affected by pathogenic exposure. For example, DBA mice are resistant to developing arthritis associated with Lyme disease following *Borrelia burgdorferi* infection, whereas C3H mice exhibit prolonged inflammation

Box 2 | **Resolution functions: specialized pro-resolving mediators**

Inflammatory exudates initially function to neutralize pathogens and local injury; however, this results in an increased presence of leukocytes and pro-inflammatory mediators that can potentially propagate tissue damage and inflammation if not resolved in a timely manner. Lipoxins comprise a family of trihydroxy eicosanoids that include lipoxin A_4 (LXA_4) and LXB_4 — as well as the aspirin-triggered epimers 15-epi-LXA_4 and 15-epi-LXB_4 (REFS 42,75) — which inhibit neutrophil recruitment via activation of the LXA_4 receptor (ALX (also known as FPR2)). Subsequently, this family of specialized pro-resolving mediators (SPMs)[8] has grown to include di- and trihydroxylated fatty acids derived from the ω-3 fish oils eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA) that have been isolated from inflammatory exudates and leukocytes. SPMs now include the EPA-derived resolvins (RvE1, RvE2 and RvE3) and DHA-derived resolvins (RvD1, RvD2, RvD3, RvD4, RvD5 and RvD6), as well as protectin D1 and the macrophage mediators in resolving inflammation (maresins) MaR1 and MaR2, which are also derived from DHA. Protectin D1 has also been termed neuroprotectin D1 (NPD1) as it was isolated from and protects retinal pigment epithelial cells from oxidative stress-induced apoptosis[76], and it has neuroprotective properties in models of brain ischaemia–reperfusion injury[77]. In addition to ALX, these mediators bind BLT1 (also known as LTB4R), ChemR23 and G protein-coupled receptor 32 (GPR32), which seem to recognize both the stereochemically specific triene or tetraene and dihydroxy or trihydroxy motifs within the fatty acid backbone. Collectively, SPMs have been shown to enhance bacterial clearance[60], downregulate pro-inflammatory cytokines and enhance clearance of apoptotic neutrophils (a process termed efferocytosis). Most recently, a sulfido-conjugate family of mediators derived from DHA was identified in mouse inflammatory exudates (maresin conjugates in tissue regeneration (MCTRs)) that promote tissue regeneration in planaria and have the classical actions of SPMs[78].

and delayed resolution despite a similar control of bacterial loads; these differences have been profiled using lipidomics[56]. Resistant DBA mice expressed high levels of protectin D1, resolvin D1 (RvD1), hepoxilin A_3 (HXA_3), PGE_2 and 15-keto-PGE_2 in ankle joints, whereas C3H mice expressed high LTE_4 levels. This eicosanoid expression pattern was largely sustained for the duration of the disease course, suggesting a defect in eicosanoid class switching and in the synthesis of anti-inflammatory and pro-resolving mediators in Lyme disease-susceptible C3H mice. These results are in line with previous studies demonstrating that inflammation resulting from *B. burgdorferi* infection fails to resolve in the context of 5-LOX[57] or COX2 (REF. 58) ablation; loss of 5-LOX in particular led to decreased macrophage-mediated efferocytosis and phagocytosis of spirochetes by macrophages and neutrophils.

An example of the importance of eicosanoids and their metabolism in the sequelae of disease-associated inflammation comes from a study that carried out transcriptomic analysis of a cystic fibrosis disease phenotype; subsequent lipidomic analysis of eicosanoids helped to identify the PGE_2 metabolite 15-keto-PGE_2 as an endogenous PPARγ agonist and a potential pharmacological therapeutic[51]. The findings suggest an important anti-inflammatory action of prostaglandin inactivation. Interestingly, another reported PPARγ agonist, 15-deoxy-PGJ_2, has recently been shown to inhibit peritoneal NOD-, LRR- and pyrin domain-containing 3 (NLRP3)-induced IL-1β secretion and leukocyte recruitment[59], although the exact mechanism has not yet been identified. The resolvins RvD1 and RvD5 have been shown to reduce antibiotic requirements for microbial clearance in mice[60] through their ability to synergize with protectins and increase bacterial phagocytosis in human macrophages (BOX 2); these data suggest that therapies derived from natural pro-resolving responses can be applied to counteract antibiotic resistance, indicating that eicosanoid-related signalling has bactericidal implications for infection. Eicosanoids such as lipoxins, together with these DHA-derived SPMs, seem to be enhancers of microbial clearance that arise after microbicidal components — including complement and neutrophils — have been deployed, partly through the actions of prostaglandins and leukotrienes.

Recent studies of influenza virus infections in mice have integrated transcriptomics, proteomics of cytokine formation and lipidomics with disease progression to isolate promising therapeutics[61] and biomarkers[62] such as protectin D1, which is one of the many pro-resolution mediators derived from ω-3 fatty acids in fish oil (BOX 2). Samples from human patients infected with influenza virus who had high clinical scores and increased levels of cytokines and chemokines also contained significantly higher percentages of eicosanoid metabolites from the LOX and CYP pathways (including those derived from EPA and DHA) than healthy individuals[62]. The amounts of individual lipid mediators — including PGE_2, LTE_4 and 17-HDHA (a 15-LOX-derived product of DHA that is a stable form of 17-HpDHA, the precursor to all D-series resolvins and to protectin D1) — were increased in nasopharyngeal lavages from patients with high cytokine levels and severe symptoms of influenza virus infection compared with patients who had low and medium levels of cytokine production and less severe symptoms[62]. More recently, 17-HDHA has been shown to increase the levels of antigen-specific antibody when administered to mice in combination with H1N1 influenza virus-derived haemagglutinin, or with ovalbumin, which resulted in increased resistance to live H1N1 infection[63]. These findings suggest that specific lipid-derived components of potential or existing adjuvants, such as in the oil of Freund's adjuvant, could be further refined for therapeutic use. Although some eicosanoids and related species are established mediators that progressively promote the neutralization and clearance of pathogens, and the trafficking and clearance of leukocytes, it remains unclear which of these molecules is responsible, how they are biosynthesized and how they signal to link innate and adaptive immunity, but the studies mentioned above highlight some potential pathways that may be used for signalling during a deluge of lipid mediators.

Therapeutic interventions

Drugs that target eicosanoid pathways (FIG. 4) have been used for more than a century; aspirin is the oldest of the numerous effective NSAIDs that have been marketed. The discovery that NSAIDs inhibit prostaglandin synthesis through the inhibition of COX enzymes[17] has led to the development of COX1- and COX2-specific NSAIDs, and these molecules have helped to determine the homeostatic importance of this enzyme family (BOX 1). In addition, the 5-LOX pathway is a major drug target for the treatment of allergic and asthmatic conditions (FIG. 4), and the 5-LOX inhibitor zileuton can inhibit all downstream metabolism.

Furthermore, leukotriene receptor antagonists (LTRAs), such as montelukast, inhibit the actions of cysteinyl leukotriene receptor 1 (CYSLT1) and reduce bronchoconstriction during inflammatory pulmonary events[64]. The inhibition of COX enzymes by NSAIDs results in a switch to arachidonic acid oxidation by 5-LOX and increased signalling by CYSLTs; therefore, combining 5-LOX inhibitors and/or LTRAs with NSAIDs may be more effective than NSAIDs alone. Other LOX pathways as well as CYP pathways, including sEH (FIG. 4), are also being pursued as drug targets for various inflammatory diseases.

The most upstream target for eicosanoid mediators is PLA2, but clinical trials for cardiovascular indications using a sPLA2 inhibitor (ClinicalTrials.gov identifiers NCT00743925 and NCT01130246) or a lipoprotein-associated PLA2 inhibitor (NCT00799903 and NCT01000727) were unsuccessful, and a clinical trial for a cPLA2 inhibitor (NCT00396955) was stopped because it exhibited the same side-effect profile as non-selective NSAIDs[16]. Future studies with plasma lipidomic monitoring should be able to identify 'problematic' side products at an earlier stage in the drug discovery process,

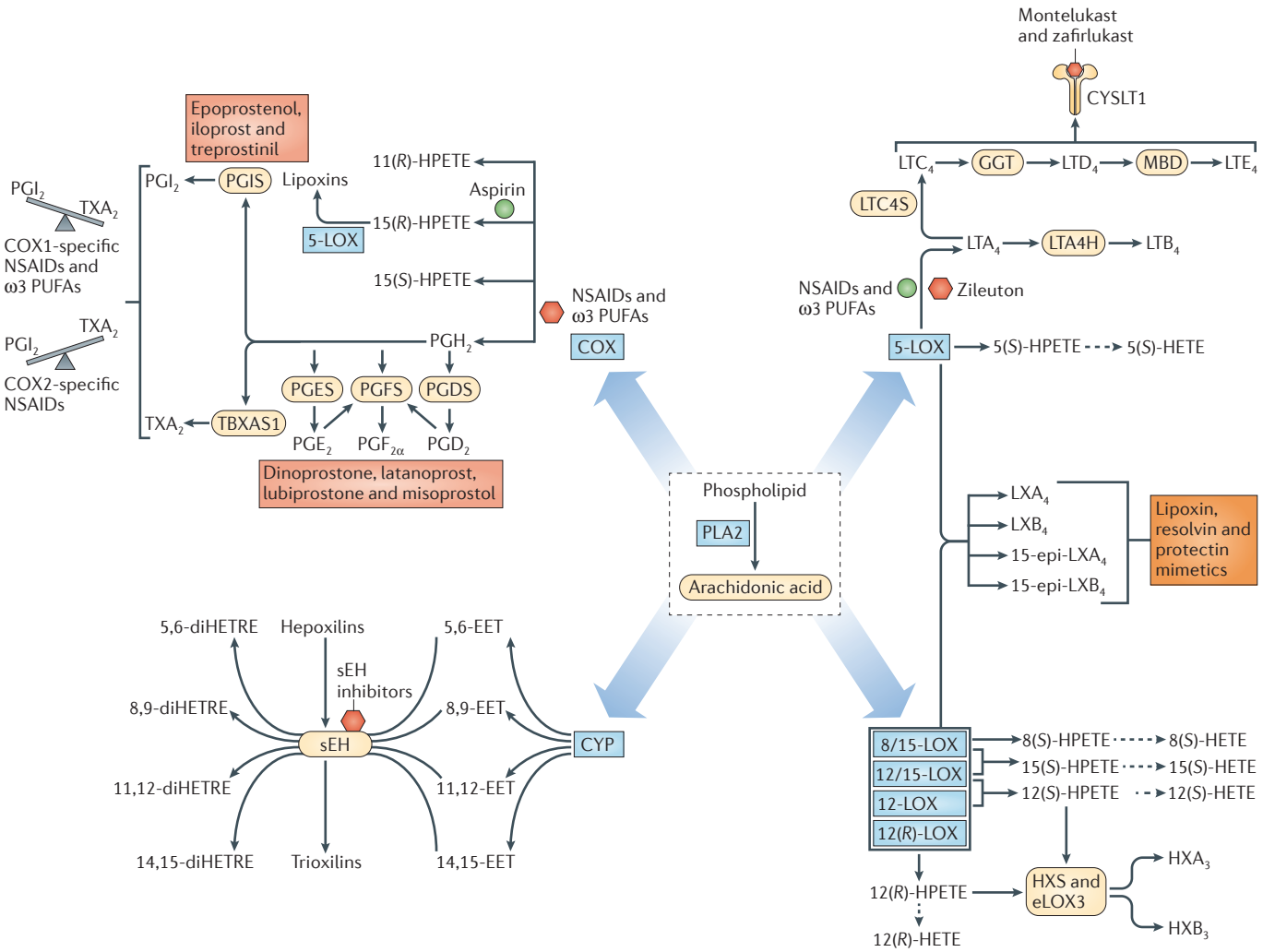

Figure 4 | **Therapeutics targeting eicosanoid pathways.** Enzymes in the cyclooxygenase (COX) pathway generate prostaglandins (PGs), thromboxanes (TXs) and lipoxins (LXs); lipoxygenase (LOX) pathway enzymes generate leukotrienes (LTs), hydroxyeicosatetraenoic acids (HETEs) and hepoxilins (HXs); and cytochrome P450 (CYP) epoxyhydrolase pathway enzymes generate epoxides and dihydroxy polyunsaturated fatty acids (PUFAs). All of the pathways have pharmacological intervention points including enzyme inhibitors and receptor antagonists (red hexagons), product enhancers (green circles) and mimetics (red boxes). Non-steroidal anti-inflammatory drugs (NSAIDs) and ω-3 PUFAs inhibit the COX-catalysed formation of each product derived from arachidonic acid, with the exception of one NSAID, aspirin, which enhances COX2-catalysed formation of 15(*R*)-hydroperoxyeicosatetraenoic acid (15(*R*)-HPETE) that can be converted into LXs by 5-LOX. COX1-specific NSAIDs and ω-3 PUFAs shift the vascular balance to higher levels of PGI_2, whereas COX2-specific NSAIDs shift the vascular balance to higher levels of TXA_2 due to coupling of platelet COX1 with TXA synthase (TBXAS1), and endothelial COX2 with PGI synthase (PGIS). PG analogues are used clinically to mimic endogenous bioactivity. Montelukast and zafirlukast specifically inhibit activation of cysteinyl leukotriene receptor 1 (CYSLT1). Zileuton inhibits 5-LOX conversion of arachidonic acid, but NSAIDs and ω-3 PUFAs can increase conversion via shunting of arachidonic acid from inhibited COX1 and COX2. Inhibitors of soluble epoxide hydrolase (sEH) reduce inactivation of epoxyeicosatrienoic acids (EETs) as well as HXs. LX, resolvin and protectin mimetics (orange box) are being developed for the treatment of ocular, periodontal and cardiovascular diseases. Dashed arrows represent non-enzymatic or subsequent transformations. eLOX3, epidermis-type LOX3; GGT, γ-glutamyl transferase; diHETRE, dihydroxyeicosatrienoic acid; HXS, HX synthase; LTA4H, LTA_4 hydrolase; LTC4S, LTC_4 synthase; MBD, membrane-bound dipeptidase; PLA2, phospholipase A2.

UPLC/MS–MS technology
Ultra-high performance liquid chromatography (UPLC) combined with tandem mass spectrometry (MS–MS) for chemical separation and quantitative analysis.

especially given the challenges of drug discovery when there is a complex network of enzymes involved. The use of mass spectrometry in helping to define the global outcomes of lipid profiles in relation to different inflammatory responses is likely to refine the specific eicosanoid targets and reveal which to avoid. For example, although inflammatory bowel disease is an inflammatory condition and standard treatments include the long-term use of salicylate derivatives, aspirin and other traditional NSAIDs are contraindicated.

Although drugs that target COX and 5-LOX pathways comprise arguably the most widely consumed drug class, they are not actually able to stop or resolve innate immune responses alone. This is understandable because cytokines, chemokines and other bioactive mediators are also crucial players in immune signalling. Furthermore, although this Review has focused on eicosanoids produced from arachidonic acid, many of the enzymes discussed are also capable of acting on the essential fatty acids containing 18 carbons (linoleic and linolenic acid), as well as on more unsaturated and longer fatty acids (docosanoids) including fish oil-derived ω-3 fatty acids (EPA, DPA and DHA). Recent UPLC/MS–MS technology[65] can accurately quantify more than 150 eicosanoid and related metabolites (if present) in a 5 minute metabolomic analysis of less than 50 µl of human plasma, and this number is increasing as new standards become available. These new methodologies are generally carried out on urine or on blood plasma and have replaced older methodologies — such as enzyme-linked immunosorbent assays (ELISAs) and traditional mass spectrometry — which lack complete specificity and quantitation capability.

Novel bioactive oxygenated lipids can also be produced by the traditional 'pro-inflammatory' enzymes — including COX, LOX and CYP enzymes — and some of these can promote the resolution of inflammation[8]. Also, several fatty acids, nitrated fatty acids, eicosanoids and related DPA- or DHA-derived oxygenated lipids seem to be agonists of the anti-inflammatory nuclear receptors PPARα, PPARδ and PPARγ[28,29,66–69]. None of these PPAR-activating eicosanoids and related species has the same very low nanomolar potencies possessed by prostaglandins, leukotrienes and lipoxins for their natural receptors, questioning the biological relevance of their proposed anti-inflammatory actions[1]. These criticisms have been voiced again more recently[70] and also in reference to the reported *in vivo* levels of transcellularly formed lipoxins and other SPMs[8]; more work is clearly needed to resolve these issues.

The number of identified PPAR agonists and SPMs continues to mount, increasing the potential for their *in vivo* relevance. Thus, the ability to measure nearly all eicosanoids and related species should become increasingly focused on their collective bioactivity rather than their activity as distinct species working in isolation. However, current analytical strategies cannot determine the precise concentration of specific eicosanoids in proximity to or bound to their receptors or in real time at confined inflammatory sites, where eicosanoids are rapidly formed and inactivated. Hopefully, future developments in lipidomics — such as secondary ion mass spectrometry (SIMS-C_{60}), which can profile lipids at the single-cell level[71] — may provide a useful platform to eventually solve the current debate regarding the presence and biological relevance of specific eicosanoids in particular cell types during infection and inflammation. Recent studies on the spatial organization of lipids in the human retina and optic nerve by matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry[72] have identified individual phospholipids, some containing DHA, in different layers of the human retina, suggesting that with future technological advances, local pools of released fatty acids and eicosanoid products may be detectable in relevant human tissues under inflammatory conditions.

Conclusions

The overall effects of the eicosanoid and cytokine storms on host survival are likely to be context dependent, and further improvements in our understanding will require greater focus on age, diet and genetic variation, in addition to the specific pathogenic assault and severity of trauma. Refined application of lipidomics methodologies and similar approaches will be crucial to advance our understanding of the role of eicosanoids in health and disease. As lipidomics now provides a means by which to assess changes in the majority of eicosanoid species simultaneously, it is likely that there will be progress in determining both the pro-inflammatory and anti-inflammatory contributions of eicosanoids in specific diseases.

The expanded view of eicosanoid signalling since the introduction of lipidomics is remarkably more complex, but the potential for improving therapeutic design is promising. Advancing treatment beyond NSAIDs may require more focus on isolating and correcting specific deficiencies in bioactive eicosanoids rather than inhibiting entire pathways. The ability to now analyse hundreds of eicosanoids and related lipid species, alongside the handful of well-characterized prostaglandins and leukotrienes, provides a wealth of possibilities to understand and develop novel treatments for inflammatory and metabolic conditions. A better understanding of the cytokine storm and its integration with the eicosanoid storm that accompanies classic inflammation and its resolution should provide new insights leading to novel strategies for the understanding and treatment of infection and inflammation.

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Competing interests statement

The authors declare no competing interests.

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ERRATUM

A brain drain

Yvonne Bordon

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The original version of this article contained a typo in the final paragraph and incorrectly stated that interstitial fluid is originally drained from the CSF via the lymphatic system – the article has been amended to indicate that interstitial fluid from the brain parenchyma is initially drained into the CSF via the glymphatic system.