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Review

Lipidomic profiling of bioactive lipids by mass spectrometry during microbial infections

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ABSTRACT

Bioactive lipid mediators play crucial roles in promoting the induction and resolution of inflammation. Eicosanoids and other related unsaturated fatty acids have long been known to induce inflammation. These signaling molecules can modulate the circulatory system and stimulate immune cell infiltration into the site of infection. Recently, DHA- and EPA-derived metabolites have been discovered to promote the resolution of inflammation, an active process. Not only do these molecules stop the further infiltration of immune cells, they prompt non-phlogistic phagocytosis of apoptotic neutrophils, stimulating the tissue to return to homeostasis. After the rapid release of lipid precursors from the plasma membrane upon stimulation, families of enzymes in a complex network metabolize them to produce a large array of lipid metabolites. With current advances in mass spectrometry, the entire lipidome can be accurately quantified to assess the immune response upon microbial infection. In this review, we discuss the various lipid metabolism pathways in the context of the immune response to microbial pathogens, as well as their complex network interactions. With the advancement of mass spectrometry, these approaches have also been used to characterize the lipid mediator response of macrophages and neutrophils upon immune stimulation *in vitro*. Lastly, we describe the recent efforts to apply systems biology approaches to dissect the role of lipid mediators during bacterial and viral infections *in vivo*.

1. Introduction

Microorganisms form most of the biomass in the world. Some of these microbes live in the environment and are innocuous, but some have evolved virulence mechanisms to infect and replicate in mammalian hosts and cause disease. The human body contains many beneficial microflora, but is also subject to frequent challenges by pathogenic bacteria and viruses. Therefore, the ability to discriminately identify and eliminate virulent microbes is essential for the host. The immune system has evolved pattern recognition receptors (PRRs) to recognize microbial components. Upon recognizing a microbial threat, the immune system induces an inflammatory response to recruit leukocytes to the site of infection. The cardinal signs of inflammation include heat, redness, swelling, pain and loss of tissue function [1]. At a molecular level, these symptoms are caused by a signaling cascade, which is promoted by collaborations between cytokines, chemokines, and eicosanoids and related lipid mediators including prostaglandins and leukotrienes. Eicosanoids are a family of bioactive lipid mediators that regulate a wide variety of physiological as well as pathophysiological inflammatory responses [1,2]. These mediators are generated from arachidonic acid (AA) after its enzymatic release from membrane phospholipids *via* complex metabolic mechanisms involving over 50 unique enzymes [3]. In addition to arachidonic acid, the same enzymes can effectively metabolize other polyunsaturated fatty acids, such as linoleic and linolenic acids. While the induction of inflammation is a highly regulated process to control microbial infection, failure to resolve inflammation can lead to chronic disease or severe tissue damage. A class of anti-inflammatory/pro-resolution lipid mediators, including lipoxins,

Abbreviations: AA, arachidonic acid; ALR, AIM2-like receptor; CFU, colony forming units; COX, cyclooxygenase; CYP450, cytochrome P450; DDXs/DHXs, DExD/H box proteins; DHA, docosahexaenoic acids; diHETrE, dihydroxy-eicosatrienoic acids; diHOME, dihydroxy-octadecenoic acids; EET, epoxy-eicosatrienoic acids; EPA, eicosapentaenoic acid; EpOME, epoxy-octadecenoic acids; ESI, electrospray ionization; GM–MS, gas chromatography coupled with mass spectrometry; HDoHE, hydroxy-docosahexaenoic acid; HEPE, hydrox-eicosapentaenoic acids; HETE, hydroxy-eicosatetraenoic acids; HODE, hydroxy-octadecadienoic acids; HOTrE, hydroxy-octadecatrienoic acids; HPLC, high performace liquid chromatography; HXA_3/B_3, hepoxilin A3/B3; IκB, inhibitor of kappa B; LC–MS, liquid chromatography coupled with mass spectrometry; LOX, lipoxygenase; LT, leukotriene; LXA_4/B_4, lipoxin A4/B4; MRM, multiple reaction monitoring; NFκB, nuclear factor kappa B; NLR, NOD-like receptor; NSAIDs, non-steroidal anti-inflammatory drugs; oxoODE, oxo-octadecadienoic acid; PAF, platelet-activating factor; PD1, protectin D1; PG, prostaglandin; PMN, polymorphonuclear cells; PPAR, peroxisome proliferator-activated receptor; PRRs, pattern recognition receptors; RLR, RIG-I like receptor; TLR, toll-like receptor; TNF, tumor necrosis factor; TXA_2/B_2, thromboxane A2/B2; UDP, uridine 5′-diphosphate.

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resolvins, protectins, and maresins, which are derived from AA, DHA and EPA, orchestrate the resolution phase of inflammation [4].

The lipid metabolism network contains multiple precursors, large enzyme families, and over one-hundred lipid species. The complexity of this network is magnified by several characteristics. Multiple enzymes can act on a single substrate, and conversely, multiple substrates can be modified by the same enzyme [1–3] allowing for crosstalk between the pathways. Inhibition or down-regulation of an enzyme within one pathway may "shunt" the substrate through another pathway [2,3,5]. Also, many lipid mediators are susceptible to lipid peroxidation, non-enzymatic oxidation, or other modifications. Often, the *in vivo* levels of a specific lipid mediator are most accurately inferred by measuring the abundance of a stable degradation product rather than the mediator itself. Lastly, transcellular biosynthesis, a process in which a substrate intermediate produced by one cell type is utilized by another to generate the final lipid mediator [3,4], requires the understanding of interactions between different cell types. For these reasons, a systematic approach is required to understand the entire lipidomic response network.

Although studying the lipid-mediated immune response(s) involves deconvoluting a complicated network, the resulting insights have significant potential for therapeutic and translational impact. Certain lipidomic metabolism pathways have been highly valuable targets for pharmacological interventions. Non-steroidal anti-inflammatory drugs (NSAIDs) have been widely used as over-the-counter analgesics that mainly target the cyclooxygenase pathway [4,6]. Besides the COX pathway, therapeutics have been developed that inhibit the lipoxygenase pathway. For example, the leukotriene receptor antagonists zafirlukast (Accolate) and montelukast (Singulair) have been shown to significantly improve the quality of life for asthmatic patients [7]. Moreover, statins, a class of therapeutics that reduce low-density lipoprotein (LDL) cholesterol levels in humans, also induce the generation of 15-epi-lipoxins, thus having an anti-inflammatory activity [8]. The use of statins and other immunoregulatory compounds may modulate inflammation during influenza [9] or other infections [10]. Lastly, the discovery of anti-inflammatory/pro-resolution lipid mediators that have the ability to modulate excessive inflammation in a wide range of animal disease models [8,11], including cystic fibrosis [12], sepsis [13] and colitis (IBD) [14], provides a potential new class of pharmacological compounds.

2. Role of eicosanoid and other bioactive lipid mediators in the induction and resolution of inflammation

Many lipid mediators have been studied individually to determine their functions in various biological contexts. Receptors recognizing lipid mediators may have one or multiple isoforms expressed differentially in distinct cell types; therefore, the effects of a lipid mediator are likely to be cell and tissue specific [15]. Due to the complexities and vast body of knowledge in the literature, we will not comprehensively discuss the characteristics, functions, and biogenesis of all the bioactive lipids but refer the reader to the many excellent reviews describing the various families of lipid mediators [3,4,16–18]. Instead, we will give an overview of the different classes of lipid mediators in the context of the immune response.

2.1. Arachidonic acid

Arachidonic acid (C20:4ω6) is released from the plasma membrane by phospholipases. The three major metabolic pathways for enzymatic arachidonic acid biogenesis are the cyclooxygenase pathway, lipoxygenase pathway, and cytochrome P450 pathway (Fig. 1A). The cyclooxygenase pathway (COX-1 and COX-2) produces prostaglandins and thromboxanes. The lipoxygenase pathway (5-LOX, 12-LOX and 15-LOX) produces leukotrienes, and numerous hydroperoxy, hydroxy fatty acids (HPETEs and HETEs), hepoxilins and lipoxins. Finally, the cytochrome P450 pathway produces epoxides and corresponding dihydroxy metabolites of arachidonic acids (EETs and diHETrEs).

The conversion from AA to prostaglandins begins with the catalytic enzymes COX-1 or COX-2. COX-1 is encoded by a constitutively expressed gene *ptgs1* and COX-2 is encoded by an immediate early response gene *ptgs2* [15]. COX-1/2 produces PGG2 and PGH2, which in turn are converted to various prostaglandins (PGE_2, PGD_2, PGI_2, and $PGF_{2\alpha}$) or thromboxanes (TXA_2 and TXB_2) by their cognate synthases. Many prostaglandins have pro-inflammatory activity due to their vasomodulatory effects [19]. PGE_2 is one of the most abundant prostaglandins produced in the body. It promotes many of the signs of inflammation due to its ability to augment arterial dilation and increase microvascular permeability; it also induces pain by acting on peripheral sensory neurons and on central sites within the spinal cord and the brain [20]. Recently, PGE_2 has been shown to possess anti-inflammatory activity, up-regulating cAMP and inducing the secretion of IL-10, an anti-inflammatory cytokine [21]. PGD_2 is synthesized in the central nervous system to regulate neurophysiological functions and is also produced by mast cells, which initiate acute allergic responses [16,22]. In addition to its pro-inflammatory activities, PGD_2 can significantly attenuate inflammation in experimental models of pleuritis and colitis [22]. Moreover, PGD_2 can be converted into its nonenzymatic degradation product 15d-PGJ_2, which inhibits NFκb signaling and activates PPARγ, both contributing to anti-inflammatory effects [23]. PGE2 along with PGD2 also upregulates 15-LOX, giving rise to lipid mediator class switching and promoting the biosynthesis of pro-resolving mediators [24]. PGI_2 regulates cardiovascular homeostasis and mediates the edema and pain that accompany acute inflammation [16]. PGI_2 exerts its effects locally and is rapidly converted to its inactive hydrolysis product, 6-keto-$PGF_{1\alpha}$, by nonenzymatic processes [16]. Elevated $PGF_{2\alpha}$ levels are reported in patients with chronic inflammatory diseases, such as rheumatoid arthritis, psoriatic arthritis, reactive arthritis, and osteoarthritis [16]. Because of its instability, the level of $PGF_{2\alpha}$ *in vivo* is reflected by its major stable metabolite 15k $PGF_{2\alpha}$ [16]. Lastly, thromboxane A2 (TXA_2) is an unstable metabolite that degrades into the biologically inactive TXB_2 [3]. TXA_2 is produced predominantly by platelets and mediates platelet adhesion and aggregation, smooth muscle contraction and proliferation, and activation of endothelial inflammatory responses [16].

Within the LOX pathway, 5-LOX-derived leukotrienes (LTB_4, LTC_4 and LTE_4) have chemoattractant activities and are potent mediators for immediate hypersensitivity, bronchoconstriction, smooth muscle contraction, and increased vascular permeability [25]. 5-HETE, another mediator produced by 5-LOX, has been shown to induce airway contraction and potentiate neutrophil transcellular migration [26]. In contrast to the 5-LOX-derived pro-inflammatory lipid mediators, 12- and 15-HETE, derived from 12-LOX and 15-LOX, have anti-inflammatory activity, blocking TNFα-induced IL-6 secretion from macrophages [27]. 12-LOX derived hepoxilins (HXA_3 and HXB_3) are highly unstable, but their stable analogs can inhibit macrophage influx as well as fibrosis in the lung [28]. Lipoxins (LXA_4 and LXB_4), derived from 15-LOX or 5-LOX/12-LOX, are the prototypic members of the endogenous anti-inflammatory/pro-resolution lipid mediators [29].

There are 57 human and 102 mouse functional enzymes within the CYP450 pathway [3]. 16-HETE, an ω-hydroxylated derivative of AA produced by CYP450 enzyme, can inhibit human PMN adhesion and aggregation, as well as decrease LTB_4 synthesis [30]. Epoxy- and dihydroxy-derivatives of arachidonic acid (EET and