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Invited review article

Eosinophil polyunsaturated fatty acid metabolism and its potential control of inflammation and allergy

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ABSTRACT

Polyunsaturated fatty acids (PUFAs) exhibit a range of biological effects, many of which are mediated through the formation and actions of their bioactive metabolites. It is well appreciated that dietary PUFA balance affects inflammation and/or allergic diseases, and recent advances in liquid chromatography tandem mass spectrometry (LC-MS/MS)-based mediator lipidomics have revealed a potential link between PUFA metabolism and biological phenotypes. This review presents insights into the emerging roles of eosinophil PUFA metabolism in controlling inflammatory responses and its potential involvement in allergy control.

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Abbreviations:

PUFA, polyunsaturated fatty acid; LC-MS/MS, liquid chromatography tandem mass spectrometry; COX, cyclooxygenase; LOX, lipoxygenase; CYP, cytochrome P450; HETE, hydroxy-eicosatetraenoic acid; EET, epoxy-eicosatrienoic acid; LT, leukotriene; PG, prostaglandin; LX, lipoxin; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; DLN, draining lymph node; PMN, polymorphonuclear leukocyte; PAF, platelet activating factor

PUFA metabolism and regulation of inflammation

PUFA metabolism is recognized as an important factor in immune regulation and disease control. In particular, the metabolic balance between n-6 and n-3 PUFAs is widely held to be important in human health and diseases. PUFA-derived bioactive metabolites are formed *in vivo* by enzymatic oxidation through the action of cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 monooxygenases (CYP). From n-6 PUFAs, e.g., arachidonic acid, the COX pathway leads to the formation of prostaglandins (PG) and

thromboxanes, the LOX pathway leads to leukotrienes (LT) and lipoxins (LX), and the CYP pathway leads to hydroxy-eicosatetraenoic acids (HETE) and epoxy-eicosatrienoic acids (EET).^{1,2} In general, COX-derived PGs and 5-LOX-derived LTs are involved in the initiation of inflammatory responses, whereas 12/15-LOX-derived LXs counter-regulate the inflammatory processes and may be involved in the resolution of inflammation. The CYP pathway generates EETs, which may have roles in regulation of inflammation and vascular tone.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are n-3 PUFAs, and dietary supplementation with such compounds is widely held to have beneficial effects in many inflammatory disorders.³ Consistently, elevation of tissue n-3 PUFA levels in fatty acid n-3 desaturase (*fat-1*) transgenic mice is protective in many disease models.^{4,5} EPA and DHA are good substrates for LOX and CYP, and thus can be efficiently converted into bioactive

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metabolites such as E-series resolvins, D-series resolvins, protectins and maresins.⁶ The n-3 PUFAs are thought to play anti-inflammatory and tissue protective roles via several mechanisms; by competing with the arachidonate cascade to attenuate the formation and action of pro-inflammatory eicosanoids (e.g., PGs and LTs), or by being converted to potent anti-inflammatory and/or pro-resolving mediators such as resolvins and protectins (Fig. 1). Thus, to elucidate the molecular mechanisms underlying the importance of PUFA balance and to gain a comprehensive understanding of their physiological and/or pathophysiological roles, it is important to know when, where and how much of these PUFA metabolites are formed in inflammatory sites. To this end, we developed a LC-MS/MS-based mediator lipidomics system that can detect and quantify more than 500 PUFA metabolites simultaneously.¹

Eosinophils control inflammatory responses via lipid signals

Acute inflammation is an important host defense mechanism, and its proper resolution is required to maintain homeostasis.⁷ The resolution of inflammation involves active cellular and molecular programs that enable inflamed tissues to return to their homeostatic state. The mechanisms by which acute inflammation is resolved are of interest to both basic scientists and clinicians, and research in recent years has uncovered novel endogenous mechanisms that involve 12/15-LOX-expressing eosinophils in controlling resolution of inflammation.⁸

LC-MS/MS-based mediator lipidomics revealed temporal changes in PUFA metabolites during the course of acute inflammation and resolution. The highest levels of COX- and 5-LOX-derived mediators such as PGE₂ and LTB₄ were observed during the initiation phase, and the formation of 12/15-LOX-derived mediators such as LXA₄ and protectin D1 was increased during the resolution phase of acute peritonitis.^{8,9} In the resolution phase, eosinophils were the major cell type expressing 12/15-LOX. The *in vivo* depletion of eosinophils significantly reduced 12/15-LOX products in resolving exudates and caused a resolution defect characterized by impaired lymphatic drainage to draining lymph nodes (DLNs),

along with increased polymorphonuclear leukocyte (PMN) numbers in inflamed sites. Furthermore, the resolution deficit caused by eosinophil depletion was rescued by adoptive transfer of eosinophils, but eosinophils deficient in 12/15-LOX were unable to rescue the resolution phenotype. These results indicated that eosinophils are recruited to the inflamed site, where they locally produce pro-resolving mediators via a 12/15-LOX-initiated biosynthetic route and play roles in promoting resolution of acute inflammation (Fig. 2).⁸

Macrophages play important roles in the resolution of inflammation by efficient clearance of apoptotic cells and/or tissue debris from inflamed sites, and thereby facilitate the return of the tissue to a state of homeostasis. 12/15-LOX-expressing eosinophils promote resolution of acute peritonitis by regulating gene expression patterns in macrophages. Microarray analysis revealed that eosinophils significantly increased the expression of macrophage CXCL13 by a 12/15-LOX-dependent mechanism.¹⁰ CXCL13 was expressed in a subset of macrophages present in the resolution phase, and mice treated with anti-CXCL13 antibody displayed a resolution deficit, with reduced lymphatic drainage to DLNs. It is well established that CXCL13 is a functional chemokine in the early development of peripheral lymph nodes.¹¹ Since lymphatic drainage is involved in promoting inflammatory cell clearance from the periphery, we questioned whether 12/15-LOX-expressing eosinophils actively contribute to this process. Analysis of the cellular composition of the inflamed DLNs revealed that eosinophils were present, and that their depletion or 12/15-LOX deficiency resulted in reduced numbers of every immune cell component examined, including lymphocytes, macrophages, PMNs and dendritic cells. Adoptive transfer of eosinophils, or administration of recombinant CXCL13, restored the inflamed DLN expansion with increased cell numbers. These results demonstrated that 12/15-LOX-expressing eosinophils control inflamed DLN hypertrophy through the CXCL13 pathway in mice (Fig. 2). It is possible that eosinophils actively promote DLN remodeling and expansion that could in turn drive lymphatic drainage and efficient clearance of inflammatory exudates from peripheral tissues.¹⁰

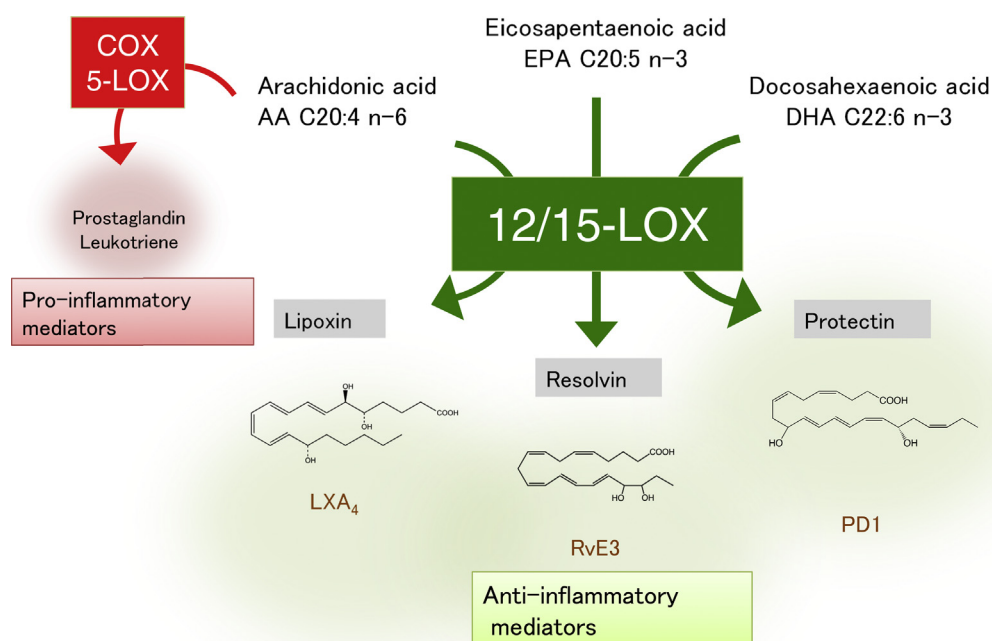


Fig. 1. Lipid mediators that regulate inflammatory responses. In addition to arachidonic acid-derived mediators such as COX-derived PGs and 5-LOX-derived LTs, 12/15-LOX-derived mediators such as LXs and n-3 PUFA-derived resolvins and protectins are also produced. These mediators play important roles in regulating inflammatory responses.

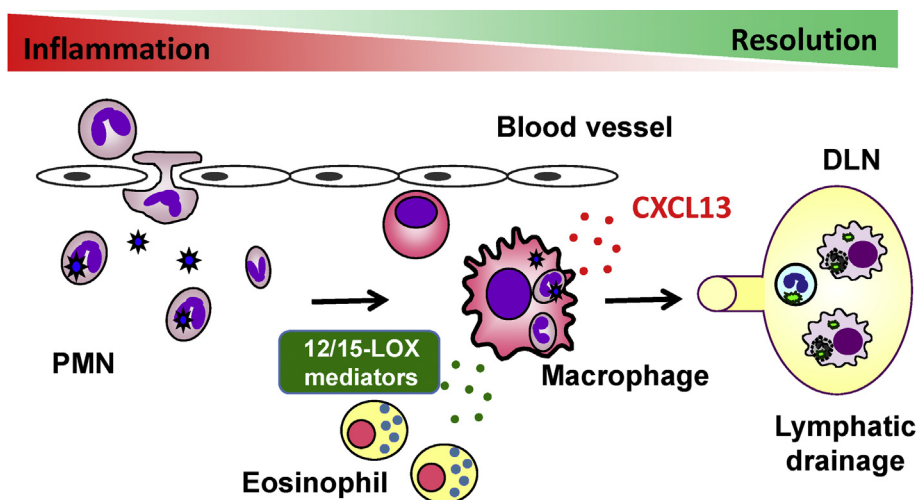


Fig. 2. Eosinophils promote resolution of acute inflammation via lipid signals. Eosinophils are recruited in the early resolution phase, where they locally produce resolution mediators via a 12/15-LOX-initiated biosynthetic route, and play roles leading to the efficient clearance of inflammatory exudates via lymphatic drainage. 12/15-LOX-expressing eosinophils control inflamed DLN hypertrophy, which is important for inflammatory cell clearance, through the CXCL13 pathway.

PUFA metabolism in health and disease

Eosinophils serve as innate immune cells by producing pro-inflammatory lipid mediators such as leukotrienes and platelet activating factor (PAF), but as described above, eosinophils also express relatively high level of 12/15-LOX and produce anti-inflammatory mediators. Therefore imbalanced PUFA metabolism in eosinophils could be involved in disease pathogenesis especially when sustained inflammation is suspected as a key component of pathogenesis. A human clinical study revealed impaired 15-LOX activity in eosinophils isolated from severe asthmatic patients as compared to healthy controls (Fig. 3).¹² This observation is consistent with other reports^{13–15} suggesting that PUFA metabolism is dysregulated in eosinophils and that this could contribute to disease pathogenesis in severe asthmatics. Further multi-omics studies are ongoing by our group to identify transcripts, proteins

and metabolites differentially expressed in eosinophils isolated from allergy patients, determining the presence of eosinophil heterogeneity (i.e. hypodense eosinophils etc.) as reported previously.^{16,17}

Mediator lipidomics have also revealed that the 12/15-LOX-mediated pathway is an important host defense mechanism to protect against influenza virus infection.¹⁸ 12/15-LOX-deficient mice were more susceptible to severe symptoms of influenza virus infection, and administration of 12/15-LOX-derived products reduced influenza virus replication by interfering with nuclear export of viral RNA in cultured cells. Protective effects were also seen *in vivo*, where administration of a PUFA-derived lipid mediator improved the pathology and survival rate. These results indicate that the 12/15-LOX pathway is protective against influenza virus infection, and that 12/15-LOX-derived PUFA metabolites could be used in the development of novel therapeutics.

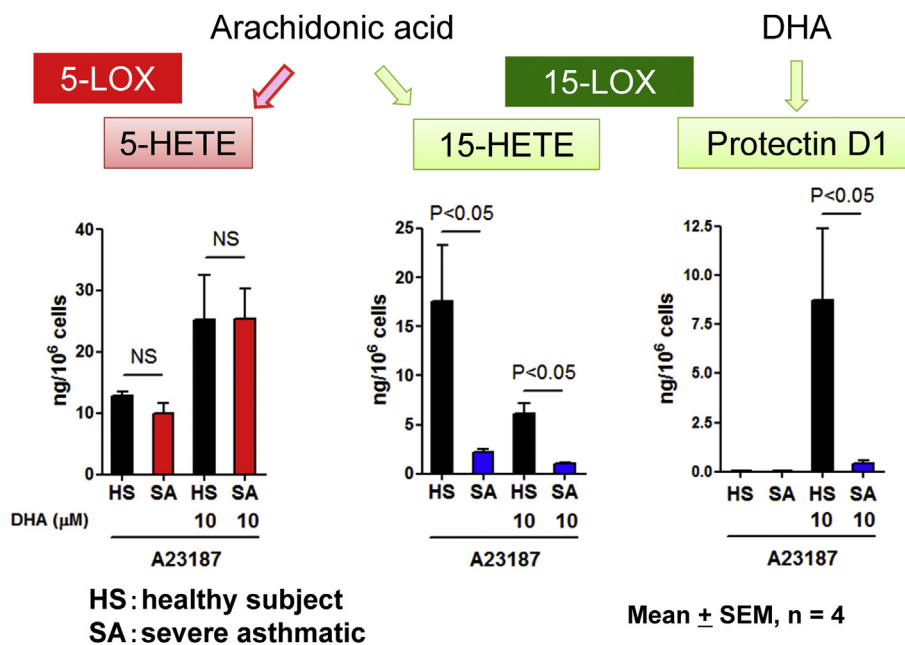


Fig. 3. Impaired 15-LOX activity in eosinophils from severe asthmatics. Peripheral blood eosinophils were stimulated with the calcium ionophore A23187 in the presence or absence of the n-3 PUFA docosahexaenoic acid (DHA). The levels of PUFA metabolites were measured by LC-MS/MS.¹²

A tissue-protective role of 12/15-LOX-expressing eosinophils is also supported by other disease models. It is well established that eosinophils reside in the colonic mucosa, and it was reported that eosinophil-deficient mice developed more severe colitis, and their colon tissues contained a greater number of neutrophils.¹⁹ Lipidomic analyses of colonic tissue from eosinophil-deficient mice identified a deficiency in 12/15-LOX-derived mediators, moreover, administration of a 12/15-LOX product to eosinophil-deficient mice reduced the disease severity.

Perspectives

Eosinophils are involved in the innate immune response to helminth infection and also in allergic responses.²⁰ Recently, eosinophils were shown to play homeostatic roles in adipose tissue, skeletal muscle, liver and small intestine.^{21–24} In this brief review, we presented insights into the emerging role of eosinophils as pro-resolving and tissue protective effector cells via the 12/15-LOX-mediated pathway. This is a novel and important endogenous mechanism that properly brings acute inflammation to a close, and is potentially related to clinical findings of dysregulated 12/15-LOX activity in eosinophils from patients with severe asthma.¹²

Besides eosinophils, 12/15-LOX is expressed in a subset of macrophages and airway epithelial cells.²⁵ 12/15-LOX-expressing macrophages are present in the peritoneal cavity of naïve mice and also during the resolution phase of acute peritonitis. These cells display competent phagocytic activity that potentially contributes to tissue homeostasis by efficient clearance of apoptotic cells.²⁶ Cells expressing 12/15-LOX may be involved in regulating inflammatory responses by locally producing anti-inflammatory and/or pro-resolving lipid mediators. This model is consistent with the findings in disease models of inflammatory phenotypes and impaired host defense in 12/15-LOX-deficient mice.^{26–29}

Mediator lipidomics deals with the simultaneous and quantitative analysis of bioactive lipid mediators in biological systems. When combined with multi-omics profiling, it can greatly assist in understanding the role of PUFA metabolism in certain physiological or pathophysiological conditions. This technology could potentially identify the metabolic fingerprint of a particular disease for clinical diagnosis and treatment. Moreover, identification of endogenous anti-inflammatory and/or pro-resolving lipid signals^{4,30,31} and elucidation of their molecular mechanisms should lead to the development of novel therapeutics for inflammatory diseases.

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Conflict of interest

The author has no conflict of interest to disclose.

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