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Review Article

Cysteinyl leukotriene metabolism of human eosinophils in allergic disease

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AERD, aspirin-exacerbated respiratory diseases; CCR3, C–C chemokine receptor type 3; Cys-LT, cysteinyl leukotriene; DPEP2, dipeptidase-2; EOS, eosinophil; FLAP, 5-LOX-activating protein; fMLP, formyl-methionyl-leucyl-phenylalanine; GGT5, gamma-glutamyl transferase-5; GM-CSF, granulocyte-macrophage colony-stimulating factor; HpETE, hydroperoxy-eicosatetraenoic acid; IFN, interferon; IL, interleukin; LIR, leukocyte Ig-like receptor; LOX, lipoxygenase; LTA₄, leukotriene A₄; LTC₄S, leukotriene C₄ synthase; LTD₄, leukotriene D₄; LTE₄, leukotriene E₄; LXA₄, lipoxin A₄; NOD2, nucleotide binding oligomerization domain containing 2; PAF, platelet activating factor; PGD₂, prostaglandin D₂;

ABSTRACT

Eosinophils are multifaceted immune cells with diverse functions that enhance allergic inflammation. Cysteinyl leukotrienes (cys-LTs), mainly synthesized in eosinophils, are a class of inflammatory lipid mediators produced via multiple enzymatic reactions from arachidonic acid. Multiple clinical studies have reported dysregulated fatty acid metabolism in severe asthma and aspirin-exacerbated respiratory diseases. Therefore, understanding the mechanism responsible for this metabolic abnormality has attracted a lot of attention. In eosinophils, various stimuli (including cytokines, chemokines, and pathogen-derived factors) prime and/or induce leukotriene generation and secretion. Cell–cell interactions with component cells (endothelial cells, epithelial cells, fibroblasts) also enhance this machinery to augment allergic responses. Nasal polyp-derived eosinophils from patients with eosinophilic rhinosinusitis present a characteristic fatty acid metabolism with selectively higher production of leukotriene D₄. Interestingly, type 2 cytokines and microbiome components might be responsible for this metabolic change with altered enzyme expression. Here, we review the regulation of fatty acid metabolism, especially cys-LT metabolism, in human eosinophils toward allergic inflammatory status.

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PGE₂, prostaglandin E₂;
 PLA2, phospholipase A2; RANTES, regulated
 on activation, normal T cell expressed and
 secreted; TNF, tumor necrosis factor;
 TLR, toll-like receptor

Introduction

Eosinophils play an important role in allergic inflammatory diseases and parasite infection. Mature eosinophils in the bone marrow accumulate in inflamed local sites through adhesion to vascular endothelial cells and migration in response to eosinophil-specific chemokines. Active cellular functions involve production of inflammatory lipid mediators represented by cysteinyl leukotrienes (cys-LTs), degranulation, super oxide generation, and release of cytokines [such as interleukin (IL)-4, IL-12, regulated on activation, normal T cell expressed and secreted (RANTES), and interferon (IFN)- γ], which amplify inflammatory responses.^{1,2} Furthermore, non-apoptotic and non-necrotic cell death, which elicits extracellular releases of DNA and granule proteins induced by interaction with microorganisms, is recently recognized as an important function of eosinophils, called ETosis.^{3,4} Endogenous Cys-LT biosynthesis is important as a pro-allergic function because of its multipotent actions in an autocrine and paracrine manner to enhance other cellular functions, including cytokine secretion, degranulation, adhesion, migration, and survival, and induce accumulation and activation of other immune cells such as lymphocytes, dendritic cells, mast cells, and basophils^{5–14} (Fig. 1). In contrast, eosinophils have a distinctive ability to produce anti-inflammatory lipid mediators such as lipoxins, resolvins, and protectins through enzymatic reactions of 15-lipoxygenase (LOX) from arachidonic acid or docosahexaenoic acid,^{15–17} suggesting the counterregulatory roles of eosinophils in the regulation of inflammation. Previous studies reported that eosinophils could negatively regulate allergic airway inflammation, which emphasizes the anti-inflammatory aspects of eosinophils.^{18,19}

This review article focuses on the metabolic regulation of cys-LTs in inflammatory eosinophils, a major cellular source of these mediators, and explains how fatty acid metabolism of eosinophils could be regulated in asthma and aspirin-exacerbated respiratory diseases (AERD), which are often complicated with eosinophilic rhinosinusitis.

Cys-LT metabolism in eosinophils

Cys-LTs are produced by complex interplay of various enzymes from arachidonic acid liberated from membrane phospholipids by phospholipase A2 in addition to prostaglandins, thromboxanes, and lipoxins (Fig. 2).^{20,21} First, 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) is produced from arachidonic acid by the action of 5-LOX and 5-LOX activating protein (FLAP) that move from the cytoplasm to the nuclear membrane. Continuous enzymatic reaction proceeds to convert 5-HpETE into the transient epoxide intermediate, leukotriene A₄ (LTA₄). LTA₄ serves as a substrate for leukotriene C₄ synthase (LTC₄S) that conjugates LTA₄ with glutathione (GSH) to synthesize LTC₄. Formation of LTD₄ from LTC₄ is catalyzed by the enzymatic action of gamma glutamyl transferase (GGT).^{22,23} Dipeptidase (DPEP) further converts LTD₄ to LTE₄ to complete the sequential peptide cleavage process.²⁴ Collectively, LTC₄, LTD₄, and LTE₄ are called Cys-LTs.

Cys-LTs bind to specific receptors, namely CysLT₁, CysLT₂, and CysLT₃ (GPR99), with different affinity as ligands.^{25,26} Signaling through these receptors exerts a wide range of immune responses observed in various inflammatory diseases including asthma. In particular, CysLT₁ is widely expressed in airway smooth muscle cells, eosinophils, mast cells, basophils, and lymphocytes, and functions as a trigger to initiate allergic inflammation. CysLT₁ antagonists are currently available options to target bronchial asthma and allergic rhinitis.^{26,27} Therapeutic effects of this drug are mediated via suppression of bronchoconstriction, hyper vascular permeability, mucus production, and infiltration of inflammatory cells.

Eosinophils, in addition to mast cells, basophils, and monocytes/macrophages, are major producers of cys-LTs. Synthesis and release of these mediators by eosinophils are controlled by various factors, including cytokines, migration factors, adhesion molecules, microbial constituents, and hormones. Briefly, modification of the cys-LT synthetic profile in eosinophils depends on four biological processes: a) priming of cells with various factors, b) inducible release

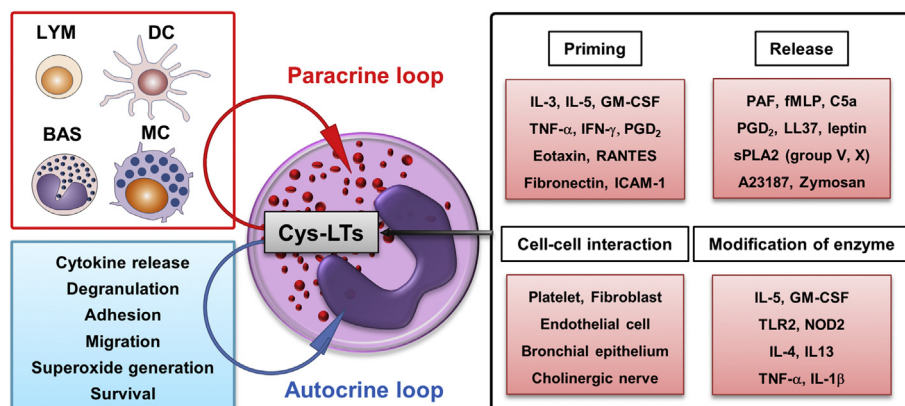


Fig. 1. Various factors promote cysteinyl leukotriene generation in autocrine and/or paracrine manners in human eosinophils. Various factors including cytokines, migration factors, adhesion molecules, microbial constituents, and hormones orchestrate biosynthesis and extracellular release of cysteinyl leukotrienes by eosinophils. Biosynthesis and extracellular release of cysteinyl leukotrienes by eosinophils are controlled through four biological processes (priming of cells with various factors, inducible release from cells, cellular interaction with other types of cells, and quantitative and/or functional changes of metabolic enzymes). Cysteinyl leukotrienes released from eosinophils enhance other eosinophilic functions and also elicit cellular activations of other immune cells to amplify inflammatory responses. LYM, lymphocyte; DC, dendritic cell; BAS, basophil; MC, mast cell.

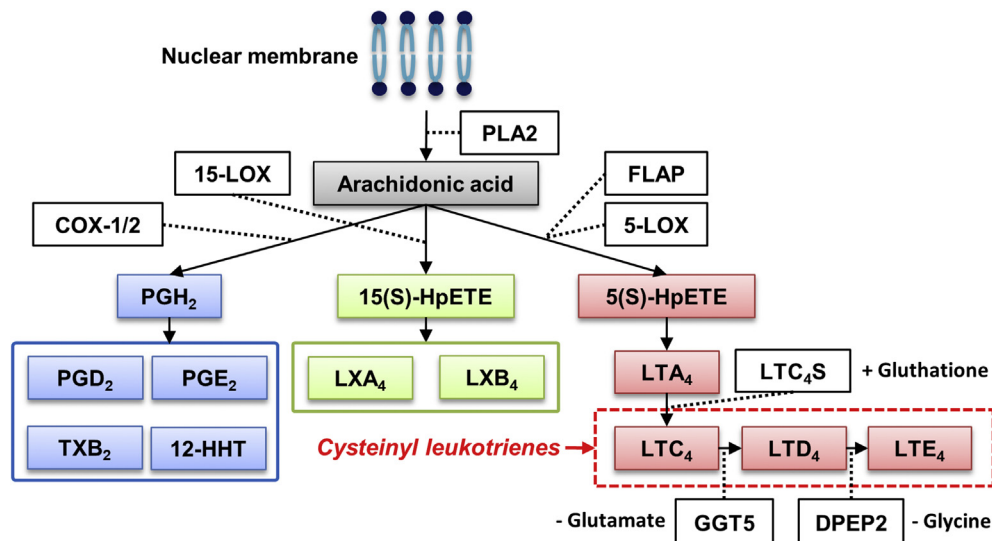


Fig. 2. The formation of cysteinyl leukotrienes through multiple enzymatic reactions in arachidonic acid metabolism. Complex interplay of multiple enzymes converts arachidonic acid liberated from the nuclear membrane by phospholipase A2 (PLA2) into cysteinyl leukotrienes. 5-Lipoxygenase (5-LOX) and FLAP (5-LOX activating protein) produce 5-hydroperoxy-eicosatetraenoic acid (5-HpETE) from arachidonic acid and subsequently convert it into leukotriene A₄ (LTA₄). Leukotriene C₄ synthase (LTC₄S) conjugates LTA₄ with glutathione (GSH) to synthesize LTC₄. Gamma glutamyl transferase (GGT) and dipeptidase (DPEP) catalyze the enzymatic transformation of LTC₄ into LTD₄ and LTD₄ into LTE₄, respectively. The three molecules, LTC₄, LTD₄, and LTE₄ are a class of lipid mediators termed as cysteinyl leukotrienes. Arachidonic acid is also metabolized into prostaglandin E₂ (PGE₂), prostaglandin D₂ (PGD₂), thromboxane B₂ (TXB₂), and 12-hydroxyheptadecatrienoic acid (12-HHT) via cyclooxygenase-1/2 (COX-1/2) or lipoxin A₄ (LXA₄) and lipoxin B₄ (LXB₄) via 15-lipoxygenase (15-LOX) in cooperation with other related enzymes.

from cells, c) cellular interaction with other types of cells, and d) quantitative and/or functional changes of metabolic enzymes (Fig. 1).

Cell priming

Type 2 cytokines, IL-3,^{28,29} IL-5,^{28–30} and granulocyte-macrophage colony-stimulating factor (GM-CSF)^{31–33}; tumor necrosis factor (TNF)- α ^{34,35}; and IFN- γ ³⁶ prime eosinophils to promote synthesis and enhance subsequent release of cys-LTs. Enhanced cys-LT synthesis is observed in hypodense eosinophils, which are converted from normal peripheral blood eosinophils by incubation with IL-3, IL-5, and GM-CSF for a long period.^{37–40}

Chemotactic factors including platelet activating factor (PAF),⁴¹ prostaglandin D₂ (PGD₂),^{42,43} eotaxin, and RANTES^{44,45} also enhance subsequent stimulation of nonphysiological stimuli, calcium ionophore, or zymosan to release cys-LTs. Adhesion molecules such as fibronectin^{46,47} and ICAM-1⁴⁸ also have priming effects on eosinophils, suggesting that adhesive cell–cell interaction triggers cys-LT production. Interestingly, major basic protein, an eosinophil granule protein, also possesses a cell-priming effect to augment LTC₄ synthesis in addition to degranulation and IL-8 production, indicative of its function as an autocrine mediator.⁴⁹

Inducible release from cells

Calcium ionophore^{50,51} and zymosan⁵² cause cellular influx of calcium ions to induce cys-LT release from eosinophils. Additionally, their physiological release is mainly induced by potent eosinophil chemotactic factors such as PAF,^{28,53} C5a,²⁸ PGD₂,⁵⁴ eotaxin, and RANTES.⁴⁴ These phenomena indicate that chemotactic responses to the inflamed sites timely initiate cys-LT synthesis to enhance eosinophilic inflammation. Interestingly, IL-16, an eosinophil and lymphocyte chemoattractant, elicits LTC₄ release through intracellular action of granule-stored RANTES,⁵⁵ indicative of autocrine regulation of this metabolism.

Additionally, C–C chemokine type 3 (CCR3)-ligands, eotaxin and RANTES, induce the release of IL-4 depending on the formation of LTC₄ within a lipid-body,^{7,56} suggesting the importance of intracellular signaling thorough cys-LTs within cells.

Furthermore, LL37,⁵⁷ an antimicrobial peptide, and formyl-methionyl-leucyl-phenylalanine (fMLP),^{28,58} a peptide released from bacteria possessing chemotactic activity toward eosinophils, also stimulate cys-LT production, suggestive of a close association between bacterial infections and leukotriene synthesis. Notably, eosinophils express LIR7, an activating class of leukocyte Ig-like receptors (LIRs), to sense microbially-cleaved immunoglobulins and its ligation to LIR7-specific antibody induces LTC₄ synthesis simultaneously with the release of IL-12, but not IL-4.^{59,60}

Various types of secretory phospholipase A2 (sPLA2) function extracellularly in autocrine or paracrine manners to specifically recognize cellular phospholipids and catalytically hydrolyze the bond releasing arachidonic acid and lysophospholipids. Thus, sPLA2 released from nearby cells also triggers cys-LT release from eosinophils.^{61–65}

Recently, leptin, a peptide hormone derived from adipocytes to regulate energy balance to inhibit hunger, has been recognized as an inducer of cys-LT release.⁶⁶ The levels of this hormone are increased in obese persons,⁶⁷ suggesting the involvement of obesity in pro-inflammatory fatty acid metabolism in allergic inflammation.

Cell–cell interaction

Cell–cell interactions are essential for eosinophils to infiltrate the local inflamed sites. Previous studies demonstrated that cellular interactions trigger cys-LT generation to facilitate inflammatory actions. Eosinophils interact with vascular endothelial cells,^{68–70} bronchial epithelial cells,^{62,71} fibroblasts,⁴⁰ or cholinergic nerves⁷² to enhance cys-LT production. These facts suggest the possibility that the sites of cys-LT synthesis are mainly in blood vessels, pulmonary interstitium, and airway.

Quantitative and/or functional change in metabolic enzyme expression

Changes in the expression levels of metabolic enzymes also regulate the amount of cys-LT produced by eosinophils. sPLA2 is increased by co-stimulation of IL-4/IL-13 or TNF- α /IL-1 β , resulting in increased production of cys-LTs.⁶⁵ IL-5, a potent eosinophil pro-surviving and activating factor, enhances the production of cys-LTs via the increased expression of FLAP, but not 5-LOX.⁷³

Eosinophils as cys-LT-producing cells in asthma and AERD (Table 1)

Previous reports confirmed the fact that eosinophils play a central role in cys-LT production in the local inflamed sites in asthma. Immunostaining experiments of airway mucus samples taken from patients with asthma revealed that the major LTC₄S-expressing cells are eosinophils.^{73,74} In addition, cell numbers of eosinophils in tissue specimen are correlated with the concentration of cys-LTs in bronchoalveolar lavage fluid.⁷³

Several studies have demonstrated that the synthetic capacity of LTC₄ from peripheral blood-derived eosinophils of patients with asthma is higher than that of healthy subjects^{75–79} (Table 1). Among the cys-LT metabolism-related enzymes, the expression levels of 5-LOX⁸⁰ and FLAP^{80,81} in eosinophils of asthmatic patients were increased, resulting in enhanced cys-LT metabolism.

Of note, severe asthmatics have more frequent aspirin intolerance, defined as AERD, than non-severe asthmatics. Importantly, genetic mutation of LTC₄S leads to upregulated mRNA expression of LTC₄S and consequently enhanced cys-LT production in eosinophils from patients with AERD.⁸² Cell-specific LTC₄S expression in eosinophils was observed in peripheral blood, airway, and nasal polyp of patients with AERD.^{12,73,82} In addition, adhesive platelets to eosinophils increase cys-LT production in patients with AERD.⁸³ These findings might denote a pathophysiological relationship between cys-LT metabolism and AERD. Interestingly, IFN- γ , a type 1 cytokine, induces the expression of LTC₄ synthase during the differentiation stage of eosinophils from bone marrow progenitors⁸⁴ and this process might be involved in the pathogenesis of AERD because of the prominent expression of this cytokine in sampled tissue from patients with AERD.

Abnormality of cys-LT metabolism in eosinophils derived from nasal polyps

Although studies have reported the abnormality in cys-LT metabolism in asthma and AERD, no comprehensive evaluation of

the relationship between fatty acid metabolism and metabolic enzyme expressions using the same samples has been reported. Therefore, we established a method to assess fatty acid metabolism in granulocytes to combine multiple omics analyses including transcriptomics, proteomics, and lipidomics.^{85,86} This technique was applied for eosinophils derived from nasal polyps of patients with chronic eosinophilic rhinosinusitis to understand their cellular characteristics.⁸⁶ Of note, AERD is often complicated with chronic eosinophilic rhinosinusitis, suggestive of shared immunological mechanisms. Chronic eosinophilic rhinosinusitis is characterized by bilateral ethmoid sinuses that are main lesions with nasal polyps causing hyposmia.^{87,88} Recently, the number of patients with this disease has been increasing, simultaneously with the increased use of inhaled corticosteroid as primary treatment for asthma.

Lipidomic analysis of metabolites released from calcium ionophore-stimulated eosinophils derived from nasal polyp demonstrated that this type of eosinophils represented dysregulated fatty acid metabolic profile with increased LTD₄ synthesis. Interestingly, release of LTC₄ and LTE₄ was decreased, suggestive of selective upregulation of LTD₄ production. In addition, the 15-LOX- and COX-mediated production of anti-allergic mediators including lipoxin A₄ (LXA₄) and prostaglandin E₂ (PGE₂) was diminished, a pattern observed in peripheral blood-derived eosinophils from patients with severe asthma.¹⁵

Proteome and transcriptome analysis revealed that expression of gamma glutamyl transferase-5 (GGT-5) was increased and that of dipeptidase-2 (DPEP-2) was decreased in eosinophils derived from nasal polyps at both protein and mRNA levels. This enzymatic change may explain why the conversion of LTC₄ to LTD₄ and LTD₄ to LTE₄ is enhanced and impaired, respectively, resulting in selective retention of LTD₄. In addition, the mRNA and protein expression levels of FLAP and 15-LOX were increased and decreased, respectively. The overall regulation of metabolic enzymes, but not the change in a single enzyme, might convert fatty acid metabolism into an inflammatory phenotype.

To identify upstream factors leading to the expression changes in metabolic enzymes, integrated assessment was performed using pathway analysis of transcriptomic data. Involvement of pattern recognition receptors for microbial components (toll-like receptor 2 (TLR2) and nucleotide binding oligomerization domain containing 2 (NOD2)) and type 2 cytokines (IL-5 and GM-CSF) was identified as a candidate factor causing abnormal fatty acid metabolism among the lists of external stimuli closely linked to disease pathophysiology. This finding was consistent with previous reports that *Staphylococcus aureus*, a gram-positive bacteria normally resident

Table 1
Cys-LT synthesis in human eosinophils of patients with asthma, AERD, and CRSwNP.

Disease	Cell	Enzyme/activation	Metabolite (stimulus)	Reference
Asthma	PB-EOS	ND	Upregulated LTC ₄ synthesis (A23187)	75
Asthma	PB-EOS	Upregulation in hypodense population	Upregulated LTC ₄ synthesis (A23187)	76
Asthma	PB-EOS	ND	Upregulated LTC ₄ synthesis (A23187)	77
Asthma	PB-EOS	ND	Upregulated LTC ₄ synthesis (A23187)	78
Asthma	PB-EOS	ND	Upregulated LTC ₄ synthesis (PAF + A23187)	79
Asthma	PB-EOS	Upregulated expression of 5-LOX and FLAP	ND	80
Asthma	PB-EOS	Upregulated expression of FLAP	ND	81
Asthma	Airway-EOS	Upregulated expression of LTC ₄ S	ND	74
Asthma/AERD	Airway-EOS	Upregulated expression of LTC ₄ S	Cys-LTs in BALF (Lys-aspirin)	73
AERD	PB-EOS	Upregulated expression of LTC ₄ S	ND	82
AERD	PB-EOS	Platelet adherence to eosinophils	Upregulated LTC ₄ synthesis (A23187)	83
CRSwNP	NP-EOS	Upregulated expression of 5-LOX and LTC ₄ S	ND	12
CRSwNP	NP-EOS	Upregulated expression of FLAP and GGT5	Upregulated LTD ₄ synthesis (A23187)	86
CRSwNP	NP-EOS	Downregulated expression of DPEP2	Downregulated LTC ₄ and LTE ₄ synthesis (A23187)	86

AERD, aspirin-exacerbated respiratory disease; BALF, bronchial alveolar lavage fluid; CRSwNP, chronic rhinosinusitis with nasal polyp; Cys-LT, cysteinyl leukotriene; DPEP2, dipeptidase-2; FLAP, 5-lipoxygenase activating protein; GGT5, gamma-glutamyl transferase-5; 5-LOX, 5-lipoxygenase; LTC₄, leukotriene C₄; LTC₄S, leukotriene C₄ synthase; LTD₄, leukotriene D₄; LTE₄, leukotriene E₄; ND, not determined; NP, nasal polyp; PAF, platelet activating factor; PB, peripheral blood.

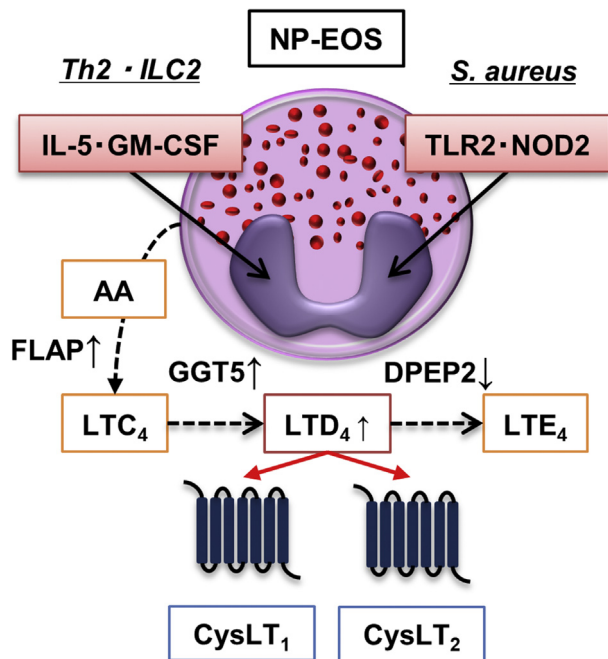


Fig. 3. Inflammatory regulation of nasal polyp-derived eosinophils with potent leukotriene D₄-synthesizing capacity. Nasal polyp-derived eosinophils (NP-EOS) acquire selective LTD₄-synthesizing capacity with upregulated expression of 5-lipoxygenase activating protein (FLAP) and gamma glutamyl transferase -5 (GGT5) and downregulated expression of dipeptidase-2 (DPEP2). Type 2 cytokines (interleukin (IL)-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF)) and pattern recognition receptors (toll-like receptor 2 (TLR2) and nucleotide binding oligomerization domain containing 2 (NOD2)) that mediate cellular interactions with *Staphylococcus aureus* (*S. aureus*) are candidate enhancers for this change of fatty acid metabolism. Leukotriene (LT) D₄ is a high-affinity ligand for two types of G protein-coupled receptors (GPCR), CysLT₁ and CysLT₂, to mediate inflammatory signals during allergic reactions. AA, arachidonic acid.

in nasal environment, is involved in the pathophysiology of chronic rhinosinusitis with nasal polyp under type 2 inflammation-dominant environment.^{89,90} In vitro experiments demonstrated that stimulation of eosinophils derived from peripheral blood of healthy subjects with type 2 cytokines (IL-5 or GM-CSF) or TLR2 and NOD2 agonists induced elevated mRNA expressions of GGT5 and FLAP and a decreased mRNA expression of DPEP2, which were consistent with changes observed in nasal polyp-derived eosinophils. These results suggested that the local environmental milieu exposed to cellular interactions with lymphocytes and microorganisms might affect the characteristic phenotypic change of eosinophils (Fig. 3).

Conclusion

Previous reports demonstrated that eosinophils are important as the major producers of cys-LT, whose production is enhanced in patients with allergic diseases, especially AERD. Basic research using *in vitro* experiments using human eosinophils has shown that various stimulators accompanied with cell–cell interactions induce hyper-production of cys-LTs; however, the regulatory mechanisms in local inflamed sites have not been sufficiently and comprehensively elucidated.

Our report showed that fatty acid metabolism is modulated to an LTD₄-producing phenotype in eosinophils derived from nasal polyps, with GGT-5 and DPEP-2 playing central roles in this inflammatory change.⁸⁶ Further integrated analysis identified type 2 cytokines or microbial components as inducers of fatty acid

metabolic abnormality, suggesting the importance of the tissue environment of nasal polyps to affect cellular inflammatory characteristics. Recent clinical trials reported the usefulness of antibody drugs for type 2 cytokines to improve disease outcomes of chronic eosinophilic rhinosinusitis.⁹¹ Collectively, these findings indicate that antibody drugs and/or control of microorganisms to correct fatty acid metabolic abnormality might possess a therapeutic potential.

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Conflict of interest

The authors have no conflict of interest to declare.

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