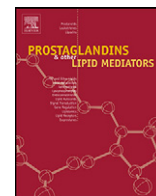




Prostaglandins and Other Lipid Mediators



Mini-Review

On the biosynthesis and biological role of eoxins and 15-lipoxygenase-1 in airway inflammation and Hodgkin lymphoma

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ABSTRACT

This mini-review is focused on the enzyme 15-lipoxygenase-1 (15-LO-1) and eoxins in airway inflammatory diseases and Hodgkin lymphoma. Several studies have demonstrated increased expression and activity of 15-LO-1 in the respiratory tissue from asthma patients, indicating a pathophysiological role of this enzyme in airway inflammation. Eoxins were recently identified as pro-inflammatory metabolites of arachidonic acid, formed through the 15-LO-1 pathway, in human eosinophils, mast cells, airway epithelial cells and Hodgkin lymphoma. Mice deficient of 12/15-LO, the ortholog to human 15-LO-1, had an attenuated allergic airway inflammation compared to wild type controls, also indicating a pathophysiological role of this enzyme in respiratory inflammation. The putative therapeutic implications of 15-LO-1 inhibitors in the treatment of asthma, chronic obstructive pulmonary disorder and Hodgkin lymphoma are discussed.

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1. Introduction

Human cells contain a family of structurally related lipoxygenases which catalyse the metabolism of arachidonic acid, released from phospholipids by inflammatory stimuli, to biologically active mediators. Human cells express three main types of lipoxygenases, which can catalyze the insertion of molecular oxygen into arachidonic acid at carbon 5, 12 and 15, and hence these enzymes are usually named 5-, 12- and 15-lipoxygenase. The 5-lipoxygenase (LO) is the key enzyme in leukotriene (LT) synthesis and catalyses the conversion of arachidonic acid to LTA₄ [1]. This metabolite can be hydrolysed enzymatically to LTB₄ or be conjugated with glu-

tathione to form LTC₄ which in turn can be cleaved sequentially to LTD₄ and LTE₄. The leukotrienes are proinflammatory mediators and LTC₄, and its metabolites, are also potent bronchoconstrictors [1]. Human platelets contain abundant amounts of 12-LO. Very little is known about the biological role of this enzyme in human [2]. There are two human forms of 15-LO, named 15-LO-1 and 15-LO-2 [3]. These enzymes have only 40% similarity in amino acid sequence and the biological function(s) of these enzymes is probably quite different [3,4].

15-LO-1 is highly transcriptionally regulated with narrow tissue distribution [5–7]. The enzyme is predominantly expressed in airway epithelial cells, eosinophils, alveolar macrophages, dendritic cells and reticulocytes (Table 1). The enzyme can catalyse the metabolism of arachidonic acid, and also certain other polyunsaturated fatty acids, to various metabolites. 15-LO-1 can also, in contrast to 5-LO and 12-LO, oxygenate fatty acids

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Table 1
Expression of 15-LO-1 and capacity to produce eoxins in human cells.

Cell type	Expression of 15-LO-1	Biosynthesis of eoxins	References
Normal cells			
Eosinophils	+++	+++	[27,28]
Airway epithelial cells	+++	+ (a)	[12,29,49]
Cornea epithelial cells	++	n.d.	[50]
Mast cells	+(+)	+	[11,28]
Alveolar macrophages	+(+)	n.d.	[51]
Dendritic cells	++	n.d.	[52]
Reticulocytes	++	n.d.	[53]
Synovial cells	+	n.d.	[54]
Neutrophils	+	n.d.	[55]
Endothelial cells	+	n.d.	[56] 56 (b)
Fibroblasts	+	n.d.	[57]
Seminal fluid	+	n.d.	[58]
Malignant cells			
Hodgkin Reed–Sternberg cells, L1236	+++	+++	[40]
Colon carcinoma cells	+	n.d.	[7]
Prostate cancer	+	n.d.	[26]

Estimated relative 15-LO-1 expression and capacity to produce eoxins, + low; ++ medium; +++ high; n.d.= not determined; (a), unpublished observation (Brunnström, Å., Tryselius, Y., Andersson, E., Backman, L. and Claesson, H.-E.); (b), only 15-LO-1 mRNA.

attached to membrane phospholipids [4,8]. Interleukin (IL)-4 and IL-13 induce the expression of 15-LO-1 in cultivated monocytes, airway epithelial cells and mast cells, and in monocytes this induction could be inhibited by interferon- γ and glucocorticoids [9–11].

The biological and medical interest for 15-LO-1 in human has mainly been focused on its potential role in airway inflammation and atherosclerosis. Several studies have demonstrated increased amounts of 15-LO-1 in the bronchial mucosa of patients with asthma compared with control subjects [12–14]. In one of these studies, it was demonstrated that increased expression of the 15-LO-1 gene was normalised after treatment with inhaled corticosteroids [14]. Patients with chronic obstructive pulmonary disease (COPD) also display an increased expression of 15-LO-1 in bronchial biopsies [15]. The major arachidonic acid metabolite produced via the 15-LO-1 pathway is 15(S)-hydroxy-eicosatetraenoic acid (15(S)-HETE) and this metabolite has been used as a biomarker of 15-LO-1 activity. Increased amounts of 15-HETE have been found in bronchoalveolar lavage (BAL) fluid and sputum from asthmatic subjects compared to control subjects [13,16]. Furthermore, osmotic activation of nasal tissue with mannitol activates 15-LO-1 leading to increased amounts of 15-HETE in nasal lavage, and the levels of 15-HETE were associated with nasal symptoms [17].

Although these studies suggest a role of 15-LO-1 in airway inflammation, our knowledge of the biological effects of various arachidonic acid metabolites, formed via the 15-LO-1 pathway, or the functional consequences of blocking 15-LO-1 activity *in vivo* was, until recently, very limited. Exogenously added 15-HETE or 13-hydroxy-octadecadienoic acid (13-HODE), a linoleic acid product formed via 15-LO-1, has been reported to stimulate certain signalling pathways in different cell types such as leukocytes, smooth muscle cells and endothelial cells [4,8]. These effects, however, are usually observed at micromolar concentrations which raise the question of whether these amounts can indeed be produced *in vivo*. However, one 15-LO-1 derived product, 5-oxo-15-hydroxy-ETA, has been reported to be a very potent chemotactic substance for human eosinophils and neutrophils [18]. There are conflicting results of the effects of inhaled 15-HETE in human airway bronchus: lack of effects as well as increased response has been reported [8].

Lipoxins constitute another group of metabolites, which possess anti-inflammatory actions [19]. The mandatory enzyme in lipoxin formation is 5-LO and the second enzymatic step could be catalysed by either 15-LO or 12-LO. The lipoxins are formed through interactions between different types of cells, via so-called transcellular metabolism.

Increased expression and/or activity of 15-LO-1 has also been demonstrated in Alzheimer's disease, indicating a possible pathophysiological role of this enzyme in this condition [20,21]. In atherosclerosis it is less clear. Several studies indicate a pro-atherogenic role of the 12/15-LO enzyme (the murine orthology to human 15-LO-1, see below) but more recent studies indicate that 15-LO-1 is not expressed in atherosclerotic lesions in man [22–24]. There are also reports indicating a role of 15-LO-1 in cell differentiation and the enzyme has been proposed to be of importance for maturation of erythrocytes and degradation of cell organelles [4,8,25]. Some studies suggest a role of 15-LO-1 in neoplasia such as prostate cancer and colon carcinoma cells [7,26].

2. Biosynthesis and biological effects of eoxins in cells related to airway inflammation

Studies on the metabolism of arachidonic acid in human eosinophils in the 1980s demonstrated that these cells convert arachidonic acid via the 15-LO pathway to 15-HETE and a series of 8,15-dihydroxy acids (DiHETEs) and 14,15-DiHETEs [27]. The biological function of these metabolites remained to be fully understood. Eosinophils have an important role in certain types of airway inflammation and since human eosinophils are a rich source of 15-LO-1, we reinvestigated the metabolism of arachidonic acid in these cells with today's modern equipment.

Human eosinophils were incubated with arachidonic acid and the products were analysed by RP-HPLC/UV-spectroscopy. In addition to the early identified 15-HETE and DiHETEs, a more polar product was also detected, possessing a conjugated triene spectrum with UV absorbance maximum at 282 nm [28]. To further analyse the structure of this metabolite, samples of human eosinophils were analysed by LC-MS/MS by using positive-ion electrospray ionization. The MS/MS-spectrum of this metabolite contained several fragment ions also found in the corresponding spectrum of LTC₄. In light of the high 15-LO activity in eosinophils and the similarities between the MS/MS-spectra of this metabolite and LTC₄, we hypothesised that the product could be 14 (R)-glutathionyl-15 (S)-hydroxy-5,8, 10,12 (Z,Z,E,E)-eicosatetraenoic acid, also named 14,15-LTC₄ (later named eoxin C₄, see below and Fig. 1). To confirm this hypothesis, synthetic 14,15-LTC₄ was prepared (Biomol Inc) and the MS/MS-spectrum of this synthetic 14,15-LTC₄ was identical to the spectrum of the unknown metabolite. In addition, this metabolite was also formed after incubation of eosinophils with 14,15-LTA₄ and eosinophils could metabolise 14,15-LTC₄ to 14,15-LTD₄. The formation of these metabolites from arachidonic acid has never before been described in human cells. Since the name leukotriene is traditionally linked to the products formed via the 5-LO pathway we thought it was misleading to call these new identified metabolites 14,15-LT, and instead we have suggested the name eoxins (EX) since eosinophils are a rich source of these mediators (Fig. 1). Eoxins have a different three dimensional structure and the cysteinyl-LT receptors 1 and 2 are not high affinity receptors for eoxins (unpublished observation). Furthermore, 5-LO inhibitors do not block the synthesis of eoxins. There seems to be different signal transduction pathways which activate leukotriene and eoxin formation in eosinophils since challenge of eosinophils with calcium ionophore led almost exclusively to the formation of leukotrienes whereas incubation of the cells with exogenous arachidonic acid favoured the formation of EXC₄ over LTC₄ [28]. The formation of EXC₄ was also

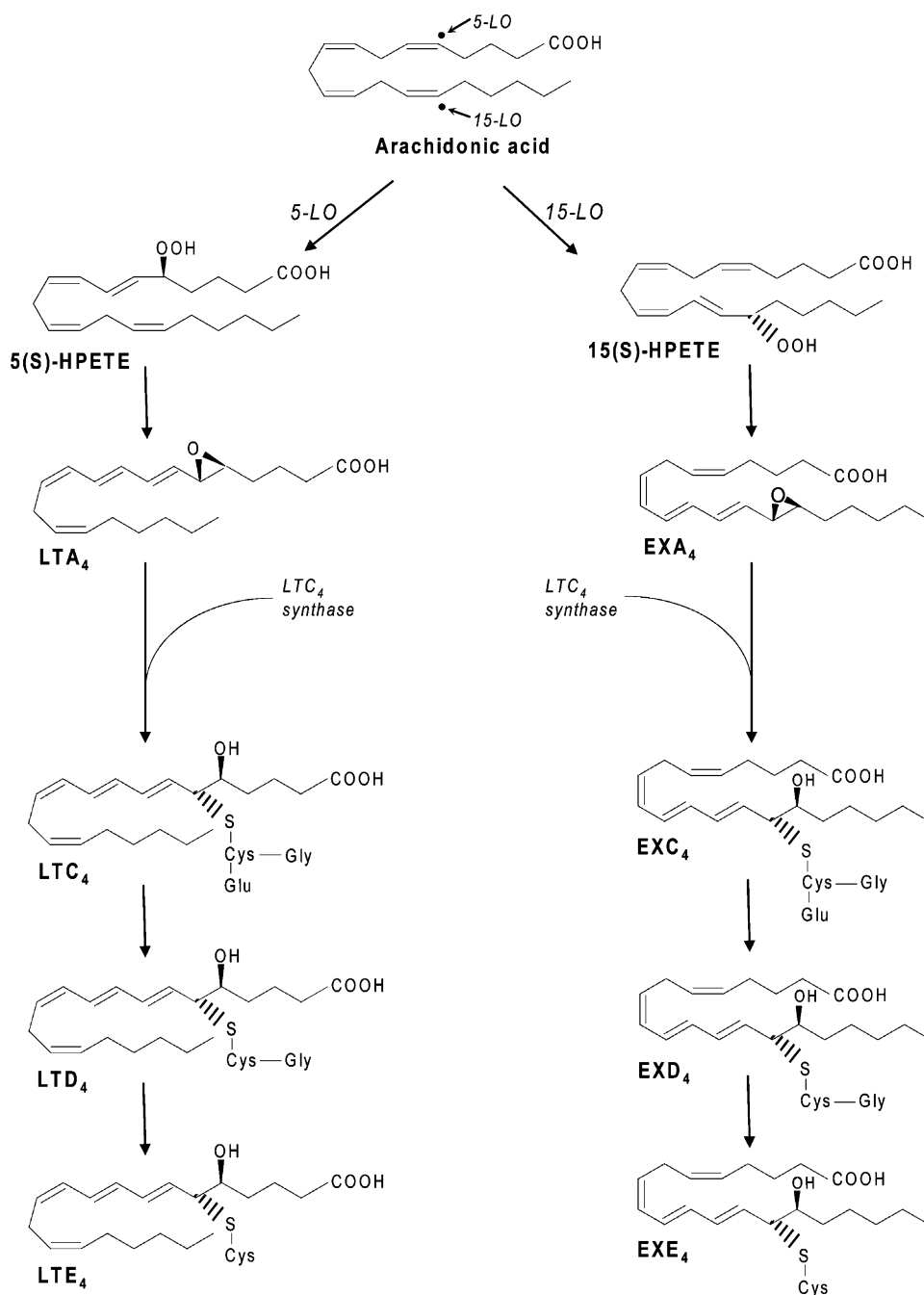


Fig. 1. Overview of the formation of leukotrienes and eoxins in human eosinophils.

observed after receptor-mediated activation of eosinophils with either LTC₄, PGD₂ or IL-5 [28]. The second step in the eoxin formation i.e. conjugation of EXA₄ with glutathione, leading to the formation of EXC₄, was shown to be catalysed by LTC₄ synthase in eosinophils [28]. In essence, human eosinophils have a high capacity to produce both cysteinyl-containing leukotrienes and eoxins (Fig. 1).

Mast cells have important functions in airway inflammation. We have recently reported that human cord blood-derived mast cells (CBMC) cultivated with IL-4 express an active 15-LO-1 [11] and that these cells produce eoxins [28]. Immunohistochemistry analysis of mast cells in skin biopsies from patients with atopic dermatitis also demonstrated expression of 15-LO-1 [11].

Human airway epithelial cells contain abundant amounts of 15-LO-1, as demonstrated by immunohistochemistry analysis [12], and

these cells have high 15-LO-1 activity *ex vivo* [29]. In agreement with this, human normal airway epithelial cells cultivated in air-liquid medium *in vitro* were found to possess high 15-LO-1 activity, and these cells also converted arachidonic acid to eoxins (unpublished observation, Table 1). In addition, nasal polyps from allergic subjects were found to spontaneously release EXC₄ *ex vivo* [28]. Several types of cells in these polyps, including eosinophils, may constitute the source of EXC₄.

Since eoxins are produced by cells which can release various bioactive mediators involved in pulmonary inflammatory processes, we investigated if also eoxins might be pro-inflammatory mediators. A marker of inflammation is edema formation and it is well-known that cysteinyl-LT and histamine can cause plasma leakage. To investigate the effects of eoxins on vascular permeability, an *in vitro* assay was used, based on assessment of changes in

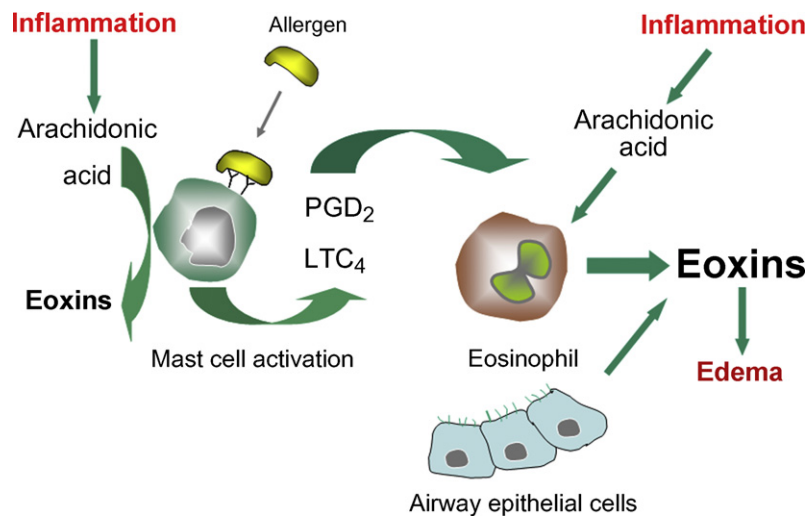


Fig. 2. A hypothetical model of biosynthesis and biological effect of eoxins.

transendothelial electric resistance across human endothelial cell (EC) monolayers [30]. The dose-response curve showed that maximum effect of histamine was obtained at 10^{-5} M in this model system. The eoxins were full agonists with about 100 times higher potency than histamine and almost as potent as LTC_4 in this model [28]. Eoxins C_4 , D_4 and E_4 induced increased EC monolayer permeability at a concentration of 10^{-7} and 10^{-8} M. The kinetics of changes in EC permeability in response to eoxins and LTC_4 indicated that these mediators were directly acting agonists. Thus, eoxins increased vascular permeability in this model of edema formation *in vitro*, demonstrating that eoxins are pro-inflammatory mediators (Fig. 2).

3. Similarities between human 15-LO-1 and the animal ortholog 12/15-LO

Human 15-LO-1 is mainly a 15-lipoxygenating enzyme when arachidonic acid is the substrate (ratio 15-HETE vs 12-HETE produced is 9:1). In contrast, all animal cells investigated so far express an enzyme which possesses mainly 12-lipoxygenating activity against arachidonic acid (except rabbit reticulocytes which also express a 15-lipoxygenating enzyme) [4,8]. This animal ortholog is usually called 12/15-LO, earlier also named leukocyte type 12-LO. Probably both 15-LO-1 and 12/15-LO exhibit their biological activity mainly through the formation of bioactive lipids. The 15-LO activity of the human enzyme and the more pronounced 12-LO activity of the animal equivalent could indicate different (patho-) physiological role since 12- and 15-lipoxygenation result in different metabolites, which may display diverse biological activities. It is important to point out, however, that *both* the human and animal enzyme possess *both* 15-LO and 12-LO activity although to different extents. In agreement with this, murine eosinophils have been shown to convert arachidonic acid to $14,15\text{-LTA}_4$ (= EXA_4) [31]. In addition, we have recently found that porcine eosinophils can produce EXC_4 which formation is also dependent on the 15-lipoxygenase activity of the porcine 12/15-LO enzyme (Brunnström Å, Backman L, Johanneson M, Forsell P, Claesson H-E, unpublished observation). This demonstrates that the 12/15-LO can catalyse the formation of EXC_4 . There are also many other similarities between the animal and human enzyme. For example, the expression pattern in the body of human 15-LO-1 and murine 12/15-LO is similar with the majority of the enzyme found in various cells and tissues in the respiratory tract [32]. The Th2 cytokines IL-4 and IL-13 induce expression of both 15-LO-1 and murine 12/15-LO [4]. Furthermore,

allergen challenge up-regulates the expression of 12/15-LO in airway epithelial cells in mice [33] and 15-LO-1 in man [12,13].

4. 12/15-LO deficient mice have reduced airway inflammation

To better understand the (patho-) physiological function of the 15-LO pathway we investigated the role of the murine 12/15-LO in an airway inflammation model. Wild type 12/15-LO mice (Wt) were compared with mice deficient in 12/15-LO (12/15-LO^{-/-}) in this study [34]. All mice were actively immunized against ovalbumin (OVA). At days 21–24 after this single immunization, the animals were exposed to daily allergen provocations. Mice deficient in 12/15-LO^{-/-} had less developed cellular response compared to Wt mice and the number of leukocytes in the BAL fluid was significantly lower in 12/15-LO^{-/-} mice. There was a marked increase of eosinophils in the lung tissue in both genotypes but the number of eosinophils was significantly lower in 12/15-LO^{-/-} mice compared to Wt mice. Also the levels of Th2 cytokines in the BAL fluid, such as IL-4, IL-13 and IL-5, were lower in 12/15-LO deficient mice compared to Wt mice. Studies on 12/15-LO in mouse macrophages have earlier demonstrated that increased activity of this enzyme leads to higher expression of IL-6, TNF- α and monocyte chemoattractant protein-1 [35,36]. Goblet cell hyperplasia and the formation and secretion of mucus into the airway luminal are important pathophysiological events in asthma and chronic obstructive pulmonary disease (COPD). There was no difference between the two groups in the proportion of the airways which developed goblet cell hyperplasia. Despite this, the luminal secretion of mucus was clearly attenuated in 12/15-LO^{-/-} mice [34]. Although it was a relatively short challenge period (4 days), there were also a markedly increased number of α -smooth muscle-actin positive alveolar cells in the peripheral airways in challenged mice. This marker of remodelling was, however, significantly lower in 12/15-LO^{-/-} mice compared to Wt mice, indicating a role of this enzyme in this pathophysiological process, which is a key event in human asthma and other respiratory diseases. Together, these data suggest that metabolites formed via the 12/15-LO pathway are important players in airway inflammation and possibly remodelling. The relative impact of eoxins versus other 12/15-LO derived metabolites in this mice model of airway inflammation remains to be clarified. In essence, the overall functional consequences of deleting 12/15-LO, and thereby blocking the activity of 12/15-LO, are reduced airway inflammation and remodelling.

5. Expression of 15-LO-1 and biosynthesis of eoxins in Hodgkin lymphoma

Hodgkin lymphoma (HL), although a malignant disorder, has many characteristics of an inflammatory/infectious disease with symptoms and signs such as fever, night sweats, itching and lymphadenopathy. The unique characteristic cells of Hodgkin lymphoma are called Hodgkin Reed–Sternberg cells (H–RS cells) and these cells are multinucleated giant cells. In contrast to most other malignant disorders, these neoplastic cells constitute only a small minority (approximately 1–5%) of the cell population in the HL-affected tissue [37,38], while the rest of the tumour consist of inflammatory cells. It is generally believed that cross-talk between H–RS cells and infiltrating inflammatory cells in the tissue plays an important pathophysiological role in this disease [39]. The inflammatory features of HL tissue triggered our interest in investigating the expression of lipoxygenases and the metabolism of arachidonic acid in H–RS cells. Incubation of the HL cell line L1236 with arachidonic acid demonstrated that these cells readily produced EXC₄ which was further converted to EXD₄ and EXE₄ [40]. Western blot and immunocytochemistry analysis of L1236 cells showed that the cells express abundant amounts of 15-LO-1 and that the enzyme was translocated to the membrane upon calcium challenge. The 15-LO-1 in L1236 cells also catalysed the formation of 5-oxo-15-hydroxy-E₂E₆, the chemotactic mediator for eosinophils which are infiltrating many HL tumours [39]. None of the other investigated HL cell lines expressed 15-LO-1. Only the L1236 cell line, among all established HL cell lines, has in fact been shown to be clonically related to the original tumour [41,42]. Immunohistochemistry analysis of primary Hodgkin lymphoma tumour tissue *ex vivo* demonstrated expression of 15-LO-1 in H–RS cells in 85% of all investigated biopsies [40] (Fig. 3). It was also found that L1236 cells and primary H–RS cells expressed the cysteinyl leukotriene receptor 1 (CysLT1) [43], which might contribute to the inflammatory characteristics of HL. In comparison, the expression of 15-LO-1 was investigated in a broad range of primary non-Hodgkin lymphomas and only one of 58 investigated cases expressed 15-LO-1 (a T cell-derived anaplastic large cell lymphoma) [44]. Thus, the expression of 15-LO-1 and putative formation of eoxins and other

15-LO-1 derived arachidonic acid metabolites by H–RS cells *in vivo* might contribute to the inflammatory features of HL. These findings may have diagnostic and therapeutic implications in HL.

The observation that Hodgkin H–RS cells contained abundant amounts of 15-LO-1 and can produce eoxins was an unexpected observation and raises the question of whether there might be any biological similarities between such different diseases as asthma and HL. Furthermore, a striking finding was also that H–RS cells, like eosinophils and mast cells, express cysLT1. Interleukin-13 is known to induce the expression of both 15-LO-1 and CysLT1 [45,46] and this interleukin could be the common link between these two very different diseases. In agreement with this, IL-13 has been proposed to be an important pathophysiological mediator of these two diseases [47,48].

6. Conclusion

Although the enzyme 15-LO-1 was discovered more than 30 years ago, our knowledge of the biological role of this enzyme in the lung remained poorly understood until recently. Several reports have demonstrated increased expression and activity of 15-LO-1 in the respiratory tissue from asthma and COPD patients and thus its involvement in respiratory inflammation has been suggested. The discovery of the potent proinflammatory eoxins, and the formation of these mediators through the 15-LO-1 pathway, supports a proinflammatory role of this enzyme. The biosynthesis of eoxins occurs primarily in human eosinophils, mast cells and airway epithelial cells indicating the involvement of eoxins in respiratory inflammatory diseases. In addition, the reduced OVA-induced airway inflammation seen in 12/15-LO deficient mice as compared to wild type mice also suggest a pathophysiological role of this enzyme in the lung. Taken together, these studies indicate that 15-LO-1 is an attractive pharmacological target for treatment of inflammatory respiratory diseases like asthma, rhinitis and COPD.

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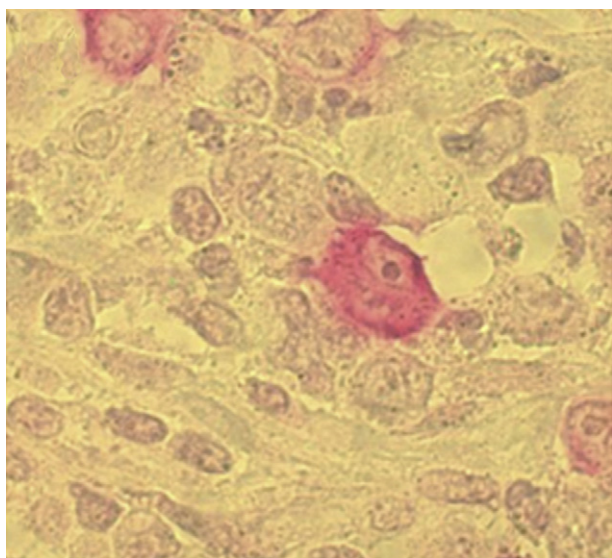


Fig. 3. Expression of 15-LO-1 in H–RS cells in a primary biopsy *ex vivo* from a patient with Hodgkin lymphoma. Red colour demonstrates positive staining with an antibody raised against 15-LO-1. The figure shows one positive binucleated H–RS cell in the middle of the figure (printed by kind permission by Dr. Frida Schain, Karolinska University Hospital, Solna, Sweden).

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