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Stina Feltenmark

Karolinska Institutet

Division of Physiological Chemistry II
Department of Medical Biochemistry & Biophysics
Karolinska Institutet, Stockholm, Sweden

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ABSTRACT

This thesis deals with the metabolism of arachidonic acid via the 5-lipoxygenase- and the 15-lipoxygenase-1 pathways in normal and malignant hematopoietic cells.

The first part of the thesis (*papers I & II*) describes the expression of genes involved in arachidonic acid metabolism and the capacity to generate leukotriene (LT) B_4 via the 5-lipoxygenase pathway in blood tumor cells from patients with precursor B-ALL (acute lymphoblastic leukemia) and AML (acute myeloid leukemia). In addition, peripheral blood $CD34^+$ pluripotent stem cells from three patients with non-myeloid malignancy were analyzed. Based on immunophenotyping, both precursor B-ALL clones and AML clones were shown to represent clones at various stages of maturation. In total, eight patients with precursor B-ALL, sixteen patients with AML and $CD34^+$ cells from three patients with non-myeloid malignancy were included. RT-PCR analyzis of the precursor B-ALL clones demonstrated that four of the investigated clones (the three most immature clones in addition to one clone with a more mature phenotype) expressed the gene coding for cytosolic phospholipase A_2 ($cPLA_2$), but not the gene coding for 5-lipoxygenase. In contrast, the remaining four clones expressed 5-lipoxygenase but not $cPLA_2$. The capacity of the precursor B-ALL clones to express the 5-lipoxygenase protein and to produce LTB_4 upon stimulation, correlated with the expression of the 5-lipoxygenase mRNA. The $CD34^+$ stem cells expressed the $cPLA_2$ protein, but not the 5-lipoxygenase protein. The enzyme $cPLA_2$ was also abundantly expressed in all sixteen AML clones and the activity of $cPLA_2$ was high in certain clones. On the contrary, 5-lipoxygenase, although expressed in all sixteen AML clones, seemed to possess low activity in general, since only the more mature clones had the ability to produce LTB_4 upon cell stimulation. Taken together, these results indicate that the capacity to produce leukotrienes via the 5-lipoxygenase pathway is under development during early hematopoiesis, and the capacity to generate leukotrienes is gained upon maturation.

The second part of the thesis (*papers III, IV & V*) deals with metabolism of arachidonic acid via the 15-lipoxygenase-1 pathway in human eosinophils, mast cells, the Hodgkin lymphoma and nasal polyps. Human eosinophils contain abundant amounts of 15-lipoxygenase-1. Also the Hodgkin lymphoma cell line L1236 was found to possess large amounts of this enzyme. Incubation of L1236 cells or eosinophils, isolated from human whole blood, with arachidonic acid led to formation of a product with an UV absorbance maximum at 282 nm and shorter retention time than LTC_4 in reverse-phase HPLC. Analyzis with positive-ion electrospray tandem mass spectrometry identified the metabolite as 14,15-LTC_4. This metabolite could be metabolized into 14,15-LTD_4 and 14,15-LTE_4. To avoid confusion with 5-lipoxygenase derived LTs and to emphasize the original finding of these metabolites in *eo*sinophils, we suggested that the 14,15-LTs should be denoted *eo*xins (EXs), and hence 14,15-LTC_4, 14,15-LTD_4 and 14,15-LTE_4 are now referred to as EXC_4, EXD_4 and EXE_4, respectively. Cord-blood derived mast cells and surgically removed nasal polyps from allergic subjects were also found to produce EXC_4. The EXC_4 synthase activity was characterized in eosinophils and in L1236 cells. The enzyme LTC_4 synthase catalyzed the conversion of EXA_4 to EXC_4 in eosinophils whereas the soluble glutathione transferase GST M1b-1b catalyzed the corresponding reaction in L1236 cells. We also provided data showing that the EXs act as proinflammatory agents with profound effects on vascular permeability leading to plasma leakage, a hallmark of inflammation, in an *in vitro* model using confluent human endothelial cells. In addition, following receptor-mediated activation by LTC_4, PGD_2 or IL-5, eosinophils were shown to produce EXC_4, and this finding supports a physiologically relevant role for EXs.

LIST OF PAPERS

I. STINA FELTENMARK, Gudmundur Runarsson, Pontus Larsson, Per-Johan Jakobsson, Magnus Björkholm, Hans-Erik Claesson
Diverse expression of cytosolic phospholipase A_2, 5-lipoxygenase and prostaglandin H synthase 2 in acute pre-B-lymphocytic leukemia cells
British Journal of Haematology, 1995, 90, 585-594

II. Gudmundur Runarsson, STINA FELTENMARK, Pontus K. A. Forsell, Jan Sjöberg, Magnus Björkholm, Hans-Erik Claesson
The expression of cytosolic phospholipase A_2 and biosynthesis of leukotriene B_4 in acute myeloid leukemia cells
Eurpoean Journal of Haematology, 2007, 79, 468-476

III. STINA FELTENMARK, Narinder Gautam, Åsa Brunnström, William Griffiths, Linda Backman, Charlotte Edenius, Lennart Lindbom, Magnus Björkholm, Hans-Erik Claesson
Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells
Proc Natl Acad Sci USA, 2008, 105, 680-685

IV. Hans-Erik Claesson, William Griffiths, Åsa Brunnström, Frida Schain, Erik Andersson, STINA FELTENMARK, Hélène A. Johnson, Anna Porwit, Jan Sjöberg, Magnus Björkholm
Hodgkin Reed-Sterberg cells express 15-lipoxygenase-1 and are putative producers of eoxins in vivo -Novel insights into the inflammatory features of classical Hodgkin lymphoma
FEBS journal, 2008, 275, 4222-4234

V. STINA FELTENMARK, Ralf Morgenstern, Tomas Bergman, Bengt Mannervik, Hans-Erik Claesson
Studies on glutathione transferases involved in the biosynthesis of eoxins
Manuscript

PUBLICATIONS NOT INCLUDED IN THE THESIS

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Jakobsson, P. J., Shaskin, P., Larsson, P., **Feltenmark, S.**, Odlander, B., Aguilar-Santelises, M., Jondal, M., Biberfeld, P. & Claesson, H. E.
Studies on the regulation and localization of 5-lipoxygenase in human B-lymphocytes
Eur J Biochem, 1995, 232, 37-46.

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The 5-lipoxygenase pathway in normal and malignant human B lymphocytes
Adv Prostaglandin Thromboxane Leukot Res, 1995, 23, 293-8.

CONTENTS

LIST OF ABBREVIATIONS

AA	Arachidonic acid
AACOCF3	Arachidonyl trifluoromethylketone
AHR	Airway hyperresponsiveness
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ATP	Adenosine triphosphate
BLT	Leukotriene B_4 receptor
cAMP	Cyclic adenosine monophosphate
cHL	Classical Hodgkin lymphoma
CLP	Coactosin-like protein
CLP	Common lymphoid progenitor
CMP	Common myeloid progenitor
COPD	Chronic obstructive pulmonary disease
COX	Cyclooxygenase
CysLT	Cysteinyl leukotriene receptor
cPLA$_2$	Cytosolic phospholipase A_2
DMSO	Dimethylsulfoxide
EIA	Enzyme immunoassay
ERK	Extracellulary regulated kinase
EX	Eoxin
FLAP	5-lipoxygenase activating protein
GM-CSF	Granulocyte macrophage colony-stimulating factor
GST	Glutathione-S-transferase
HETE	Hydroxyeicosatetraenoic acid
HL	Hodgkin lymphoma
HPETE	Hydroperoxyeicosatetraenoic acid
H-RS	Hodgkin Reed-Sternberg
IFN-γ	Interferon-γ
Ig	Immunoglobulin
IL	Interleukin
IP-10	Interferon-inducible protein 10
iPLA$_2$	Calcium independent phospholipase A_2
JAK	Janus kinase
LC	Liquid chromatography
LO	Lipoxygenase
LT	Leukotriene
MAPEG	Membrane-associated proteins in eicosanoid and glutathione metabolism
MAPKAPK	Mitogen-activated protein kinase-activated protein kinase
MIP	Macrophage inflammatory protein
MRP-1	Multidrug resistance-associated protein 1
MGST	Microsomal glutathione-S-transferase
mPGEs	Microsomal prostaglandin E synthase
MS	Mass spectrometry

NHL	Non Hodgkin lymphoma
NSAID	Non-steroidal anti-inflammatory drug
OAG	1-oleoyl-2acetyl-glycerol
OVA	Ovalbumin
PG	Prostaglandin
PKA	Protein kinase A
PKC	Protein kinase C
PLA_2	Phospholipase A_2
PMA	Phorbol 12-myristate 13-acetate
PMBCL	Primary mediastinal B-cell lymphoma
PMNL	Polymorphonuclear leukocytes
PPARα	Peroxisome proliferator receptor α
RANTES	Regulated upon activation, normal T-cell expressed, and secreted
ROS	Reactive oxygen species
RP-HPLC	Reverse phase-high performance liquid chromatography
RT-PCR	Reverse transcription-polymerase chain reaction
SRS-A	Slow reacting substance of anaphylaxis
STAT6	Signal and activator of transcription 6
Th	T helper cell
TX	Thromboxane
TGFβ	Tumor growth factor β

1 INTRODUCTION

1.1 PHOSPHOLIPIDS & EICOSANOIDS

The phospholipid bilayer constitutes the fundamental building structure of all cellular membranes and compartments. The most common phospholipids consist of a glycerol backbone with two fatty acid chains and one phosphorylated alcohol, resulting in the amphipathic character of phospholipids. Eicosanoids constitute a family of biologically active lipids derived from polyunsaturated fatty acids with 20 carbon atoms. Major members of the eicosanoid family are prostaglandins, leukotrienes, thromboxanes and lipoxins. The precursors of eicosanoid biosynthesis are dihomo-γ-linolenic acid (20:3, ω6), arachidonic acid (20:4, ω6) and eicosapentaenoic acid (20:5, ω3), obtained from the diet or by desaturation and elongation of linoleic (18:2, ω6) and α-linolenic acid (18:3, ω3). In mammalian cells, the most abundant precursor of eicosanoid biosynthesis is arachidonic acid (AA). Arachidonic acid is incorporated into the membranes of all cells in the body. The bulky size of AA may be one possible explanation to why this fatty acid is predominantly found in the *sn-2* position of membrane phospholipids. Eicosanoids are synthesized *de novo* from released AA. Various signals stimulate the membranes to release AA, and this process is a prerequisite for further transformation into biologically active eicosanoids, and hence the initial step in this synthesis. Release of AA is catalyzed by phospholipase A_2, an enzyme present in different isoforms in various cell types [1].

Once liberated, AA can follow three major metabolic routes in the cell. In the first major pathway, prostaglandin endoperoxide synthases (cyclooxygenase (COX) I and II) catalyzes the formation of prostaglandin (PG)G_2 and PGH_2, which can be further metabolized to yield biologically active prostaglandins and thromboxane A_2 [2-4]. The second pathway involves cytochrome P-450 enzymes, which convert AA into hydroperoxides and *cis*-epoxides [5]. The third pathway is governed by lipoxygenases, non-heme-iron containing enzymes, which stereospecifically introduce molecular oxygen into AA, leading to formation of hydroperoxy acids. In mammalian cells, three main types of lipoxygenases exist; 5-, 12- and 15-lipoxygenase, which are distinguished by their preferred position of oxygen insertion at C5, C12 and C15, respectively, in AA [6]. The 5-lipoxygenase pathway generates leukotrienes (LTs), and the 15-lipoxygenase pathway generates eoxins (EXs). The emphasis of this thesis will be held at the 5- and 15- lipoxygenase pathways. An

overview of biologically important lipid mediators derived from AA is shown in *figure 1*.

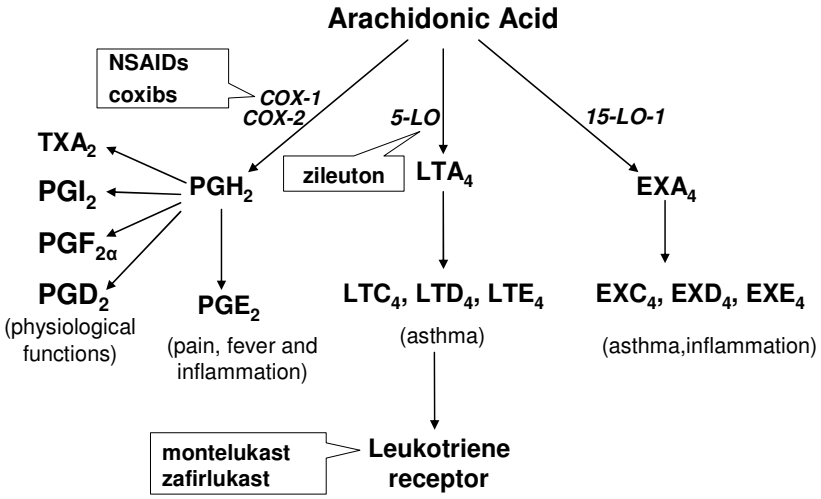

Figure 1. Arachidonic acid metabolism. The boxes contain information on available inhibitors.

1.2 OVERVIEW OF LEUKOTRIENE BIOSYNTHESIS

The key enzyme in LT biosynthesis is 5-lipoxygenase, which in a two step reaction catalyzes the formation of LTA_4 from free AA [7]. In the resting cell, 5-lipoxygenase is located in the cytosol or in soluble compartments inside the nucleus. Upon activation, 5-lipoxygenase translocates to the nuclear envelope where also 5-lipoxygenase activation protein (FLAP) and AA reside. FLAP is thought to facilitate the transfer of AA to 5-lipoxygenase. The catalysis of AA to LTA_4 by 5-lipoxygenase is generally considered to follow a free radical mechanism. This mechanism is initiated by a stereospecific hydrogen abstraction at C7 of AA and followed by a

radical migration to C5 and formation of a Δ^6- *trans* double bond. Molecular oxygen is introduced at C5 to form 5(S)-hydroperoxy-6-*trans*-8,11,14-*cis*-eicosatetraenoic acid (5(S)-HPETE). A subsequent abstraction of hydrogen at C10 of 5(S)-HPETE is followed by a radical migration to C6. Rearrangements of the double bonds yield a conjugated triene structure. Finally, the radical combines with the hydroperoxy group, resulting in dehydration to form the unstable epoxide LTA_4 [8, 9]. Thus, 5-lipoxygenase possesses two catalytical activities, i.e. dioxygenase activity and LTA_4 synthase activity. LTA_4 may undergo enzymatic transformation into 5(S),12(R)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (LTB_4), a reaction catalyzed by the widely distributed enzyme LTA_4 hydrolase. Alternatively, LTA_4 can be enzymatically conjugated to glutathione to form 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-*trans*-11,14-*cis*-eicosatetraenoic acid (LTC_4). This reaction is mediated by the membrane associated glutathione transferase LTC_4 synthase. Once formed, LTC_4 can be further metabolized to yield LTD_4 and LTE_4. The cysteinyl LTs (i.e. LTC_4, LTD_4 and LTE_4) were previously known as the slow reacting substance of anaphylaxis (SRS-A). The biosynthesis of LTs from AA is depicted in *figure 2*.

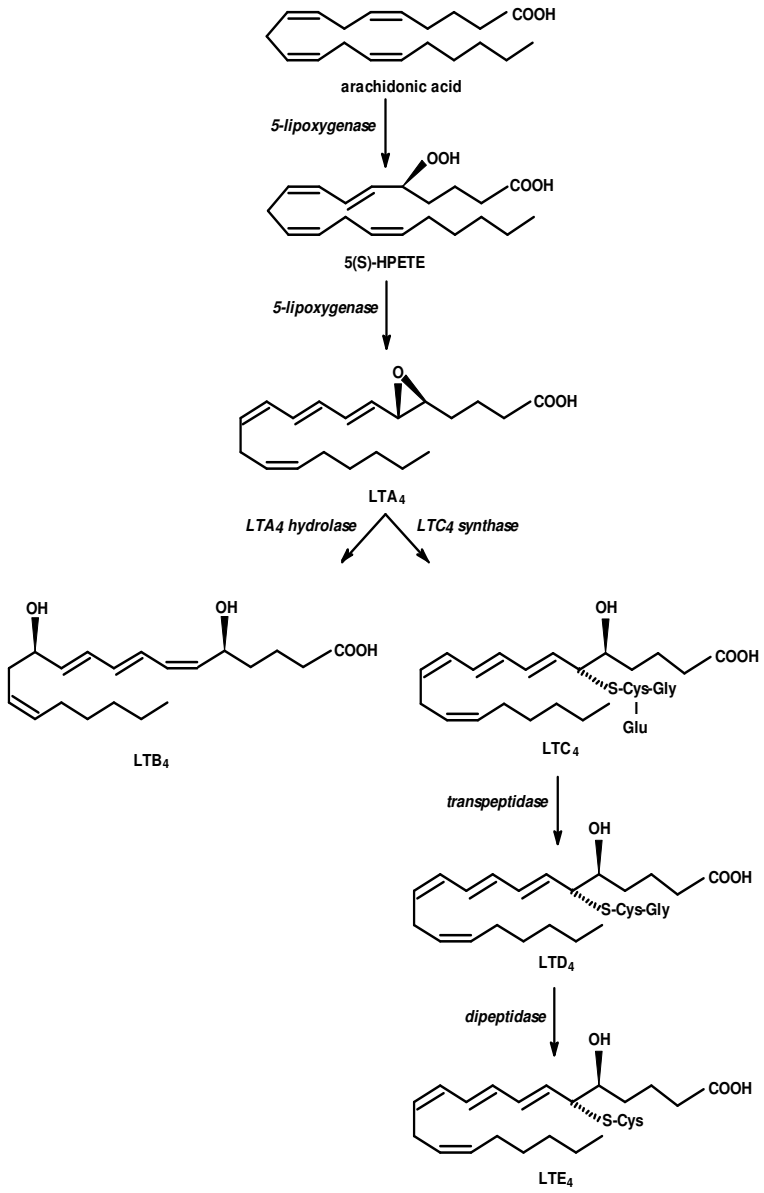

Figure 2. Biosynthesis of leukotrienes from arachidonic acid

1.3 ENZYMES/PROTEINS INVOLVED IN LEUKOTRIENE BIOSYNTHESIS

1.3.1 Phospholipases A_2

Phospholipases A_2 (PLA_2) is a family of enzymes involved in a variety of physiological and pathophysiological processes. They are defined by the catalytic hydrolysis of the *sn-2* ester bond of phospholipid substrates. The products of the PLA_2 catalysis are one free fatty acid and one lysophospholipid. Phospholipases A_2 can be divided into secretory low molecular mass enzymes (10-14 kD) which are cell associated or extracellular, and intracellular high molecular mass enzymes (85-110 kD) localized in the cytosol of cells. The intracellular PLA_2s can be subdivided into Ca^{2+} dependent PLA_2 ($cPLA_2$) and Ca^{2+} independent PLA_2 ($iPLA_2$). Among all the PLA_2 enzymes, $cPLA_2$ is unique in showing a marked substrate preference for glycerolphospholipids containing AA esterified at the *sn-2* position. Cytosolic PLA_2 is active in the reductive milieu and rapidly translocates to intracellular membranes upon elevation of the cytosolic Ca^{2+} concentration. The enzyme was originally cloned from the monocytic cell line U937 [10] and the crystal structure of $cPLA_2$ [11] revealed an N-terminal C2-domain, found in several membrane active enzymes such as PKC (protein kinase C). The C2-domain of $cPLA_2$ is the Ca^{2+} binding domain, and recombinant $cPLA_2$ lacking the C2 domain has been shown to be unable to translocate to membranes in response to Ca^{2+} as well as to release AA [12]. The C-terminal domain of $cPLA_2$ contains the catalytic site with three functionally important serine residues (ser505, ser727 and ser515) which are phosphorylated upon cell activation and involved in increased AA release [13]. Phosphorylation of $cPLA_2$ is catalyzed by mitogen activated protein kinases. Calcium dependent PLA_2 is implicated in a variety of inflammatory diseases, such as allergy, pulmonary fibrosis and arthritis. The central role that $cPLA_2$ plays in the production of eicosanoids is supported by extensive experimental data, and it has been shown that transgenic mice deficient in this enzyme showed an almost complete inability to synthesize prostaglandins or LTs in response to a variety of stimuli [14-16].

1.3.2 5-lipoxygenase

Human 5-lipoxygenase is expressed mainly in leukocytes, in line with the function of LTs as lipid mediators of immune reactions. Granulocytes, monocytes/macrophages, mast cells, dendritic cells and B-cells express 5-lipoxygenase, whereas platelets, erythrocytes, endothelial cells and T-cells do not. The enzyme has been purified from

leukocytes and cloned [17-19]. Five-lipoxygenase is soluble and approximately 78 kD. Although cloned more than 20 years ago, the three dimensional structure for 5-lipoxygenase has not yet been solved. Based on the crystal structure of rabbit reticulocyte 15-lipoxygenase, the 5-lipoxygenase structure has however been modeled, revealing a monomeric enzyme with two domains. The small N-terminal C2-like domain is critical to membrane binding of the enzyme upon activation. This domain resembles the C2 domain present in $cPLA_2$, with binding of two Ca^{2+} ions. The C2-like domain of 5-lipoxygenase is highly negatively charged, and binding of Ca^{2+} is thought to neutralize this charge and thereby making the enzyme more hydrophobic which facilitates membrane insertion [20]. After Ca^{2+} binding, 5-lipoxygenase (as well as $cPLA_2$) gains an increased affinity towards certain membrane lipids typically found within the nuclear membrane, especially glycerophosphocholine [21]. Other factors besides Ca^{2+} known to regulate 5-lipoxygenase activity through the C2-like domain are phosphatidylcholine vesicles, 1-oleoyl-2acetyl-glycerol (OAG) and coactosin-like protein (CLP). ATP binds to 5-lipoxygenase and increases enzyme activity, but hydrolysis of ATP is not required. Instead, ATP in this perspective seems to have an enzyme stabilizing effect [22].

The C-terminal of 5-lipoxygenase contains the catalytic site. As for all other lipoxygenases, the catalytic site for 5-lipoxygenase contains one non heme iron atom (Fe^{2+}) essential for enzyme activity [23]. Based on modeling studies, three histidine residues (His367, His372 and His550) as well as Asn554 and the Ile673 carboxy group at the C-terminus have been shown to chelate this iron [24]. During 5-lipoxygenase catalysis, the iron cycles between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states, and because of this feature the cellular redox tone is an important parameter for 5-lipoxygenase activity. Upregulation of 5-lipoxygenase product formation is achieved by conditions that promote lipid peroxidation, such as formation of reactive oxygen species (ROS), addition of peroxides or depletion of glutathione, whereas suppression of 5-lipoxygenase product formation is obtained by reducing lipid peroxidation processes.

Also phosphorylation of 5-lipoxygenase plays an important role in the regulation of the enzymatic activity. Three separate phosphorylation sites have been identified: Ser271 (a MAPKAPK site, activated by oxidative stress), Ser663 (an ERK-dependent site) and Ser523 (a cAMP/PKA-dependent site). Phosphorylation of the first two sites leads to activation of 5-lipoxygenase, in contrast to phosphorylation of Ser523, which leads to inactivation of the enzyme. The genomic structure of 5-lipoxygenase has

been determined [25]. The gene is located on chromosome 10 and comprises more than 82 kb DNA consisting of 14 exons. Nuclear import sequences are present both in the N-terminal domain and close to the C-terminus of 5-lipoxygenase [26, 27]. In addition, also nuclear export sequences have been identified [28]. Interestingly, both nuclear import and nuclear export of 5-lipoxygenase are modulated by phosphorylation. The promoter region for 5-lipoxygenase contains no TATA or CCAAT boxes, instead eight GC-boxes have been found. This is characteristic for housekeeping genes, which are constitutively expressed. Since 5-lipoxygenase is primarily expressed in myeloid cells, this finding is somewhat surprising. Studies of the promoter region of both 5-lipoxygenase positive and 5-lipoxygenase negative myeloid cell lines revealed that the 5-lipoxygenase promoter region is completely methylated in 5-lipoxygenase negative cells, whereas the promoter region of 5-lipoxygenase positive cells is unmethylated [29]. Possibly, DNA methylation is responsible for suppression of 5-lipoxygenase expression in non-myeloid cell types and tissues. Upregulation of the 5-lipoxygenase protein may be induced in response to cellular activation by agents such as dimethyl sulfoxide (DMSO), PMA, TGFβ, 1,25(OH)2D3 and GM-CSF [30, 31]. This upregulation is not related to transcriptor initiation by induction of the 5-lipoxygenase promoter activity, but rather to posttranscriptional events such as transcript elongation and maturation [32].

In addition to the above mentioned mechanisms for regulating the 5-lipoxygenase activity, this enzyme is also capable of self-inactivation. The 5-lipoxygenase products 5-HPETE and LTA_4 can inactivate the enzyme through a suicide mechanism [33, 34].

1.3.3 5-Lipoxygenase Activating Protein

In resting cells, 5-lipoxygenase is predominantly localized in the cytosol. In the presence of Ca^{2+}, 5-lipoxygenase translocates to the nuclear membrane, where it interacts with FLAP (5-lipoxygenase activating protein) [35-38]. The human gene coding for FLAP is localized on chromosome 13 and comprises 31 kb, consisting of five small exons and four large introns. The promoter region contains a TATA box, thereby displaying little similarity to the 5-lipoxygenase promoter. FLAP is an 18 kD membrane protein found to be present in the nuclear envelope [38]. The protein is a member of the MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) protein superfamily [39]. In contrast to the other five members of the MAPEG family (LTC_4 synthase, mPGEs, MGST1, MGST2 and MGST3), FLAP does not bind glutathione and has no known enzymatic activity. FLAP is necessary

for 5-lipoxygenase activity in intact cells, but appears not to be needed for the membrane association of 5-lipoxygenase. It has been shown that the FLAP inhibitor MK886 does not alter 5-lipoxygenase translocation to the membrane, even though LTA_4 production is inhibited, suggesting that 5-lipoxygenase-FLAP association is not a major mechanism for the translocation process [40]. The x-ray structures of human FLAP in complex with two LT synthesis inhibitors [41] show that these inhibitors bind in membrane embedded pockets of FLAP. This is in accordance to the suggestions that FLAP inhibitors (like MK886) inhibit LT biosynthesis by binding to the FLAP protein and thereby prevent AA from binding and subsequently being transferred to 5-lipoxygenase. This is in line with the finding that MK886 only inhibits LT biosynthesis in intact cells and not in cell homogenates or cell free systems [42]. Moreover, MK886 only inhibits the utilization of the endogenous pool of AA, and exogenously added arachdonic acid override the inhibitory effect of MK886 on LT biosynthesis.

1.3.4 LTA_4 hydrolase

LTA_4 hydrolase catalyzes the final step in the biosynthesis of the proinflammatory compound LTB_4 in a reaction without any cofactor requirement [43]. The enzyme was originally purified from human leukocytes and is a soluble monomer of approximately 69 kD. The gene is located on chromosome 12 and comprises 35 kb DNA consisting of 19 exons [44, 45]. The promoter region does not contain a TATA box; instead several transcription factor-binding sites are present. LTA_4 hydrolase is a widely distributed enzyme that has been detected in almost all cells, organs and tissues in man as well as in other species. In addition to the epoxide hydrolase activity, LTA_4 hydrolase also displays an aminopeptidase activity [46]. As other aminopeptidases, LTA_4 hydrolase is a zinc-binding protein [47]. The presence of this catalytic zinc is necessary for both the epoxide hydrolase activity and the peptidase activity, and the molar ratio is determined to one atom of zinc per enzyme molecule. Bestatin, a peptidase activity inhibitor, was found to also inhibit the conversion of LTA_4 to LTB_4, suggesting the involvement of the same active site for both enzymatic mechanisms, i.e. the peptidase and epoxide hydrolase activities. The three dimensional structure of LTA_4 hydrolase in complex with the competitive inhibitor bestatin has been solved by x-ray crystallography [48]. The monomeric enzyme is folded into three domains: N-terminal, catalytic and C-terminal. The domains are

packed in a flat triangular arrangement, creating a deep cleft in between. At the bottom of this interdomain cleft, the zinc site is located, and the zinc atom is bound, as predicted, to His295, His299 and Glu318. In close vicinity of the zinc, the catalytic residues Glu271, Glu296 and Tyr383 resides. The binding pocket for the substrate LTA_4 is constituted of an L-shaped hydrophobic cavity.

The epoxide hydrolase activity of LTA_4 hydrolase i.e. the transformation of LTA_4 into LTB_4 is highly substrate specific. The biological role of the aminopeptidase activity of LTA_4 is not known. However, since the two enzymatic activities of LTA_4 hydrolase in terms of catalytic parameters are very similar, with kcat/Km values of about 10^6 M^{-1}/s^{-1}, important physiological roles for both activities are suggested.

LTA_4 hydrolase is inactivated and covalently modified by its substrate LTA_4, i.e. suicide inactivated. The amino acid residue Tyr378 has been identified as the site of attachment between LTA_4 and the enzyme [49].

1.3.5 LTC_4 synthase

The second faith for the unstable intermediate LTA_4, apart from hydrolysis into LTB_4, is conjugation with glutathione to form the cysteinyl containing leukotriene LTC_4. This reaction is catalyzed by the enzyme LTC_4 synthase and constitutes the key step in cysteinyl LT formation. Once formed, LTC_4 can be transported to the cytoplasm from where it can be secreted via the glutathione adduct transporter MRP-1 (multidrug-resistance protein-1) [50] and further metabolized into LTD_4 and LTE_4. These three cysteinyl LTs were previously known as the slow reacting substance of anaphylaxis (SRS-A). LTC_4 synthase is mainly expressed in myeloid cells, such as eosinophils, basophils, mast cells and monocytes/macrophages. In addition, also platelets, although lacking 5-lipoxygenase, contain LTC_4 synthase and are capable of LTC_4 production through transcellular metabolism [51]. LTC_4 synthase is an 18 kD integral membrane protein found on the endoplasmatic reticulum and on the nuclear envelope. The enzyme is a member of the MAPEG family and the amino acid identity between LTC_4 synthase and FLAP is approximately 31% [52]. The three dimensional structure of LTC_4 synthase has been solved by x-ray crystallography and the protein was found to form homotrimers [53, 54]. Each LTC_4 synthase monomer has four transmembrane α-helices and forms a threefold symmetric trimer as a unit with functional domains across each interface. LTA_4 would fit into the interface so that Arg104 of one monomer activates glutathione to provide the thiolate anion that

attacks C6 of LTA_4 to form a thioether bond, and Arg31 in the neighboring monomer donates a proton to form a hydroxyl group at C5, resulting in LTC_4.

Transcriptional regulation of LTC_4 synthase occurs through binding of Sp1 and Sp3 to the Sp1 binding site located in the promoter region of the LTC_4 synthase gene [55]. In addition, upregulation of this enzyme after treatment with PMA, TGFβ or IL-4 has been reported [56-58].

In addition to LTC_4 synthase, also the two MAPEG family members MGST2 and MGST3 have the ability to catalyze the synthesis of LTC_4 from LTA_4, although LTC_4 synthase is considered the major source of LTC_4 production. Endothelial cells, lacking both 5-lipoxygenase and LTC_4 synthase, readily convert LTA_4 to LTC_4 by MGST2 through transcellular metabolism of LTA_4 [59].

1.4 LEUKOTRIENE RECEPTORS

Leukotrienes exert their action by binding to specific, heptahelical receptors located on the outer plasma membrane of inflammatory and structural cells. The LT receptors could be sub classified into the LTB_4 receptors (BLTs) [60] and the cysteinyl LT receptors (CysLTs) [61]. Once ligated by the LT, the receptors interact with G proteins in the cytoplasm, thereby eliciting increase in intracellular Ca^{2+} and reduction in intracellular cAMP. These proximal signals activate downstream kinase cascades which alter various cellular activities.

Two receptors for LTB_4 have been identified: BLT1 and BLT2. BLT1 is a high affinity receptor [62], mediating chemoattractant and proinflammatory actions, whereas BLT2 is a low affinity receptor which binds also other lipoxygenase products. The physiological functions of this receptor is not completely understood, but it has been shown that 12-HHT is a high affinity ligand for BLT2 [63].

In addition to BLT1 and BLT2, LTB_4 has been shown to bind to peroxisome proliferator-activated receptor alpha (PPARα) *in vitro* [64]. Binding leads to activation of PPARα, resulting in the transcription of genes that promote fatty acid degradation, and there are data supporting an important role for PPARα in the clearance of lipid mediators during inflammation [65].

Regarding the CysLTs, two high affinity receptors with different affinity prevalence have been identified: CysLT1 and CysLT2. CysLT1 [66] recognizes cysteinyl LTs in the descending affinity order LTD_4>LTC_4=LTE_4, and mediates sustained bronchoconstriction, mucus secretion and edema in the airways. CysLT1 is the target

of antileukoteriene agents such as montelukast, zafirlukast and pranlukast. CysLT2 [67] recognizes cysteinyl LTs in the descending affinity order LTC_4=LTD_4>LTE_4, and mediates inflammation, vascular permeability and tissue fibrosis. There are no known specific CysLT2 antagonists. Certain reported actions of cysteinyl LTs can not be readily explained by binding to CysLT1 or CysLT2, and this has raised the possibility of additional receptors. Recent findings suggest that P2Y12, an adenosine diphosphate receptor, is also acting as a selective LTE_4 receptor [68]. GPR17, a G protein coupled orphan receptor with homology to CysLT1 and P2Y, has been suggested to be a ligand-independent, constitutive negative regulator for the CysLT1, suppressing CysLT1 mediated function at the cell membrane [69].

1.5 BIOLOGICAL ROLES FOR LEUKOTRIENES

The cellular events activated by binding of LTs to their receptors are many and diverse, depending on the nature of the affected cells. A number of diseases have been associated with the action of LTs, for example asthma, rhinitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, psoriasis, inflammatory bowel disease, rheumatoid arthritis, leukemias, lymphomas and atherosclerosis. Leukotrienes are also involved in host defense processes. Emphasis here will be on the roles for LTs in airway inflammation.

In response to stimuli like stress or infection, LTB_4 is synthesized by leukocytes residing in the tissue. Being an inflammatory mediator, LTB_4 is one of the most potent chemotaxins for neutrophils, acting to promote recruitment of these cells to sites of inflammation [70]. At nanomolar concentrations, LTB_4 induces chemokinesis and neutrophil adhesion to vascular endothelial cells [71-73]. When present at higher concentrations, LTB_4 also mediates Ca^{2+} mobilization, superoxide anion generation, aggregation and release of lysosomal enzymes in neutrophils. Also inhibition of neutrophil apoptosis is caused by LTB_4. Concomitant activation of recruited leukocytes by LTB_4 also triggers additional synthesis of LTB_4 in these cells, so called paracrine activation. The result is a dramatic increase in tissue cellularity, a hallmark of inflammation. In severe asthma conditions, an overproduction of LTB_4 as a consequence of neutrophilic inflammation of the airways can be observed, suggesting that LTB_4 may contribute to asthma pathogenesis in certain situations [74]. BLT1-deficient mice have been shown to manifest reduced levels of serum IgE, reduced airway hyperresponsiveness (AHR) and reduced levels of Th2 cytokines in lung lavage

fluid in a model of allergic asthma [75]. In idiopathic pulmonary fibrosis, elevated levels of LTB_4 have been detected in bronchoalveolar lavage fluid and lung tissue. Recent results showed that a non selective BLT1 receptor antagonist was capable of preventing bleomycin-induced pulmonary fibrosis in mice by decrease of inflammation and altering TGF-β, IL-6, IL-13 and IFN-γ, factors known to be strongly associated with the pathogenesis of pulmonary fibrosis [76].

The cysteinyl LTs (LTC_4, LTD_4 and LTE_4) are potent bronchoconstricting agents with the ability to contract airway smooth muscle cells [77]. This airway constriction is a common feature in asthma. Other features caused by cysteinyl LTs are mucus secretion by bronchial mucosa, vasoconstriction as well as vascular leakage, resulting in edema formation [78]. There are studies suggesting that cysteinyl LTs are also implicated in airway remodeling. The CysLT1 antagonist montelukast has been shown to dramatically reverse established parameters of airway remodeling (airway collagen deposition and smooth muscle thickening) in a chronic allergen-induced asthma model [79]. In addition cysteinyl LTs have been suggested to have a role in tissue fibrosis of lung parenchyma [80].

In summary, the LTs act like pleiotropic lipid mediators with the capacity to influence almost every aspect of asthma pathogenesis.

1.6 15-LIPOXYGENASE

1.6.1 Properties and Regulation

The 15-lipoxygenase pathway constitutes a second branch in the metabolism of AA. Fifteen-lipoxygenase-1 is a lipid peroxidizing enzyme predominantly expressed in airway epithelial cells, eosinophils, reticulocytes, activated monocytes and in subsets of mast cells and dendritic cells (*Table 1*). A second form of human 15-lipoxygenase also exists, which is named 15-lipoxygenase-2 [81]. The amino acid sequence similarity between these enzymes is only 40% and the biological function of these enzymes is probably quite different [82]. In this thesis, focus will be held at 15-lipoxygenase-1.

Cell type	Expression of 15-LO-1	References
Normal cells		
Eosinophils	+++	[83]
Airway epithelial cells	+++	[84-86]
Cornea epithelial cells	++	[87]
Mast cells	+(+)	[88]
Alveolar macrophages	+(+)	[89]
Dendritic cells	++	[90]
Reticulocytes	++	[91]
Synovial cells	+	[92]
Neutrophils	+	[93]
Endothelial cells	+	[94] (a)
Fibroblasts	+	[95]
Seminal fluid	+	[96]
Malignant cells		
Hodgkin Read-Sternberg cells, L1236	+++	[97]
Colon carcinoma cells	+	[98]
Prostate cancer	+	[99]

Table 1. Expression of 15-lipoxygenase-1 in human cells.
Estimated relative 15-lipoxygenase-1 expression, + low; ++ medium; +++ high;
(a), only 15-lipoxygenase-1 mRNA.

The only three dimensional crystal structure solved for mammalian lipoxygenases is the rabbit reticulocyte 15-lipoxygenase-1 [100]. Apart from rabbit reticulocytes, the animal orthologue to 15-lipoxygenase-1 is called 12/15-lipoxygenase, formerly named leukocyte 12-lipoxygenase. The 12/15-lipoxygenase has similar enzymatic properties, expression, distribution and regulation as the human 15-lipoxygenase-1, but converts AA mainly to 12-HETE [82], whereas the human orthologue converts AA to 15-HETE and 12-HETE in a 9:1 ratio.

Human 15-lipoxygenase-1 is a 75 kD protein consisting of a single polypeptide chain with one non-heme iron atom per molecule. The structure reveals a two-domain structure with a large C-terminal and a small N-terminal. The C-terminal comprises 550 amino acids and contains the catalytic domain, with the non-heme iron buried deeply inside the enzyme molecule. The N-terminal C2-domain comprises 110 amino acids and has been implicated in membrane binding [101]. Upon Ca^{2+} stimulation, 15-lipoxygenase-1 translocates from the cytosol to the plasma membrane [102] leading to the activation of the enzyme's oxygenase activity in eosinophils. The affinity to Ca^{2+}

has been estimated to be relatively low, with a Kd of 0.2-0.5 mM [103]. It has been proposed that the Ca^{2+} ions form salt bridges between the negatively charged amino acids at the enzyme-membrane interphase [102].

In contrast to 5-lipoxygenase, which strongly prefers free C20-fatty acids but hardly accepts C18-derivatives or esterified fatty acids, 15-lipoxygenase-1 oxygenates a variety of polyenoic fatty acids regardless of chain length. In addition, 15-lipoxygenase-1 readily accepts also ester lipids, even if they are incorporated in biomembranes or lipoproteins [104]. However, the reaction rates with various substrates are quite different. Setting the rate of free linoleic acid oxygenation to 100%, linoleic acid-containing phospholipids are oxygenated with a rate of about 20%, and biomembranes with about 5%. The oxygenation rate of low-density lipoprotein is even lower (1-2%).

The expression of 15-lipoxygenase-1 is strictly regulated at a transcriptional, translational, post-translational and epigenetical level [82]. Interleukin (IL)-4 and IL-13 have been shown to induce 15-lipoxygenase-1 transcription via the signal and activator of transcription (STAT) 6 / Janus kinase (JAK) signalling pathway [105]. Furthermore, Ca^{2+}-dependent membrane association has been implicated as an important post-translational control mechanism [102]. Another post-translational control mechanism is the suicide inactivation of 15-lipoxygenase-1, where the product 15-HPETE has been shown to covalently modify the enzyme in the active site [106].

1.6.2 Biological Significance

Arachidonate 15-lipoxygenase-1 has been implicated in various physiologic and pathophysiologic processes including the breakdown of mitochondria during erythropoiesis, tumorigenesis, apoptosis, and inflammation-related diseases including asthma, COPD, diabetic vascular disease and atherosclerosis [82, 107, 108]. The inflammatory response in asthma is characterized by a type II immune reaction with high tissue levels of T helper cell type 2 (Th2) cytokines, including IL-4 and IL-13, which are known inducers of 15-lipoxygenase-1 expression [109-111], and many investigators have reported increased amounts and activity of 15-lipoxygenase-1 in the airway tissues of patients with asthma compared to control subjects [107, 112, 113]. In line with these reports it has been shown that mice lacking 12/15-lipoxygenase, the animal ortholog to human 15-lipoxygenase-1, have attenuated airway inflammation and airway remodelling after systemic sensitization with OVA [114]. With AA as substrate, the most abundant metabolite generated by 15-lipoxygenase-1 is 15-hydroxy

eicosatetraenoic acid (15-HETE) and this metabolite has been used as a biomarker of 15-lipoxygenase-1 activity. Increased concentrations of 15-HETE has been demonstrated in bronchoalveolar lavage fluid after allergen challenge in severe atopic asthmatics, accompanied by increased 15-lipoxygenase-1 expression in bronchial epithelium [115]. It has recently been shown in cultured human lung epithelia cells overexpressing 15-lipoxygenase-1 that this enzyme induces expression and release of chemokines such as MIP-1α, RANTES and IP-10. These cytokines are implicated in the recruitment of inflammatory cells like mast cells, activated T-cells and immature dendritic cells [116]. Th1 immune response plays an important role in the development of non-eosinophilic inflammation induced by airway exposure of an allergen plus double stranded (ds) RNA (virus associated/non-atopic asthma). A recent report suggests that 15-lipoxygenase metabolites play important roles in the development of non-eosinophilic allergic inflammation induced by the Th1 immune response [117]. It was shown that dsRNA-induced Th1-allergic inflammation was significantly impaired in 12/15-lipoxygenase-knockout mice. Moreover, the 15-lipoxygenase inhibitor (PD 146176) blocked the production of 15-HETE and the development of dsRNA-induced Th1 allergic response in wild type mice.

Recently, we reported on the discovery of eoxins (EXs), biologically active lipid mediators synthesized from AA via the 15-lipoxygenase-1 pathway in eosinophils, mast cells and in the Hodgkin lymphoma cell line L1236 (See *papers III, IV* and *V* in this thesis).

Apart from the pro inflammatory aspects of 15-lipoxygenase-1, this enzyme is also involved in processes to reduce inflammation and promote resolution. Lipoxins are anti inflammatory lipid mediators derived from AA and formed through transcellular metabolism. The first enzymatic step in the biosynthesis of lipoxins is catalyzed by 5-lipoxygenase and the second step is catalyzed by either 12-lipoxygenase or 15-lipoxygenase-1 [118].

1.7 BLOOD CELLS

1.7.1 Hematopoietic Stem Cells

Hematopoiesis (from Ancient Greek: *haima* blood; *poiesis* to make) is the formation of blood cells. An overview of hematopoiesis is depicted in *figure 3*. All blood cells originate from hematopoietic stem cells which reside in the medulla (bone marrow) and these cells have the unique ability to give rise to all of the different mature blood cell

types. Hematopoietic stem cells are self renewing: when they proliferate, at least some remain as stem cells, so the pool does not become depleted. Proliferation of stem cells gives rise to common lymphoid progenitor (CLP) cells and common myeloid progenitor (CMP) cells.

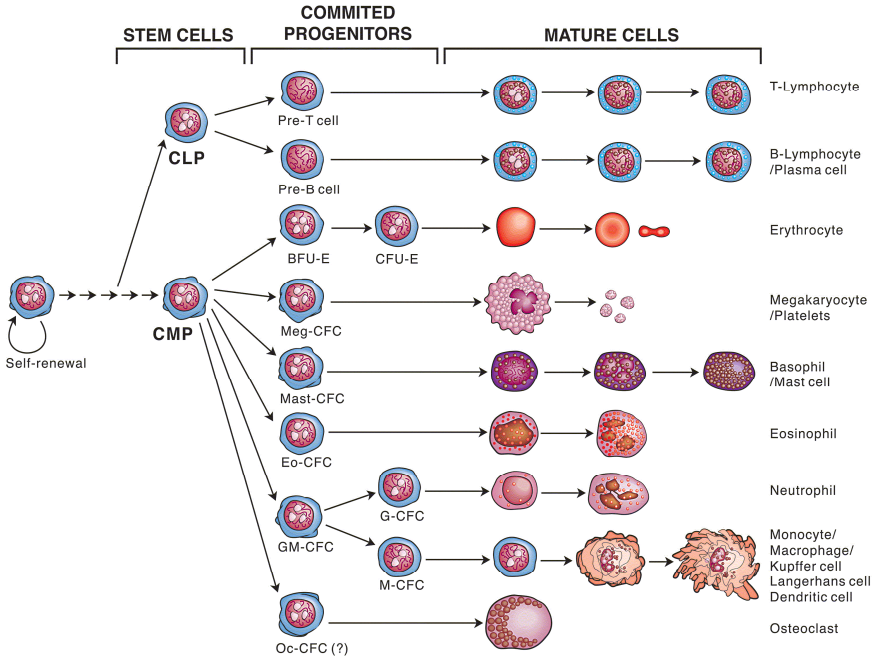

Figure 3. An overview of hematopoiesis. CLP: common lymphoid progenitor, CMP: common myeloid progenitor.

1.7.2 Lymphoid Cells

The lymphoid cells are derived from the common lymphoid progenitor and are later divided into B-cells and T-cells. B-cells play important roles in the humoral immune response, with the principal function to synthesize antibodies against antigens. B-cells are derived from the B-cell precursor linage in the bone marrow. There they rearrange their immunoglobulin heavy chain and enter the blood circulation with immunoglobulins expressed on the cell surface. Upon activation in the lymph nodes they mature further. In this milieu B-cells proliferate, rearrange their light chains, undergo heavy chain class switching and develop into memory cells or

immunoglobulin-producing plasma cells. T-cells have a central role in cell-mediated immunity.

1.7.3 Myeloid Cells

The myeloid cells are derived from the common myeloid progenitor and are divided into monocytes/macrophages, eosinophils, neutrophils, basophils and mast cells. Also erythrocytes and platelets stem from this common myeloid progenitor. The LT synthesis in myeloid cells has been extensively studied, and the 15-lipoxygenase-1 pathway in eosinophils was investigated in *paper III* and *paper V* of this thesis.

1.7.4 Acute Leukemia

Acute leukemia is characterized by a neoplastic expansion of immature hematopoietic cells in the bone marrow. These immature cells accumulate in the bone marrow, leading to suppression of normal hematopoiesis. This leads to anaemia, neutropenia and thrombocytopenia, causing fatigue, infections and bleeding disorders. The leukemic cells enter the circulation to a varying degree, and leukemic infiltration in organs can occur, with concurrent symptoms. Hyperleukocytosis (extremely high white blood cell count) can occur, resulting in disturbed microcirculation with multiorgan failure and bleeding disorders.

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a neoplasm of immature lymphoid precursor cells (lymphoblasts) committed to the B-cell lineage, whereas *Acute Myeloid Leukemia (AML)* is a neoplasm of immature myeloid precursor cells (myeloblasts). The precursor B-ALL and AML types of acute leukemia were characterized with regards to LT biosynthesis capabilities in *paper I* and *paper II*, respectively, of this thesis.

1.7.5 Lymphomas

Lymphomas are cancers originating from lymphoid cells and often forming tumors in the lymph nodes. Lymphomas are traditionally classified as either Hodgkin lymphoma (HL) or non-Hodgkin lymphomas (NHL). Hodgkin lymphoma is well characterized, whereas non-Hodgkin lymphomas constitute a heterogeneous group of several different subtypes. Studies of the 15-lipoxygenase pathway in biopsies of tumor affected lymph nodes from patients with classical Hodgkin lymphoma (cHL) and the cHL cell line L1236 [119] were performed in *paper IV* and *paper V* of this thesis.

2 AIMS OF THE STUDIES

2.1 PAPERS I & II

- To characterize the gene expression of enzymes involved in the metabolism of AA via the 5-lipoxygenase pathway in precursor B-ALL and AML in relation to the stage of cell differentiation
- To elucidate the capacity of precursor B-ALL and AML to produce LTB_4 in relation to the stage of cell differentiation

2.2 PAPERS III, IV & V

- To delineate the metabolism of AA via the 15-lipoxygenase-1 pathway in eosinophils, mast cells, nasal polyps, platelets as well as in the classical Hodgkin lymphoma cell line L1236
- To describe biological effects of EXs
- To characterize enzymes with EXC_4 synthase activity in eosinophils and L1236 cells

3 METHODOLOGY

For details on experimental conditions and assays, please see the Material and Methods sections in *papers I-V*.

4 RESULTS

4.1 PAPER I

Diverse expression of cytosolic phospholipase A_2, 5-lipoxygenase and prostaglandin H synthase 2 in acute pre-B-lymphocytic leukaemia cells

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is a neoplasm of immature lymphoid precursor cells (lymphoblasts) committed to the B-cell lineage. In acute precursor B-ALL, the differentiation of B-lineage cells is blocked at an early stage. The disease is characterized by a clonal expansion of immature B-cells and suppression of normal hematopoietic stem cells. Since several reports indicate that phospholipases and AA metabolites have a role in proliferation and differentiation of certain cell types, we thought it would be of interest to study the gene expression of enzymes involved in the biosynthesis of LTB_4 in different precursor B-ALL clones in relation to the stage of B-cell differentiation. When this study was designed, the capability of B-cells to produce LTB_4 had recently been described [120].

Cells from eight patients diagnosed with precursor B-ALL were collected and characterized. By use of flow cytometric analyzis by FACScan, the eight different clones could be arranged into two different groups; $CD10^-$ clones representing a very immature phenotype and $CD10^+$ clones representing a more mature phenotype.

The capacity of cells isolated from six patients to produce LTB_4 and 5-HETE was investigated. Intact cells (10^7 cells / sample) were stimulated with AA, Ca^{2+} ionophore A23187 and the thiol-reactive substance diamide (axodicarboxylic acid bis (dimethylamide)) for 10 minutes at 37°C. After termination of reactions, the products were subsequently analyzed by reverse-phase high performance liquid chromatography (RP-HPLC). In addition, the capacity of cell sonicates from these patients to synthesize LTB_4 and 5-HETE was studied. The $CD10^-$ cells, representing a more immature phenotype (patients A and B), were unable to synthesize LTB_4 and 5-HETE under these conditions, whereas the $CD10^+$ cells, representing a more mature phenotype (patients E-H), produced both LTB_4 and 5-HETE in amounts similar to those produced by normal B-cells, polymorphonuclear leukocytes (PMNL) and monocytes. The most immature of the $CD10^+$ clones (patient E) produced relatively small amounts of both LTB_4 and 5-HETE, whereas the most mature $CD10^+$ clone (patient H) produced the highest amounts of these AA metabolites.

By use of total RNA isolation, conversion to cDNA by moloney murine leukemia virus reverse transcriptase (M-MLV RT) and PCR analyzis, enzymes involved in the biosynthesis of LTB_4 and prostaglandins were investigated. In addition to the patients mentioned above, two additional patients were included in this study. The expression of mRNA coding for $cPLA_2$ was detected in all three $CD10^-$ patients as well as in one $CD10^+$ patient, whereas the remaining patients (all $CD10^+$) did not express $cPLA_2$ mRNA. Concerning 5-lipoxygenase, the signal pattern was the opposite: patients expressing $cPLA_2$ did not express mRNA coding for 5-lipoxygenase, whereas the patients without $cPLA_2$ expression did express 5-lipoxygenase. These results were totally in agreement with the capacity of the various precursor B-ALL clones to produce LTB_4 and 5-HETE.

In contrast to the inverse mRNA expression of $cPLA_2$ and 5-lipoxygenase in the precursor B-ALL clones, cell clones from all eight patients expressed the genes coding for FLAP and LTA_4 hydrolase.

In light of the reported effects of prostaglandins/thromboxanes and the expression of COX-1 and COX-2 (PGH synthase 1 and PGH synthase 2, respectively) during cell growth and differentiation, we also examined the expression of these genes in the precursor B-ALL clones. To our surprise, all clones expressed mRNA for COX-1, and seven of the eight investigated clones also expressed COX-2.

To confirm that 5-lipoxygenase mRNA expression correlated to expression of the 5-lipoxygenase protein, Western blot analyzis of one 5-lipoxygenase mRNA negative clone (patient B, $CD10^-$) and one 5-lipoxygenase mRNA positive clone (patient H, $CD10^+$) was performed. A polyclonal anti-human 5-lipoxygenase antiserum was used for detection, and the 15,000 x g supernatant of sonicated cells from patient B did not reveal any immunoreactive band corresponding to 5-lipoxygenase, whereas in the sample from patient H, a band corresponding to the recombinant 5-lipoxygenase standard was detected. The results correlated with the capacity of these cell clones to express the 5-lipoxygenase mRNA and to produce LTB_4.

4.2 PAPER II

The expression of cytosolic phospholipase A_2 and biosynthesis of leukotriene B_4 in acute myeloid leukemia cells

Acute myeloid leukemia (AML) is a neoplasm of immature myeloid precursor cells. AML is a heterogenous disease, with great variability in the degree of differentiation and lineage in the expanding clone. In the FAB classification of AML, clones are classified according to stage of maturation and lineage based on morphology, cytochemistry and immunophenotyping. In light of the results presented in *paper I* and in addition to the earlier findings indicating a stimulatory role for LTs in normal and malignant myelopoiesis, we thought it would be of interest to study the gene expression of enzymes involved in the biosynthesis of LTB_4, and also the ability to synthesize LTB_4 in relation to maturation stages.

In this study, 16 AML clones representing different maturation stages (M0-M5) were selected. Also peripheral blood $CD34^+$ pluripotent stem cells from three patients with non-myeloid malignancy were included.

The $CD34^+$ progenitor cells were analyzed for $cPLA_2$, 5-lipoxygenase, FLAP and LTA_4 hydrolase mRNA expression by RT-PCR. All three cell samples were positive for $cPLA_2$, FLAP and LTA_4 hydrolase. In contrast, none of the $CD34^+$ clones expressed mRNA coding for 5-lipoxygenase. Cells from one of the three patients were also subjected to Western blot analyzis of $cPLA_2$ and 5-lipoxygenase, and the results were confirmed; $CD34^+$ cells from this patient expressed the $cPLA_2$ protein but not the 5-lipoxygenase protein.

Cells from the 16 AML clones were analyzed for $cPLA_2$, 5-lipoxygenase, FLAP and LTA_4 hydrolase protein expression by Western blot. PMNL cells were used as control in these experiments. The results showed that the $cPLA_2$ protein was expressed in all AML clones tested, and that this expression was generally stronger than in PMNL cells from normal subjects. In contrast, the expression of 5-lipoxygenase, FLAP and LTA_4 hydrolase was generally lower than in PMNL. The quantities of 5-lipoxygenase protein were to some extent related to the maturation stage of the clones, whereas the quantities of FLAP and LTA_4 hydrolase could not be related to the degree of cell differentiation.

The capacity of the different AML clones to produce LTB_4 and 5-HETE in relation to PMNL was investigated, and this capacity was generally much lower than in PMNL, except for the most mature AML clones where the levels of LTB_4 and 5-HETE production were comparable to PMNL. Maturational stage of the AML clones seems to

be related to the capacity to synthesize LTB_4 and 5-HETE, with the greatest relative capacity found among the most mature clones.

As release of AA is required for LT synthesis, we investigated the effect of Ca^{2+} ionophore A23187 on ^{14}C labelled AA release from 11 of the AML clones. All investigated clones released substantially less AA than PMNL, regardless of the strong expression of $cPLA_2$.

Since the AML clones released less AA and produced lower amounts of LTB_4 and 5-HETE than PMNL, despite the fact that these clones expressed higher amounts of the $cPLA_2$ protein in comparison to PMNL, we performed a PLA_2 activity assay to elucidate the magnitude of PLA_2 activity in the AML clones. The results showed that in five of the six investigated AML clones, PLA_2 activity was higher than in PMNL. The PLA_2 inhibitor AACOCF3 partly inhibited the enzyme activity.

4.3 PAPER III

Eoxins are proinflammatory arachidonic acid metabolites produced via the 15-lipoxygenase-1 pathway in human eosinophils and mast cells

Human eosinophils contain abundant amounts of 15-lipoxygenase-1. In a two step reaction, 15-lipoxygenase-1 converts AA into 15(S)-HETE via the formation of 15(S)-HPETE. Alternatively, 15(S)-HPETE has been shown to undergo dehydration to form 14,15-epoxy-eicosatetraenoic acid (14,15-LTA_4). Another AA metabolite, formed via the 15-lipoxygenase-1 pathway is 5-oxo-15-hydroxy-6,8,11,13-eicosatetraenoic acid. This metabolite is produced by eosinophils and has been reported to constitute a potent chemotactic agent for human eosinophils. In addition, 15-lipoxygenase-1 is also implicated in anti-inflammatory processes by catalyzing the formation of the anti-inflammatory lipid mediators called lipoxins. The biological role of 15-lipoxygenase-1 is far from clear. In this study, we described the formation and biological effects of cysteinyl 14,15-LTs (eoxins (EXs)), formed via the 15-lipoxygenase-1 pathway in human eosinophils, mast cells and in nasal polyps.

Incubation of eosinophils, isolated from human whole blood, with AA led to formation of a product with an UV absorbance maximum at 282 nm and shorter retention time than LTC_4 in RP-HPLC. Analyzis with positive-ion electrospray tandem mass spectrometry (MS) identified this eosinophilic metabolite as 14,15-LTC_4. This metabolite could be metabolized into 14,15-LTD_4 and 14,15-LTE_4 in eosinophils. To avoid confusion with 5-lipoxygenase derived LTs and to emphasize the original finding

of these metabolites in *eo*sinophils, we suggested that the 14,15-LTs should be denoted *eo*xins (EXs), and hence 14,15-LTC_4, 14,15-LTD_4 and 14,15-LTE_4 are now referred to as EXC_4, EXD_4 and EXE_4, respectively. Cord-blood derived mast cells and surgically removed nasal polyps from allergic subjects were also found to produce EXC_4. Incubation of eosinophils with AA favoured the biosynthesis of EXs, whereas incubation with ionophore A23187 led to exclusive formation of LTC_4.

Also production of EXs from the endogenous pool of AA in eosinophils was demonstrated. Challenge of eosinophils with the proinflammatory agents LTC_4, prostaglandin D_2 and IL-5 led to biosynthesis of EXC_4, as detected with an EXC_4-EIA (developed by Cayman Chemical).

In addition, in an *in vitro* model system for vascular permeability, a hallmark of inflammation, the EXs induced increased permeability of endothelial cell monolayer. The EXs were 100 times more potent than histamine and almost as potent as LTC_4 and LTD_4 in this model, indicating that the EXs can act as potent proinflammatory lipid mediators.

4.4 PAPER IV

Hodgkin Reed-Sternberg cells express 15-lipoxygenase-1 and are putative producers of eoxins in vivo *–Novel insight into the inflammatory features of classical Hodgkin lymphoma*

Hodgkin lymphoma has many features of an infection/inflammatory condition, although classified as a true neoplastic disease. The large majority of classical Hodgkin lymphomas (cHL) are B-cell lymphomas, characterized by the presence of Hodgkin Reed-Sternberg cells (H-RS). These giant cells constitute only a small portion (normally <1%) of the cell population in the affected tissue, and the rest of the inflammatory cell infiltrate is rather heterogeneous, consisting of lymphocytes, macrophages, eosinophils, mast cells, plasma cells, stromal cells and fibroblasts.

In light of the characteristic inflammatory features of cHL, this study was designed to investigate the expression of lipoxygenases in H-RS cells and to explore the capacity of these cells to metabolize AA.

Incubation of the cHL cell line L1236 with AA for 5 minutes led to formation of a major product that cochromatographed with synthetic 15-HETE on RP-HPLC. A minor 12-HETE peak was also identified. The ratio between 15-HETE and 12-HETE formation was approximately 9:1, which is in line with 15-lipoxygenase-1 catalyzed

formation of these products. When an acetonitrile based RP-HPLC mobile phase was used to improve separation of more polar lipids, two major peaks with a conjugated triene spectrum and UV absorbance maximum at 282 nm were detected. The material in these peaks coeluted with synthetic EXC_4 and EXD_4, respectively. The identity was confirmed with positive ion LC-MS/MS. After incubation of L1236 cells with EXA_4, also a third peak with a conjugated triene spectrum and UV absorbance maximum of 282 nm was detected. The material in this peak coeluted with synthetic EXE_4, and the identity was also confirmed with positive ion LC-MS/MS.

RT-PCR revealed expression of 15-lipoxygenase-1 but not 15-liopxygenase-2 in L1236 cells. Western blot and immunohistochemistry analyzes demonstrated that 15-lipoxygenase-1 was present mainly in the cytosol and that the enzyme translocated to the membrane upon Ca^{2+} challenge. By immunohistochemistry of Hodgkin lymphoma tumor tissue, 15-lipoxygenase-1 was found to be expressed in primary H-RS cells in 17 of 20 investigated biopsies. In contrast, 15-lipoxygenase-1 was not expressed in any of the 10 investigated non-Hodgkin lymphoma biopsies, representing nine different subtypes of non-HL. In essence, the expression of 15-lipoxygenase-1 and the putative formation of EXs by Hodgkin Reed-Sternberg cells *in vivo* are likely to contribute to the inflammatory features of Hodgkin lymphoma. Furthermore, the finding that the Hodgkin lymphoma cell line L1236 expresses highly active 15-lipoxygenase-1 demonstrates that this cell line comprises a useful model system to study chemical and biological roles of 15-lipoxygenase-1.

4.5 PAPER V

Studies on glutathione transferases involved in the biosynthesis of eoxins

The EXs are synthesized from AA and this biosynthesis is initiated by oxidation of AA to 15-HPETE, followed by dehydration to form EXA_4. Both these steps are catalyzed enzymatically by 15-lipoxygenase-1. EXA_4 can be further conjugated with glutathione, leading to the formation of EXC_4. Human eosinophils, mast cells, nasal polyps and the human classical Hodgkin lymphoma cell line L1236 express 15-lipoxygenase-1 and generate EXs upon stimulation.

In this study we further explored the EX pathway with characterization of the enzymes involved in the transformation of EXA_4 into the biologically active EXC_4 in isolated human eosinophils and L1236 cells. In eosinophils, the capacity to convert EXA_4 to EXC_4 was almost exclusively found in the 100,000 x g pellet demonstrating that a

membrane bound enzyme catalyzed the reaction. Furthermore, MK886 (at a concentration of 10 μM) was found to inhibit the conversion of EXA_4 to EXC_4, indicating that the enzyme LTC_4 synthase was responsible for the formation of EXC_4 in eosinophils. In agreement with this conclusion, purified LTC_4 synthase and human platelets, which express LTC_4 synthase, readily converted EXA_4 to EXC_4. Other MAPEG proteins expressed by eosinophils, namely MGST2, MGST3 and FLAP, did not possess significant EXC_4 synthase activities. In contrast, the enzyme responsible for EXC_4 biosynthesis in L1236 cells was identified as the soluble glutathione transferase (GST) M1b-1b by means of subcellular fractionation, protein purification, protein N-terminal sequence analyzis, as well as cDNA sequence analyzis. By use of recombinant enzymes, it was found that also GST P1-1 (Val105 and Ile105 allelic variants) readily converted EXA_4 to EXC_4, whereas GST A1-1, GST A2-2, GST A3-3, GST A4-4, GST M2-2, GST M3-3, GST M4-4, GST M5-5 and GST T1-1 possessed only minor EXC_4 synthase activity. In essence, both LTC_4 synthase and certain soluble GST, in particularly GST M1-1 and GST P1-1, can catalyze the conversion of EXA_4 to EXC_4.

5 DISCUSSION

5.1 PAPERS I & II

In these papers we studied the expression of genes involved in AA metabolism as well as the capacity to generate LTB_4 via the 5-lipoxygenase pathway in blood tumor cells from patients with precursor B-ALL (acute lymphoblastic leukemia) and AML (acute myeloid leukemia). In addition, peripheral blood $CD34^+$ progenitor stem cells were analyzed. Based on immunophenotyping, both precursor B-ALL clones and AML clones were shown to represent clones at various lymphoid and myeloid maturation stages, respectively. In total, eight patients with precursor B-ALL, sixteen patients with AML and $CD34^+$ cells from three patients with non-myeloid malignancies were included. Whether the pattern of mRNA expression, protein expression and capacity to synthesize LTB_4 is linked to the normal lymphoid and myeloid differentiation processes or to aberrant enzyme expression related to the malignant process, is not possible to establish from the results of these studies.

Among the precursor B-ALL clones, the three most immature clones, in addition to one clone with a more mature phenotype expressed mRNA coding for $cPLA_2$. All sixteen AML clones as well as all three $CD34^+$ cell samples expressed the $cPLA_2$ protein, and the enzyme activity in the AML clones was high. The high expression of $cPLA_2$ suggests a central function for this enzyme in these very immature cells. However, the role for $cPLA_2$ in these most immature precursor B-ALL clones, the AML-clones and the $CD34^+$ cell population seems not to be connected to AA metabolism and LTB_4 generation since the $CD34^+$ cells and three most immature precursor B-ALL clones, in addition to the clone with a more mature phenotype, did not express mRNA coding for 5-lipoxygenase. These cells were also unable to synthesize LTB_4 or 5-HETE upon stimulation. In addition, the AML clones with the most immature phenotypes had an extremely low capacity to produce LTB_4 and 5-HETE upon stimulation, despite the fact that these cells expressed proteins coding for 5-lipoxygenase, FLAP and LTA_4 hydrolase. Thus, also other factors must be involved in the cellular regulation of LT synthesis. The high expression of $cPLA_2$ in these cells could be related to cell proliferation since a study performed on the myeloid cell line U937 suggested that Ca^{2+}/Calmodulin-dependent protein kinase II-induced activation of $cPLA_2$ resulted in cell proliferation of these cells. Furthermore, the proliferation signals induced by $cPLA_2$

activation were not exerted by AA or its metabolites, but rather by lysophosphatidyl-choline [121].

The aberrant capacity of immature AML clones to produce LTB_4 despite the protein expression of all necessary enzymes ($cPLA_2$, 5-lipoxygenase, FLAP and LTA_4 hydrolase) could be a result of elevated protein kinase A (PKA) activity in these cells. In acute lymphoblastic leukemia cells, an elevated total PKA cellular activity has been shown [122], and another study described the inhibitory effects of PKA on LT synthesis by phosphorylation of ser523 of 5-lipoxygenase [123]. This phosphorylation leads to reduced activity and a shift in the cellular distribution of the enzyme [124]. By inhibiting the nuclear import of 5-lipoxygenase, the association to FLAP and thereby interaction with AA is prevented, ultimately leading to reduced LTB_4 biosynthesis. In light of these reports, it would be interesting to determine the subcellular localization of 5-lipoxygenase in activated AML cells.

The four precursor B-ALL clones with a more mature phenotype expressed the mRNA coding for 5-lipoxygenase, and these clones, together with the more mature AML clones, had the capacity to generate LTB_4 and 5-HETE upon stimulation. From the results of these studies, it seems that the capacity to generate LTB_4 is gained upon maturation of cells. Both AML and precursor B-ALL constitute very heterogeneous types of leukemias. In addition to conventional markers (morphology, immunohistochemistry, flow cytometry and cytogenetics), biochemical markers that make it possible to subdivide these patients into separate groups could be of importance when evaluating the antitumor effect of different chemotherapeutic agents. It has been reported that the FLAP inhibitor MK886, at a concentration of 100 nM, inhibited the DNA synthesis in a subset of AML cells [125]. On the other hand, MK886 failed to inhibit DNA synthesis in normal bone marrow cells. In addition, we have in another study shown that LTB_4 plays a pivotal role in CD40-dependent activation of B-cell chronic lymphocytic leukemia cells, with inhibition of DNA synthesis in these cells upon treatment with MK886 or BWA4C, a specific 5-lipoxygenase inhibitor [126]. Taken together, it is possible that LTB_4 has an intrinsic role in cellular differentiation and growth of leukemia clones and that LTB_4 has an endogenous role in cellular proliferation during specific differentiation stages.

5.2 PAPERS III, IV & V

In these papers we described the novel finding of the lipid mediators eoxins (EXs; 14,15-LTs). The name eoxins instead of 14,15-LTs for these 15-lipoxygenase-derived

metabolites was suggested to avoid confusion with the classical, 5-lipoxygenase-derived LTs. The name *eo*xins also reflects the original finding in *eo*sinophils, in analogy to *leuko*trienes from *leuko*cytes. The EXs are generated via the 15-lipoxygenase-1 pathway in eosinophils, mast cells and in surgically removed nasal polyps from allergic subjects. Also the cHL cell line L1236 was found to express abundant amounts of 15-lipoxygenase-1 and to efficiently synthesize EXs upon stimulation. However, L1236 cells displayed no 5-lipoxygenase activity and had no capacity to produce LTs. This is in contrast to eosinophils, which contain 5-lipoxygenase and readily convert AA into cysteinyl LTs. Incubation of eosinophils with exogenous AA favoured the formation of EXC_4 over LTC_4; whereas incubation of eosinophils with the Ca^{2+} ionophore A23187 led to exclusive formation of LTC_4, i.e. the activation of 15-lipoxygenase-1 in eosinophils is not Ca^{2+} dependent. In the L1236 cells, the 15-lipoxygenase-1 enzyme translocated from the cytosol to the membrane upon Ca^{2+} stimulation. However, the cytosolic fraction of the enzyme was found to be more active than the membrane associated enzyme fraction. This is in line with the results in eosinophils, where Ca^{2+} was found to be unnecessary for EXC_4 formation. Possibly, also in eosinophils the 15-lipoxygenase-1 is active in the cytosol, at least when the cells are provided with exogenous AA. The role of $cPLA_2$ in the formation of EXs under physiological conditions has not been examined in these studies. If liberation of AA via $cPLA_2$ activity is a committed step in EX biosynthesis, Ca^{2+} would be needed for $cPLA_2$ activity. Alternatively, AA may be metabolized into EXA_4 within the membrane, since 15-lipoxygenase-1 has the ability to metabolize both free AA, as well as AA attached to biological membranes. Incubation of eosinophils with AA led to the formation of EXC_4, with slow subsequent metabolism into EXD_4 and EXE_4. In L1236, the EXC_4 produced after AA stimulation was rapidly further converted into EXD_4 and EXE_4, indicating that L1236 cells express higher amounts, or more active, γ-glutamyltransferase and dipeptidase, which probably catalyze the further reaction of EXC_4 into EXD_4 and EXE_4. In addition, L1236 cells may contain a more efficient EXC_4 transport system, responsible for the transfer of the intracellularly produced EXC_4 out to the γ-glutamyltransferase and dipeptidase, attached to the extracellular side of the cell membrane.

We also characterized enzymes with EXC_4 synthase activity, responsible for glutathione conjugation of EXA_4 into EXC_4 in eosinophils and L1236 cells, respectively. In eosinophils, the EXC_4 synthase activity totally resided in the membrane fraction of subcellular fractionated cells. Further characterization revealed that the

EXC_4 synthase activity in eosinophils belonged to LTC_4 synthase, the enzyme responsible for the conversion of LTA_4 into LTC_4. Thus, LTC_4 synthase is a key enzyme both for cysteinyl LT formation via the 5-lipoxygenase pathway and for EX formation via the 15-lipoxygenase pathway (*figure 4*).

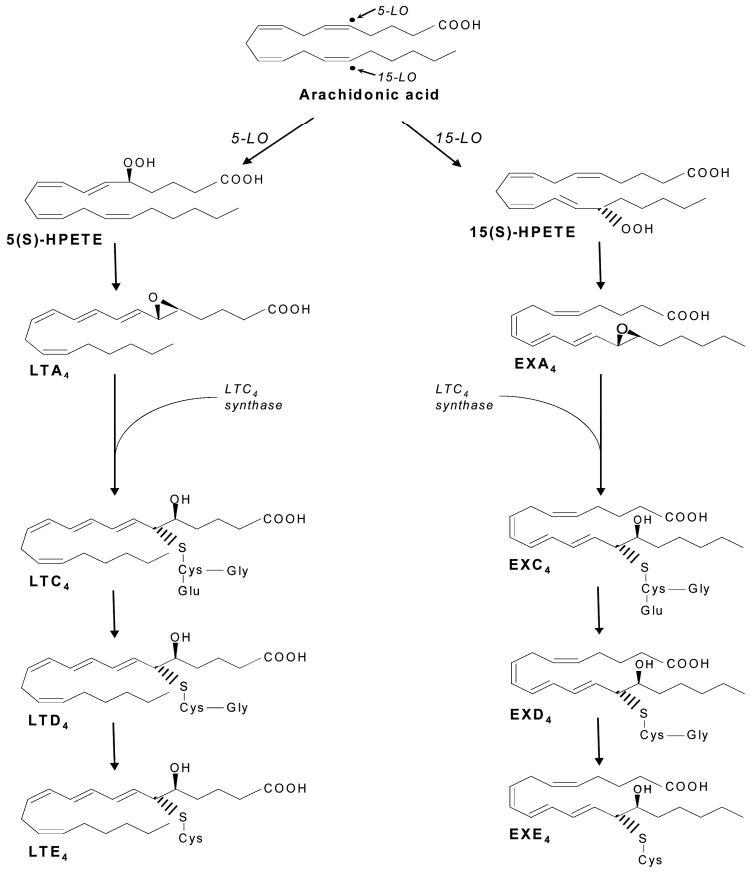

Figure 4. Overview of the formation of leukotrienes and eoxins in human eosinophils

Since eosinophils express both 5-lipoxygenase and 15-lipoxygenase-1 it is likely that the actual type of cellular stimuli decides which lipoxygenase pathway is to be activated. The transcription of 15-lipoxygenase-1 is dependent on activation of the transcription factor STAT-6, which is activated by ligation of IL-4 and IL-13 to the shared receptor IL-4Ralpha [127]. Since IL-4 and IL-13 are both important mediators

of the Th2 immune response implicated in asthma, and numerous publications describe elevated amounts of 15-lipoxygenase-1 and production of 15-HETE in airway inflammation, we speculate that one possible way for activation of the EX pathway in eosinophils proceeds via IL-4 and IL-13 binding to its surface receptor. In line with this suggested mechanism of activation, it has been demonstrated that IL-13 induced AHR, eosinophilia and mucus production are critically dependent on the IL-4Ralpha and STAT-6 [128]. Another piece of information regarding the possible activation of the EX pathway came from recent research performed in our laboratory. Primary mediastinal B-cell lymphoma (PMBCL) and Hodgkin lymphoma share many biological and clinical characteristics supporting a common pathogenic pathway. In this study, treatment of a PMBCL-derived cell line with IL-4 or IL-13 led to a HL-like phenotype of the cells, with the development of giant, multinucleated cells expressing 15-lipoxygenase-1, CysLTs and a capacity to produce EXs (Andersson, E. *et al* 2009, *in press*).

In the L1236 cells, the EXC_4 synthase activity totally resided in the soluble fraction, which was in contrast to the data generated in eosinophils. Further characterization revealed that the EXC_4 synthase in L1236 cells belonged to the human soluble glutathione transferase GST M1b-1b. Screening of other enzymes belonging to the human soluble glutathione transferase family [129] showed that in addition to GST M1b-1b, also the two isoforms of GST P1-1 (Val105 and Ile105) had great capacity to convert EXA_4 to EXC_4. Interestingly, we have recently found that GST P1-1 is coexpressed with 15-lipoxygenase-1 in primary airway epithelial cells and subcellular fractionation of these cells demonstrated that the 100,000 g supernatant, but not the 100, 000 pellet, had the capacity to produce eoxins (*manuscript in preparation*). Glutathione S-transferase P1-1 is the most expressed GST enzyme in the lung [130], and in the literature there is accumulating evidence for a role of GST M1 and GST P1 in the lung. It has been shown that asthma is associated with increased oxidative stress and that polymorphism in GSTs may influence susceptibility to asthma, atopy and AHR. Glutathione S-transferase P1-1 has been shown to protect against oxidative stress and also to be implicated in inflammatory responses in allergy [131, 132].

It is well known that human platelets possess LTC_4 synthase and are capable of LTC_4 biosynthesis through transcellular metabolism of LTA_4 [51, 133]. Since our investigations on EXC_4 biosynthesis in eosinophils revealed that LTC_4 synthase is acting also as EXC_4 synthase in these cells, we thought it was of interest to examine the capacity of platelets to synthesize EXC_4. Indeed, platelets had capacity to produce both

EXC_4 and LTC_4 to similar extent when incubated with EXA_4 and LTA_4, respectively. Interestingly, subcellular fractionation of platelets revealed the presence of EXC_4 synthase activity both in membrane and supernatant fractions, whereas the LTC_4 synthase activity was found exclusively in the membrane fraction. Since platelets contain both LTC_4 synthase as well as soluble glutathione transferases from the Mu and Pi classes [133, 134], we hypothesize that the membrane EXC_4 synthase activity stems from LTC_4 synthase, whereas the soluble EXC_4 synthase activity stems from GSTM and GSTP enzymes in platelets. In summary, both the microsomal glutathione transferase LTC_4 synthase, as well as the soluble glutathione transferases, in particularly GST M1b-1b and GST P1-1, possess EXC_4 synthase activity, and readily convert EXA_4 to EXC_4.

In these studies, we also provided data showing that the EXs act as proinflammatory agents with profound effects on vascular permeability leading to plasma leakage, a hallmark of inflammation, in an *in vitro* model using confluent human endothelial cells (EC). The permeability-increasing activity of the inflammatory mediators histamine and the cysteinyl LTs has been known since long. These mediators exert their effect on EC barrier function by triggering intracellular Ca^{2+} mobilization and cytoskeleton rearrangement [135], leading to paracellular gap formation and enhanced macromolecular permeability. The EXs were 100 times more potent than histamine and almost as potent as cysteinyl LTs in increasing permeability in this model system with confluent human EC. Whether also the EXs exert their proinflammatory effects via Ca^{2+} signalling or via an alternative second messenger system remains to be elucidated. In contrast to the 5-lipoxygenase derived cysteinyl LTs, EXC_4 and its metabolites do not appear to have contractile effects on guinea pig pulmonary parenchymal strip or ileum [136, 137].

We also provided data suggesting endogenous activation of the EX pathway in eosinophils. Following receptor-mediated activation by LTC_4, PGD_2 or IL-5, the cells were shown to produce EXC_4, and this finding supports a physiologically relevant role for EXs.

The finding that the HL cell line L1236 contained abundant amounts of 15-lipoxygenase-1 and was capable of EX biosynthesis led to further studies aiming to elucidate if this pathway is a common theme for HL. Immunohistochemical analyzes of primary Hodgkin lymphoma tumor tissue *ex vivo* demonstrated expression of 15-lipoxygenase-1 in H-RS cells in 85% (17/20) of all investigated biopsies. In comparison, the expression of 15-lipoxygenase-1 has later been investigated in a broad

range of primary non-Hodgkin lymphomas, and only one out of 58 investigated cases expressed 15-lipoxygenase-1 [138]. In addition, it has also been shown that both L1236 cells and primary H-RS cells express CysLT1, which might contribute to the inflammatory characteristics of HL [139]. As also eosinophils and mast cells express CysLT1 and 15-lipoxygenase-1 and are capable of EX biosynthesis, this raises the question if there might be biological similarities between such different diseases as asthma and HL. As IL-13 is proposed to be an important pathophysiological mediator of both asthma and Hodgkin lymphoma [140, 141], and this interleukin is known to induce expression of both 15-lipoxygenase-1 and CysLT1, maybe IL-13 could constitute a common link between these two apparently different diseases.

6 CONCLUSIONS

6.1 PAPERS I & II

- In precursor B-ALL cells, the capacity to express 5-lipoxygenase and to synthesize LTB_4 is gained upon maturation of cells. On the contrary, the expression of $cPLA_2$ mRNA is lost upon differentiation of the precursor B-ALL cells.
- In AML cells, the expression of $cPLA_2$ as well as the activity of the enzyme is high, regardless of the maturation stage of the cells. However, the strong $cPLA_2$ expression seems not to be connected to AA metabolism into LTB_4. Despite the fact that AML cells of all investigated maturational stages expressed proteins coding for 5-lipoxygenase, FLAP and LTA_4 hydrolase, only the more mature cells had the capacity to generate LTB_4 upon stimulation.

6.2 PAPERS III, IV & V

- Eoxins are metabolites formed from AA via the 15-lipoxygenase-1 pathway in eosinophils, mast cells, nasal polyps, platelets as well as in the classical Hodgkin lymphoma cell line L1236.
- The eoxins are proinflammatory lipid mediators with profound effects on vascular permeability, causing edema formation in inflammatory conditions.
- Both microsomal and soluble glutathione transferases can act as EXC_4 synthases: In eosinophils the microsomal glutathione transferase LTC_4 synthase readily converts EXA_4 to EXC_4, whereas in the Hodgkin lymphoma cell line L1236 the soluble glutathione transferase GST M1b-1b possesses this activity. Platelets possess both microsomal and soluble EXC_4 synthase activities.

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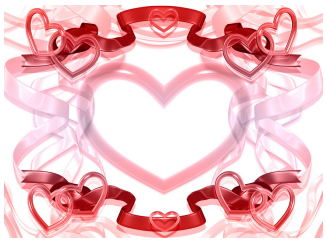

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