

Reduced lipoxygenase and cyclooxygenase mediated signaling in PBMC of atopic dermatitis patients

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ABSTRACT

Lipoxygenases (LOX) and cyclooxygenases (COX) are the main enzymes for poly-unsaturated fatty acid (PUFA) metabolism to highly bioactive prostaglandins, leukotrienes, thromboxanes and protectins. LOX and COX pathways are highly important for the regulation of pro- and anti-inflammatory active metabolite synthesis and metabolism in various inflammatory diseases like atopic diseases (AD). In this study using QRT-PCR, we found that in PBMCs the expression of 5-LOX, 12-LOX, 15-LOX and COX pathways and further enzymatic pathways like various leukotriene-hydroxylases, leukotriene-, prostaglandin-, and thromboxane-synthases as well as various of their membrane based receptors are mainly significantly down-regulated in AD-patients vs. healthy volunteers. In addition, using HPLC MS–MS we determined up to 19 different metabolites originating from eicosapentaenoic acid (EPA), docosapentaenoic acid (DHA) and arachidonic acid (AA) ranging from hydroxylated-PUFA derivatives and further bioactive derivatives like thromboxanes, leukotrienes, prostaglandins and protectins originating from LOX and COX metabolism. In PBMCs from AD-patients LOX and COX pathways were down-regulated. We conclude from this study, that in PBMCs from AD-patients in comparison to healthy volunteers, a systemic down-regulation of LOX- and COX-responses occurs to generally reduce eicosanoid/docosanoid synthesis during the current allergic inflammatory status.

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1. Introduction

Poly-unsaturated fatty acids (PUFAs) are present in high concentrations in the human organism. They are mainly bound as fatty acid esters in various forms but also freely available in serum and various organs. Multiple studies investigated the fatty acid composition in the field of atopic diseases and significant changes in their intake and endogenous concentrations could be seen between healthy and diseased individuals [1–5]. In this study we focused more on hydroxylated-PUFA derivatives and their further more bioactive derivatives the prostaglandins (PG), thromboxanes, leukotrienes (LT) and protectins [6,7]. A limited number of enzymes is responsible for hydroxylation of various n3- and n6-PUFAs like the 5-, 12-, 15-lipoxygenases

(LOX) and cyclooxygenases (COX). Specific hydroxy-derivatives are further metabolized by prostaglandin-, thromboxane- or leukotriene-synthases and leukotriene-hydroxylases to potent bioactive prostaglandins, leukotrienes, thromboxanes and protectins which are known anti- or pro-inflammatory lipid mediators [8]. These eicosanoids/docosanoids are binding and activating either nuclear hormone receptors like the peroxisome proliferator-activated receptors (PPARs) or various G-protein coupled receptors (GPCRs) [8].

In atopic dermatitis (AD) various studies have been performed showing that mainly increased concentrations of prostaglandins and leukotrienes were present in plasma, PBMCs or skin of AD-patients mainly using non specific ELISA and RIA as well as simple HPLC techniques [9–14]. Leukotrienes and prostaglandins are responsible for various adverse reactions observed in AD like induction of itch/scratch, degranulation, chemotaxis, immune/epidermal cell hyper-proliferation and histamine release, reviewed in [15]. Multiple treatment strategies blocking the receptor activation and the synthesis of various eicosanoids especially for leukotriene B4 (LTB4) and prostaglandin D2 (PGD2) are available for therapy of

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Table 1

Clinical, immunological and basic demographic data from healthy volunteers and AD-patients. Total IgE – plasma total IgE levels in kilounits (KU)/L; EOS % – number of eosinophils as percentage from PBMCs.

	healthy volunteers	AD-patients	Sign.
Age in years	21 ± 1	20 ± 5	–
Gender	70% female	60% female	–
SCORAD	0 ± 0	35.2 ± 16.9	*
Total IgE (KU/L)	32 ± 9.2	2941 ± 1134	*
EOS %	2.5 ± 0	7.3 ± 1.2	*
IFN γ (pg/ml)	0.84 ± 0.58	3.09 ± 0.93	*
IL5 (pg/ml)	1.81 ± 0.99	9.40 ± 1.55	*

* $p < 0.05$.

various atopic diseases to interfere mainly with pro-inflammatory lipid metabolite mediated signaling [16–18].

In our study we used human PBMCs and plasma from healthy volunteers and AD-patients to compare QRT-PCR based expression profile of various enzymes and receptors involved in PUFA-metabolite signaling as well as HPLC MS–MS based detection of PUFAs, hydroxylated-PUFA metabolites and bioactive eicosanoids/docosanoids to determine COX and LOX mediated signaling in plasma and PBMCs of human AD-patients.

2. Materials and methods

2.1. Study population

After informed consent and the approval of the local Ethics Committee of the University of Debrecen, Hungary, Medical and Health Science Center, peripheral blood was collected from 20 AD patients (8 male, 12 female; mean age 20 years, range 15–32 years). A group of 20 healthy age-matched volunteers (6 males, 14 females, mean age 21 years, range 19–24 years) served as controls in this study. All AD patients fulfilled the diagnostic criteria established by Hanifin and Rajka [19]. The severity and activity of the disease was determined by the SCORAD (SCORAD Atopic Dermatitis) index [20] the mean SCORAD was 35.15 (range 13–64). In 8 of 20 patients the disease started in the first year of life, in 2 patients between ages 3–4, in 7 patients between 6 and 18 years and in 3 patients in adulthood. In patients' history 10 patients of 20 had rhinitis, 3 had asthma, and 3 had both rhinitis and asthma (Table 1). Patients have not been treated with oral glucocorticosteroids, non-steroidal anti-inflammatory drugs or other systemic immunomodulatory agents for at least 4 weeks, also did not receive antihistamines and topical corticosteroids for at least 5 days prior to blood sampling.

2.2. Measurement of plasma IgE level and SCORAD

Patients were tested for plasma total IgE by ELISA (ADALTIS Italia S.p.A., Casalecchio di Reno, Italy) according to the manufacturer's instructions. The severity of the disease was determined with the SCORAD index [20].

2.3. Eosinophil cell count

The rate and absolute count of eosinophils as in whole blood were determined by Advia 120 hematology analyzer (Siemens, Deerfield, USA), as part of the routine clinical investigation of the patients and calculated as percentage (EOS %). The eosinophils in the analyzer were identified based on their scatter properties and myeloperoxidase positivity.

2.4. PBMC preparation and plasma samples

Peripheral blood was collected into EDTA-containing Vacutainer tubes (Bechton-Dickinson, Cedex, France) and transferred to a 15 ml falcon tube immediately after collection and then centrifuged at room temperature, 2500 rpm for 15 min. The plasma was removed after centrifugation and kept on -80°C . On the top of 5–10 ml Ficoll-Paque TM Plus the cell/plasma suspension was slowly slid down and afterwards centrifuged carefully at room temperature, 2500 rpm, for 20 min. Peripheral blood mononuclear cells (PBMCs) were removed after centrifugation and washed with physiological salt solution. Another centrifugation was performed at room temperature, 1800 rpm, for 10 min, the supernatant was discarded and this step was repeated 2 more times, with 7 min centrifugation at the given conditions. The cell pellet was suspended in 500 μl physiological salt solution and transferred into a 1.5 ml Eppendorf tube, centrifuged at room temperature, 13,000 rpm, for 6 min and the physiological salt solution was removed from the top in order to dry the pellet.

2.5. Analysis of mRNA expression

PBMC samples were homogenized in Tri[®] reagent solution and total RNA was isolated according to the manufacturer's instructions. Concentration and purity of RNA were measured by means of NanoDrop spectrophotometer (Thermo). For quantitative real-time PCR (QRT-PCR), total RNA was reverse transcribed into cDNA using the Super Script II First-Standard Synthesis System (Invitrogen). QRT-PCR was carried out in triplicate using pre-designed MGB assays from Applied Biosystems, on an ABI Prism 7900. Relative mRNA levels were calculated using either the comparative C_T or standard curve methods normalized to cyclophilin A mRNA. Sequence Detector Software (version 2.1) was utilized for data analysis and relative fold induction was determined by the comparative threshold cycle method.

2.6. Protein luminex assay procedure

All samples and standards were assayed in duplicate after bringing them to room temperature. The filter-bottomed microplate has been pre-wetted by filling each well with wash buffer and the liquid were removed by a vacuum manifold. The microparticle mixture was added to each well of the microplate and 50 μl of standard or sample was added per well. The assays were pipetted within 15 min and covered securely with a foil plate sealer. The assays were incubated for 3 h at room temperature on a horizontal microplate shaker at 500 rpm, each well was filled with wash buffer and the liquid was removed again, repeating the wash procedure three times. 50 μl of diluted Biotin Antibody Cocktail was added to each well and the assays were incubated for 1 h at room temperature on the shaker. 50 μl of diluted Streptavidin-PE was added to each well after repeating the washing step and the assays were incubated for 30 min at room temperature on the shaker. The washing step was repeated and the microparticles were resuspended by adding 100 μl of wash buffer to each well and the assays were incubated for 2 min on the shaker. The results were read within 90 min using a Luminex analyzer. The average of the duplicate readings has been calculated for each standard and sample. For data acquisition and evaluation ACS StarStation software (version 3.0) was used. Significantly increased protein concentrations of IFN γ and the Th2 marker IL5 were observed in the plasma of AD-patients by Luminex assay (Table 1).

Semi-quantification – no further validation of the extraction procedure and analysis has been performed. This method was just used to determine the “area under the curve” (AUC) of these specific compounds based on the same origin of the analyzed material and same amount of analyzed material used for the comparative analysis. The compounds were identified by comparable MS–MS characteristics and comparable retention time. Displayed in the figures are the AUC of the analysis and these AUCs can be used for semi-quantification and comparison in this study. Due to the

limited amount of PBMCs we just used ca. 20 mg of PBMCs for the analysis to get a sufficient amount of data.

2.8. Statistics

Data are shown as mean and standard error mean of values of three measurements per data point in case of the QRT-PCR results and as mean and standard deviation of values of three measurements per data point in case of the HPLC MS–MS results. Statistical analysis was performed using the program SPSS 16.0. A *p* value of less than 0.05 was considered significant by using Student's *t*-test for independent samples.

3. Results

Various LOX and COX pathways are regulated in plasma and PBMCs from AD-patients in comparison to healthy volunteers. Using QRT-PCR based analysis of enzymes and receptors of various eicosanoids/docosanoids and HPLC MS–MS analysis of plasma and PBMC lipids we try to get more details about systemic eicosanoid/docosanoids/PUFA regulation. QRT-PCR was performed using 40 PBMC samples 20 from healthy volunteers and 20 from AD-patients (Fig. 2), while the HPLC MS–MS analysis was performed in 20 plasma samples from healthy volunteers and 20 AD-patients (Table 2B) as well as in PBMC-samples (Table 2A) from 10 healthy volunteers and 10 AD-patients were analyzed.

3.1. Characterization of the study cohort

AD-patients and healthy volunteers did not differ in age and gender. Clinical markers of atopic dermatitis like SCORRAD, total IgE and percental amount of eosinophils from PBMCs were significantly increased in addition to increased protein concentrations of the TH1-marker IFN γ and the Th2 marker IL5 that were observed in the plasma of AD- patients (Table 1).

3.2. 5-lipoxygenase pathway

5-Lipoxygenase (5-LOX) is the key enzyme in the synthesis of leukotrienes (LTB4, LTC4 and LTD4) which are pro-inflammatory mediators originating from AA. 5-LOX expression in our experimental setup was slightly, but not significantly increased in case of AD-patients in comparison to healthy volunteers. The expression of 5-LOX activating protein (FLAP), a transporter protein for 5-LOX products, leukotriene A4 hydrolase (LTA4H), which catalyzes the conversion of semi-stable leukotriene A4 to the stable leukotriene B4 (LTB4), leukotriene C4 synthase (LTC4S), which converts leukotriene A4 to leukotriene C4 (LTC4), were all significantly decreased in AD-patients (Fig. 2). In addition the LTB4 metabolizing enzymes CYP4F3 and CYP4F22 were also significantly down-regulated in the PBMCs of AD-patients. Derivatives originating from the 5-LOX metabolism like LTB4 were in general lower in PBMCs from AD-patients (the values reach significance just for LTB4) (Table 2B). AA-derived 5-LOX metabolites (5-HETE and LTB4) were higher in the plasma of AD-patients; while the n3-PUFA derived 5-LOX metabolite 4-HODHA was lower in AD-patients (Table 2A).

3.3. 12/15-Lipoxygenase pathway

12-Lipoxygenase (ALOX12A) displayed a decreased expression in AD-patients PBMCs, while the other 12-lipoxygenase, the epidermal ALOX12B, and the 15-lipoxygenase 15-LOX were always under detection limit in all PBMC samples (Fig. 2). Metabolites originating from the 12-LOX and 15-LOX in the PBMCs were all lower in AD-patients: 12-HETE and 14-HODHA were significantly lower

Table 2
Relative concentrations in AUC including standard deviation of PUFAs and PUFA metabolites in (A) human plasma and (B) human PBMCs. UDL – under detection limit; all other abbreviations see Fig. 1. n3 derived PUFA metabolites are marked with (n3).

(A) PLASMA	Healthy volunteers (n = 20)	AD-patients (n = 20)	Sign.
COX pathway			
PGD2	1.14 ± 0.40	1.19 ± 0.31	0.44
15dPGJ2	4.86 ± 1.77	7.19 ± 2.94	0.24
TXB2	10.3 ± 4.87	7.69 ± 2.84	0.29
5-LOX pathway			
5-HETE	44.3 ± 17.5	51.0 ± 10.9	0.37
4-HODHA (n3)	10.7 ± 4.1	2.50 ± 1.50	0.20
LTB4	8.19 ± 3.94	12.6 ± 3.8	0.21
12-LOX pathway			
9-HODE	40.3 ± 11.1	39.0 ± 9.2	0.50
12-HETE	590 ± 126	637 ± 106	0.39
12-HEPE (n3)	5.64 ± 1.66	13.4 ± 3.8	0.03
14-HODHA (n3)	18.1 ± 5.0	27.8 ± 6.0	0.16
15-LOX pathway			
13-HODE	34.0 ± 8.7	32.7 ± 10.0	0.46
15-HETE	16.6 ± 7.2	11.8 ± 5.40	0.15
17-HODHA (n3)	5.69 ± 1.70	4.50 ± 1.89	0.30
PD1 (n3)	0.94 ± 0.42	2.58 ± 1.63	0.09
Unknown pathway			
8-HETE	22.1 ± 4.7	14.1 ± 10.6	0.05
n3-PUFAs			
DHA	6887 ± 1719	6165 ± 2107	0.36
EPA	898 ± 243	565 ± 137	0.09
n6-PUFAs			
LA	33,383 ± 6624	24,585 ± 4477	0.10
AA	55,415 ± 14,591	36,145 ± 10,067	0.11
(B) PBMCs	Healthy volunteers (n = 10)	AD-patients (n = 10)	Sign.
COX pathways			
PGD2	2.67 ± 1.15	1.20 ± 0.55	0.05
15dPGJ2	7.72 ± 1.95	4.45 ± 2.04	0.17
TXB2	228 ± 62	91.6 ± 47.5	0.01
5-LOX pathway			
5-HETE	77.8 ± 28.0	41.4 ± 24.1	0.21
4-HODHA (n3)	UDL	UDL	
LTB4	112 ± 41	40.9 ± 22.2	0.04
12-LOX pathway			
9-HODE	74.4 ± 36.1	37.7 ± 12.6	0.11
12-HETE	51,447 ± 33,330	20,210 ± 13,321	0.04
12-HEPE (n3)	516 ± 277	180 ± 125	0.11
14-HODHA (n3)	806 ± 489	339 ± 193	0.05
15-LOX pathway			
13-HODE	50.3 ± 13.8	28.3 ± 11.5	0.07
15-HETE	32.7 ± 16.6	13.4 ± 7.33	0.07
17-HODHA (n3)	7.06 ± 4.20	0.50 ± 0.00	0.07
PD1 (n3)	2.17 ± 1.12	1.75 ± 1.04	0.36
Unknown pathway			
8-HETE	126 ± 79	57.2 ± 40.8	0.05
n3-PUFAs			
DHA	216 ± 79	205 ± 92	0.41
EPA	53.6 ± 26.4	44.3 ± 19.1	0.30
n6-PUFAs			
LA	1708 ± 483	1366 ± 439	0.06
AA	8482 ± 4108	9033 ± 4626	0.48

* *p* < 0.05.

while the others were lower and close to significance in PBMCs from AD-patients (Table 2B). The 12-LOX metabolites (12-HEPE, 12-HETE and 17-HO-DHA) were all higher in plasma of AD-patients, while the EPA-derived metabolite 12-HEPE was significantly higher (Table 2A). The 15-LOX metabolites were all in the same range or slightly lower in plasma samples from AD-patients (Table 2A). Just the 15-LOX and further epoxygenation n3-PUFA metabolite PD1 was significantly higher in plasma of AD-patients.

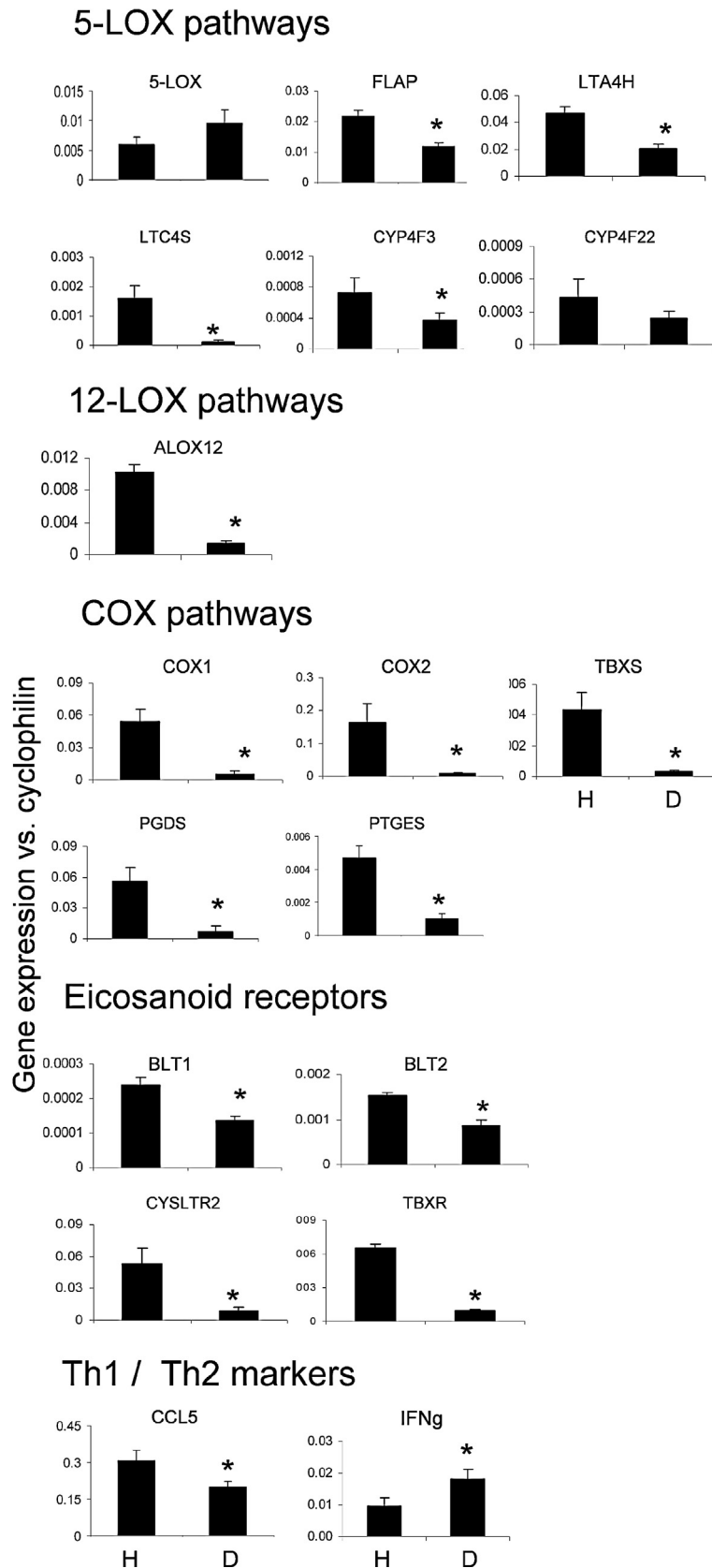


Fig. 2. QRT-PCR data of various enzymes and receptors involved in PUFA-metabolite signaling. PBMCs from healthy volunteers (H, $n=20$) vs. AD-patients (D, $n=20$) were compared. * $p < 0.05$; 5LOX – 5-lipoxygenase, FLAP – 5-LOX activating protein, LTA4H – leukotriene A4 hydrolase, LTC4S – leukotriene C4 synthase, BLT1 – leukotriene B4 receptor 1, BLT2 – leukotriene B4 receptor 2, CYSLTR2 – cysteinyl leukotriene receptor 2, COX1 – cyclooxygenase 1, COX2 – cyclooxygenase 2, TBXS – thromboxane synthase, TBXR – thromboxane receptor, PGDS – prostaglandin D synthase, PTGES – prostaglandin E synthase, ALOX12 – lipoxygenase 12, CYP4F3 – cytochrome P450, family 4, subfamily f, polypeptide 3, CYP4F22 – cytochrome P450, family 4, subfamily f, polypeptide 22.

3.4. Unknown 8-hydroxylation pathway

The metabolite 8-HETE which originates in human by a still unknown pathway was significantly lower in PBMCs AD-patients (57.2 ± 40.8 AUC) in comparison to healthy volunteers (126 ± 79 AUC) (Table 2B). 8-HETE was also significantly lower in the plasma of AD-patients (Table 2A).

3.5. Cyclooxygenase pathway

Cyclooxygenases are the responsible enzymes for the formation of prostaglandins and thromboxanes. The expression of both COX1 and COX2 showed a remarkable decrease in the PBMCs of the AD-patients in comparison to healthy volunteers. Thromboxane synthase (TBXS), prostaglandin E synthase (PTGES) and prostaglandin D synthase (PGDS) are the enzymes responsible for thromboxane or prostaglandin E/D synthesis and their expression in PBMCs was also significantly lower in AD-patients (Fig. 2). In PBMC samples the prostaglandin (PGD2) and thromboxane B2 (TXB2) levels were significantly lower in AD-patients, while 15dPGJ2 concentration was just non-significantly lower in AD-patients (Table 2B). In the plasma samples all detected COX-metabolites were in general in the same range (Table 2A).

3.6. Eicosanoid receptors

The three leukotriene receptors leukotriene B4 receptors BLT1 and BLT2, the cysteinyl leukotriene receptor 2 (CYSLR2) as well as the thromboxane receptor (TXB2) were all significantly lower expressed in PBMCs of AD-patients, while CYSLR1 was always under quantification limit.

3.7. Fatty acids

With our HPLC MS–MS method we detected also free fatty acids because the used sample preparation was without prior saponification like in various other studies. Relative PUFA concentration showed an inconsistent tendency; n3-PUFAs (DHA and EPA) were both non-significantly lower in PBMCs and plasma samples from AD-patients, comparable to lower levels of the n6-PUFAs linoleic acid (LA) (Table 2A and 2B). The precursor of the majority of prostaglandins and leukotrienes the arachidonic acid was higher in PBMCs and lower in plasma from AD-patients (Table 2A and 2B).

3.8. Th1/Th2 pathways

Using QRT-PCR assay of PBMCs (Fig. 2) we found that the mRNA expression of the Th2 marker CCL5/RANTES is significantly decreased while the Th1 marker IFN γ is significantly increased in PBMCs of AD-patients.

4. Discussion

Lipoxygenase and cyclooxygenase enzymatic pathways as well as their corresponding lipid products, the potent anti- or pro-inflammatory lipid mediators (eicosanoids and docosanoids) are strongly down-regulated in PBMCs of AD-patients (Fig. 2).

This study shows for the first time a pathway analysis and a connection between the QRT-PCR based expression of lipid metabolizing enzymes and various specific lipid mediator receptors and the HPLC MS–MS based concentration determination of various main substrate and product lipid mediators in human PBMCs and plasma from AD-patients. 5-, 12-, 15-LOX and COX metabolic pathways, leading to hydroxylated-PUFA metabolites of linoleic acid (LA), arachidonic acid (AA), eicosapentaenoic acid

(EPA) and docosahexaenoic acid (DHA) and further more bioactive PUFA-metabolites, were mainly significantly down-regulated in PBMC samples from human AD-patients in comparison to healthy volunteers (Table 2A and B; Fig. 2). This down-regulation of pro-inflammatory lipid signaling in PBMCs can be a fact of feedback mechanism to chronic topical or systemic inflammation and in addition by an altered distribution of different cell types in PBMCs originating from healthy volunteers or AD-patients [24].

In our AD-patients a chronic atopic dermatitis skin phenotype was partly confirmed by a strong increased Th1 response, which is an indicator of chronic atopic dermatitis [25], and an increased Th2 response (Table 1). There was no significant correlation between Th1 marker IFN γ as well as the Th2 marker CCL5 expression with any LOX/COX pathway. In chronic inflammation/dermatitis a strong down-regulation of lipid attraction, processing and metabolism in the skin occurs [26]. In our case the n3-PUFAs EPA and DHA displayed a tendency of lower levels in human PBMCs and plasma samples in comparison to healthy volunteers. The n6-PUFAs LA was, comparable to the n3-PUFAs, significantly lower in plasma and PBMCs of AD-patients, while AA was higher in human PBMCs from AD-patients (Tables 2A and 2B). In summary, we observed a general down-regulation of n3-PUFAs and the n6-PUFA LA as free fatty acids in PBMCs and plasma samples from AD-patients. To specifically investigate PUFA-metabolite regulation we need a specific and more detailed investigation for COX and LOX mediated pathways using QRT-PCR and HPLC MS–MS studies to elucidate a specific PUFA/eicosanoid pathway regulation in AD-patients.

Specific COX and LOX pathways are altered in PBMCs as well as partly in plasma samples from AD-patients leading to various bioactive eicosanoids/docosanoids. Starting with the pro-inflammatory eicosanoid leukotriene B4 (LTB4) originating from the 5-LOX pathway, which is significantly lower in PBMCs from human AD-patients and a tendency of higher levels in the plasma of AD-patients. Various enzymes and binding proteins involved in leukotriene synthesis and also degradation are significantly lower also in PBMCs from AD-patients (Fig. 2). This lower level of LTB4 and 5-LOX pathways is quite surprising because this pathway is mainly associated with pro-inflammatory signaling in atopic diseases [11,12,27,28]. These data are coherent to various studies performed before in neutrophils from AD-patients [29], which also display reduced LTB4-release. Other studies which were mainly performed in the AD target organ, the affected skin, displayed an increase in LTB4 concentration, using mainly non-specific ELISA/RIA and simple HPLC techniques [11–13]. Previously it was shown using various animal studies (mice and dogs) with acute atopic inflammation that an increased expression of 5-LOX pathway enzymes for the synthesis of LTB4 as well as elevated LTB4 concentration are present in affected AD-skin [27] and we could confirm these data in mice also in our laboratories (Rühl et al. in preparation). We propose that in acute AD increased 5-LOX activity and LTB4-synthesis is mainly localized in the skin.

In contrast to the PBMCs where all LOX and COX pathways were down-regulated, in the plasma samples of AD-patients not all LOX/COX pathway derived lipids were down-regulated and in the plasma these eicosanoids/docosanoids remained more stable and even were increased like the 12-LOX metabolites 12-HEPE. This up-regulation of the 12-LOX metabolites in the plasma may origin from the inflamed skin especially the infiltrated immune cells inside the skin. 12/15-lipoxygenase is usually associated with the generation of pro- and anti-inflammatory lipid mediators and can negatively [30,31] or positively influences inflammation processes [32]. 12/15-LOX possesses an anti-inflammatory and tissue protective role in chronic inflammatory disorders [32] and that may be the reason of the selective up-regulation of the EPA derived

12-LOX metabolites in the plasma. This could mean that these hydroxylated-PUFAs and their further down-stream and more complex and bioactive metabolites from n3-PUFAs maybe involved in anti-inflammatory or even resolving activities [33].

COX and LOX pathways are pathways to enable and trigger pro-inflammatory processes [15] and in addition to enable and trigger further resolution of the inflammation in the affected site [33]. The problem of this down-regulation of COX and LOX pathways in chronic dermatitis is that these pathways also lead to down-regulation of various, mainly n3-PUFA derived, anti-inflammatory lipids and potential resolving lipids like protectins, resolvins, maresins and poxotriins [34], which is in accordance with significantly lower n3-PUFAs in plasma and PBMCs from AD-patients (Table 2A). These n3-PUFA derived anti-inflammatory derivatives occur just in very low concentrations in highly inflamed tissues of the human organism [6,35] and were under the quantification limit in all our examined samples in the current study. In addition, in this study we did not determine lipoxygenase/cyclooxygenase pathways leading to bioactive eicosanoids/docosanoids in inflamed skin samples from humans, but this project is partly in process in our laboratories. Here we focused on easier accessible biological materials from humans, the PBMC and plasma samples. In the plasma samples the relative concentrations of fatty acids and the majority of eicosanoids/docosanoids is much lower, taking in consideration that for plasma analysis 100 µl (corresponding to 100 mg) were used and for PBMCs 20 mg material was needed based for our comparative AUC-calculation (Table 2A and 2B).

For therapy of atopic diseases various derivatives inhibiting leukotriene signaling are used mainly for topical therapeutic approaches [16,17]. These treatments were effectively for asthma but not in general for AD. In this study we see that all pathways involved in leukotriene signaling are already strongly down-regulated on systemic level in PBMCs of AD-patients. Considering from our data we doubt that a systemic approach for inhibition of leukotriene pathways may lead to improvement of atopic disease, this explains that various treatment strategies for inhibition of leukotriene signaling lack success confirmed by various studies.

5. Conclusion

COX and LOX mediated pathways and their corresponding products, the arachidonic acid derived pro-inflammatory lipid mediators, are mainly significantly down-regulated in PBMCs from AD-patients. These results let us conclude the plasma reflects the eicosanoids/docosanoids present from the whole organism, while the PBMCs seem to counteract this current inflammatory status by a general down-regulation of eicosanoid/docosanoids synthetic pathways.

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References

- [1] Rühl R, Koch C, Marosvolgyi T, et al. Fatty acid composition of plasma lipid classes in mice following allergic sensitisation with or without dietary docosahexaenoic acid-enriched fish oil substitution. *Br J Nutr* 2008;99:1239–46.
- [2] Weise C, Heunemann C, Loddenkemper C, Herz U, van Tol EA, Worm M. Dietary docosahexaenoic acid in combination with arachidonic acid ameliorates allergen-induced dermatitis in mice. *Pediatr Allergy Immunol* 2011;22:497–504.
- [3] Schafer L, Kragballe K. Abnormalities in epidermal lipid metabolism in patients with atopic dermatitis. *J Invest Dermatol* 1991;96:10–5.
- [4] Kankaanpää P, Nurmela K, Erkkilä A, et al. Polyunsaturated fatty acids in maternal diet, breast milk, and plasma lipid fatty acids of infants in relation to atopy. *Allergy* 2001;56:633–8.
- [5] Solvoll K, Soyland E, Sandstad B, Drevon CA. Dietary habits among patients with atopic dermatitis. *Eur J Clin Nutr* 2000;54:93–7.
- [6] Dennis EA, Deems RA, Harkewicz R, et al. A mouse macrophage lipidome. *J Biol Chem* 2010;285:39976–85.
- [7] Quehenberger O, Armando AM, Brown AH, et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 2010;51:3299–305.
- [8] Buczynski MW, Dumlao DS, Dennis EA. Thematic review series: proteomics. An integrated omics analysis of eicosanoid biology. *J Lipid Res* 2009;50:1015–38.
- [9] Elias PM, Williams ML, Holleran WM, Jiang YJ, Schmuth M. Pathogenesis of permeability barrier abnormalities in the ichthyoses: inherited disorders of lipid metabolism. *J Lipid Res* 2008;49:697–714.
- [10] Proksch E, Jensen JM, Elias PM. Skin lipids and epidermal differentiation in atopic dermatitis. *Clin Dermatol* 2003;21:134–44.
- [11] Fogh K, Herlin T, Kragballe K. Eicosanoids in skin of patients with atopic dermatitis: prostaglandin E2 and leukotriene B4 are present in biologically active concentrations. *J Allergy Clin Immunol* 1989;83:450–5.
- [12] Ruzicka T, Simmet T, Peskar BA, Ring J. Skin levels of arachidonic acid-derived inflammatory mediators and histamine in atopic dermatitis and psoriasis. *J Invest Dermatol* 1986;86:105–8.
- [13] Reilly DM, Parslew R, Sharpe GR, Powell S, Green MR. Inflammatory mediators in normal, sensitive and diseased skin types. *Acta Derm Venereol* 2000;80:171–4.
- [14] Sampson AP, Thomas RU, Costello JF, Piper PJ. Enhanced leukotriene synthesis in leukocytes of atopic and asthmatic subjects. *Br J Clin Pharmacol* 1992;33:423–30.
- [15] Boyce JA. Mast cells and eicosanoid mediators: a system of reciprocal paracrine and autocrine regulation. *Immunol Rev* 2007;217:168–85.
- [16] Veien NK, Busch-Sorensen M, Stausbol-Gron B. Montelukast treatment of moderate to severe atopic dermatitis in adults: a randomized, double-blind, placebo-controlled trial. *J Am Acad Dermatol* 2005;53:147–9.
- [17] Silverberg NB, Paller AS. Leukotriene receptor antagonists are ineffective for severe atopic dermatitis. *J Am Acad Dermatol* 2004;50:485–6.
- [18] Arai I, Takano N, Hashimoto Y, et al. Prostanoid DP1 receptor agonist inhibits the pruritic activity in NC/Nga mice with atopic dermatitis. *Eur J Pharmacol* 2004;505:229–35.
- [19] Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatovenereol Suppl (Stockh)* 1980;92:44–7.
- [20] European-Task-Force-on-Atopic-Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. *Dermatology* 1993;193:23–31.
- [21] Rühl R. Method to determine 4-oxo-retinoic acids, retinoic acids and retinol in plasma and cell extracts by liquid chromatography/diode-array detection atmospheric pressure chemical ionisation tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2006;20:2497–504.
- [22] Csanky E, Rühl R, Scholtz B, Vasko A, Takacs L, Hempel WM. Lipid metabolite levels of prostaglandin D2 and eicosapentaenoic acid recovered from bronchoalveolar lavage fluid correlate with lung function of chronic obstructive pulmonary disease patients and controls. *Electrophoresis* 2009;30:1228–34.
- [23] Flachs P, Rühl R, Hensler M, et al. Synergistic induction of lipid catabolism and anti-inflammatory lipids in white fat of dietary obese mice in response to calorie restriction and n-3 fatty acids. *Diabetologia* 2011;54:2626–38.
- [24] Yona S, Jung S. Monocytes: subsets, origins, fates and functions. *Curr Opin Hematol* 2010;17:53–9.
- [25] Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;113:651–7.
- [26] Saaf AM, Tengvall-Linder M, Chang HY, et al. Global expression profiling in atopic eczema reveals reciprocal expression of inflammatory and lipid genes. *PLoS ONE* 2008;3:e4017.
- [27] Schlötter YM, Riemers FM, Rutten VP, Knol EF, Willemse T. Enzymes involved in the conversion of arachidonic acid to eicosanoids in the skin of atopic dogs. *Exp Dermatol* 2010;19:e317–9.
- [28] Okano-Mitani H, Ikai K, Imamura S. Leukotriene A4 hydrolase in peripheral leukocytes of patients with atopic dermatitis. *Arch Dermatol Res* 1996;288:168–72.
- [29] Schafer L, Kragballe K, Jepsen LV, Iversen L. Reduced neutrophil LTB4 release in atopic dermatitis patients despite normal fatty acid composition. *J Invest Dermatol* 1991;96:16–9.
- [30] Hajek AR, Lindley AR, Favoreto Jr S, Carter R, Schleimer RP, Kuperman DA. 12/15-Lipoxygenase deficiency protects mice from allergic airways inflammation and increases secretory IgA levels. *J Allergy Clin Immunol* 2008;122:633–9, e3.

- [31] Andersson CK, Claesson HE, Rydell-Tormanen K, Swedmark S, Hallgren A, Erjefalt JS. Mice lacking 12/15-lipoxygenase have attenuated airway allergic inflammation and remodeling. *Am J Respir Cell Mol Biol* 2008;39:648–56.
- [32] Kronke G, Katzenbeisser J, Uderhardt S, et al. 12/15-lipoxygenase counteracts inflammation and tissue damage in arthritis. *J Immunol* 2009;183:3383–9.
- [33] Serhan CN, Brain SD, Buckley CD, et al. Resolution of inflammation: state of the art, definitions and terms. *FASEB J* 2007;21:325–32.
- [34] Serhan CN, Gotlinger K, Hong S, Arita M. Resolvins, docosatrienes, and neuroprotectins, novel omega-3-derived mediators, and their aspirin-triggered endogenous epimers: an overview of their protective roles in catabasis. *Prostaglandins Other Lipid Mediat* 2004;73:155–72.
- [35] Gombolka B, Siegert E, Blossey K, Schunck WH, Rothe M, Weylandt KH. Analysis of omega-3 and omega-6 fatty acid-derived lipid metabolite formation in human and mouse blood samples. *Prostaglandins Other Lipid Mediat* 2011;94:81–7.