

Received: 2004.XX.XX  
Accepted: 2004.XX.XX  
Published: 2005.XX.XX

## Expression regulation of MAO isoforms in monocytic cells in response to Th2 cytokines

### Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Pavlos Chaitidis<sup>1ABCDEF</sup>, Ellen Billett<sup>2ABCDEF</sup>, Ralf J. Kuban<sup>1,3ABCDEF</sup>,  
Ute Ungethuen<sup>3ABCDEF</sup>, Hartmut Kuhn<sup>1ABCDEF</sup>

<sup>1</sup> Institute of Biochemistry, University Medicine Berlin Charité, Berlin, Germany

<sup>2</sup> School of Science, Division of Biomolecular Sciences, The Nottingham Trent University, Clifton Lane, Nottingham, U.K.

<sup>3</sup> Laboratory of Functional Genome Research, University Medicine Berlin Charité, Berlin, Germany

**Source of support:** Financial support for this study was provided by the German Research Society (DFG) (Ku 961/8-1)

### Background:

Th2-cytokines, such as interleukins-4 and -13 (IL-4, IL-13), have been identified as alternative stimuli of monocytes/macrophages. We have recently profiled the gene-expression pattern of IL-4-treated human peripheral monocytes and found that 15-lipoxygenase-1 (15-LOX1) and monoamine oxidase A (MAO-A) are among the five most strongly upregulated gene products in IL-4-treated cells. Transfection of monocytic cells (U937) with 15-LOX1 also induced MAO-A expression. These data suggested that 15-LOX1 products might play a role in the IL-4-induced signaling cascade leading to expression of MAO-A in human monocytes.

### Material/Methods:

To test this hypothesis we incubated wild-type and 15-LOX1-transfected U937 cells with different concentrations of either IL-4 or 15-LOX-products [13S-H(p)ODE, 15S-H(p)ETE] and quantified the expression of 15-LOX1, MAO-A, and MAO-B by activity assays and real-time RT-PCR.

### Results:

Wild-type U937 cells express neither MAO-A nor MAO-B, but after three days of IL4 treatment, MAO-A mRNA was detected. A similar isoform-specific expression of MAO-A mRNA was observed when U937 cells were transfected with 15-LOX1 or when the cells were incubated with primary 15-LOX1 products (hydroperoxy fatty acids) or H<sub>2</sub>O<sub>2</sub>. In contrast, the corresponding hydroxy fatty acids were ineffective.

### Conclusions:

These data indicate that increased intracellular peroxide concentrations (oxidative stress) induce MAO-A expression in monocytes/macrophages, which normally do not express the enzyme. Our findings also suggest that IL-4-induced upregulation of MAO-A expression in human peripheral monocytes may proceed via 15-LOX1-dependent and 15-LOX1-independent pathways. The biological role of MAO-A expression for monocyte function is discussed.

### key words:

monocytes • cytokines • lipid mediators • inflammation • eicosanoids

### Abbreviations:

**MAO-A(B)** - monoamine oxidase A(B); **LOX** - lipoxygenase; **IL-4/-13** - interleukin-4/-13; **STAT** - signal transducer and activator of transcription; **13S-H(p)ODE** - 13S-hydro(pero)xy-9Z,11E-octadecadienoic acid; **15S-H(p)ETE** - 15S-hydro(pero)xy-5Z,8Z,11Z,13E-eicosatetraenoic acid; **PBS** - phosphate buffered saline

### Full-text PDF:

[http://www.MedSciMonit.com/pub/vol\\_11/no\\_5/6629.pdf](http://www.MedSciMonit.com/pub/vol_11/no_5/6629.pdf)

### Word count:

XXXX

### Tables:

–

### Figures:

4

### References:

53

### Author's address:

Dr. Hartmut Kühn, Institut für Biochemie, Universitätsmedizin Berlin Charité, Monbijoustr. 2, 10117 Berlin, Germany, e-mail: hartmut.kuehn@charite.de

## BACKGROUND

Cytokines are powerful mediators of inflammatory and immune reactions [1–3] and sometimes they are classified as pro- or anti-inflammatory messengers [4]. However, this classification is quite simplistic and there is convincing evidence indicating that a given cytokine may exhibit pro- and/or anti-inflammatory activities depending on its concentration, the nature of the target cell, and even on the experimental inflammation model [5]. Lymphocyte-derived Th2 cytokines, such as interleukins-4 and -13 (IL-4, IL-13), regulate gene expression of inflammatory and/or immune competent cells. In human peripheral monocytes they appear to induce an anti-inflammatory response [6]. Expression of classic pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and TNF, is down-regulated by these cytokines and the pattern of CD cell-surface antigens is dramatically altered [6]. It has been reported previously that 15-LOX1 is strongly upregulated during treatment of peripheral monocytes with IL-4 [7] and recent microarray studies indicate that 15-LOX1 constitutes the most strongly upregulated gene product. [8]. LOXs form a diverse family of lipid peroxidizing enzymes capable of oxygenating polyunsaturated fatty acids to the corresponding hydroperoxides [9]. They have been implicated in the biosynthesis of inflammatory mediators [10], in cell development [11,12], and in the pathogenesis of various diseases [13–15]. The mechanism of IL-4/-13-induced upregulation of 15-LOX1 expression involves functional IL-4/-13 cell surface receptors [16], various protein kinases [17,18], and members of the STAT transcription factor family [19,20]. Under resting conditions the STAT-responsive *cis*-regulatory elements in the 15-LOX1 promoter [21] appear to be blocked by nuclear histones so that activated transcription factors are prevented from binding. However, IL-4 treatment activates nuclear acetyltransferases catalyzing acetylation of nuclear histones, and this covalent modification induces conformational alterations in the nucleosome structure rendering the *cis*-regulatory elements of the 15-LOX1 promoter accessible for activated *trans*-acting proteins [21].

15-LOX1 is a pro-oxidative enzyme and its cytokine-dependent upregulation increases the intracellular oxidizing potential [22]. Another pro-oxidative enzyme is monoamine oxidase (MAO, EC 1.4.3.4). This enzyme has been characterized as an FAD-containing protein converting biogenic or dietary amines to the corresponding aldehydes [23,24]. The enzymatic reaction requires molecular dioxygen and produces stoichiometric amounts of hydrogen peroxide [23]. In mammalian cells the aldehyde intermediates are rapidly oxidized via aldehyde dehydrogenase to the corresponding acid or, alternatively, are reduced to alcohols. The hydrogen peroxide formed via MAO-catalyzed oxidative deamination increases the cellular oxidizing potential. MAO is located in the outer mitochondrial membrane as well as in the cytosol [25], and there are two genetic isoforms, MAO-A and MAO-B [23,24]. They are encoded by separate genes, have different protein-chemical properties, and exhibit distinct substrate and inhibitor specificities [26–28]. Recent investigations into the molecular reasons for the enzymatic differences suggested that a single amino acid exchange is responsible [29,30]. Although MAO-A and MAO-B occur in many cells, there are differences in tissue- [31,32] and development-specific expression patterns [33,34]. For instance,

MAO-A is expressed at a high-level in placenta [35], but in pre-eclamptic patients its expression level is reduced [36].

The two genes encoding MAO-A and MAO-B are localized on the X-chromosome [37,38]. They contain 15 exons and the exon-intron organization is very similar [39]. The 5'-flanking regions of the two genes share a high degree of sequence similarity, but there is a distinct pattern of *cis*-regulatory elements [40]. The basic promoter of the MAO-A gene contains four SP1 binding sites and reporter gene assays indicated that three of them are functional [41]. More recently it was reported that MAO-B, but not MAO-A, expression is upregulated by phorbol esters and that the signal transduction cascade involves c-Jun and Erg-1 [42]. Inspecting the 5'-flanking region of the MAO-A gene we failed to detect consensus sequences for members of the STAT family (signal transducer and activator of transcription), suggesting that these transcription factor may not be directly involved in the expression regulation of the MAO-A gene.

Investigating cytokine-dependent alterations in the gene expression of human peripheral monocytes using the microarray technology, we recently found that the expressions of both 15-LOX1 and MAO-A are strongly upregulated in IL-4-treated cells [8]. In addition, fibronectin-1, the Fc-fragment of IgE (CD23A), the CD1C cell surface antigen, and the coagulation factor XIII belonged to the most strongly upregulated gene products [8]. Interestingly, this expression regulation was isoform-specific since neither MAO-B nor other LOX isoforms were upregulated [43]. In contrast, we even observed significant downregulation of various enzymes of the 5-LOX pathway. Mechanistic investigations with transfected U937 cells suggested that 15-LOX1 may play a role in the IL-4-induced signal transduction cascade leading to MAO-A expression [43]. Since 15-LOX is involved in the biosynthesis of lipid signal transducers [10], there was the possibility that products of the 15-LOX pathway might be involved in the signaling cascade. To test this hypothesis we incubated wild-type U937 cells with various products of the 15-LOX pathway and found that 13S-HpODE and 15S-HpETE, the primary products of 15-LOX1-catalyzed oxygenation of linoleic acid and arachidonic acid, respectively, induced MAO-A expression in a dose-dependent manner.

## MATERIAL AND METHODS

### Materials

The chemicals used were from the following sources: Dulbecco's modified Eagle's medium (DMEM), RPMI 1640, penicillin-streptomycin solution, geneticin (G418 sulphate), and L-glutamine from PAA Laboratories GmbH (Colbe, Germany); fetal bovine serum (FCS) from Biochrom AG (Berlin, Germany); recombinant human IL-4 and IL-13 from Strathmann Biotech AG (Hannover, Germany) or Promega (Mannheim, Germany); M-MLV reverse transcriptase and agarose from Promega (Mannheim, Germany); Pyra exo(-) DNA polymerase from Q BIO gene (Illkirch Cedex, France); dNTPs from Carl Roth GmbH (Karlsruhe, Germany); DNA molecular weight markers (100bp, 1kb) from New England Biolabs GmbH (Schwalbach, Germany); arachidonic acid, 12(S)-hydroxy-(5Z,8Z,10E,14Z)-eicosatetraenoic acid (12S-HETE), 15(S)-hydroxy-(5Z,8Z,11Z,13E)-eicosatetraenoic acid (15S-HETE), and soybean LOX-1 (grade 1) from

Sigma (Deisenhofen, Germany). PCR primers were custom-synthesized by BIOTEZ (Berlin, Germany).

### Cell culture

U937 (human promyelomonocytic) cells were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). Cells were maintained in RPMI-1640 or DMEM medium supplemented with 10% (v/v) fetal calf serum containing L-glutamine and antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin) at 37°C under 5% CO<sub>2</sub>.

### Reverse transcriptase polymerase chain reaction

Total RNA was isolated using the RNeasy mini kit (Qiagen, Hilden, Germany). 3 µg of total RNA was reverse-transcribed at 37°C for 170 min in 45 µl 28 mM Tris-HCl buffer, pH = 8.3, containing 1.7 mM MgCl<sub>2</sub>, 42 mM KCl, 5.5 mM dithiothreitol, 100 µg/ml BSA, 277 µM dNTP's, 33 ng/µl oligo d(T)<sub>18</sub> primer, and 200 U reverse transcriptase. To stop the reaction, the samples were heated to 94°C for 10 min. The RT-sample was diluted 1: 5 and 5 µl were used for amplification. RT-PCR products were separated by 2% agarose gel electrophoresis and the DNA bands were stained with ethidium bromide. The signal intensity was quantified densitometrically and normalized for expression of glyceraldehyde-3-phosphate dehydrogenase mRNA (GAPDH). The following primer sequences were used: human GAPDH (forward) – 5'-CCA TCA CCA TCT TCC AGG AGC GA-3', human GAPDH (reverse) – 5'-GGA TGA CCT TGC CCA CAG CCT TG-3', rabbit 15-LOX1 (forward) – 5'-ACT GAA ATC GGG CTG CAA GGG-3', rabbit 15-LOX1 (reverse) – 5'-GGG TGA TGG GGG CTG AAA TAA-3', human MAO-A (forward) - 5'-GCC AAG ATT CAC TTC AGA CCA GAG-3', human MAO-A (reverse) – human MAO-A (reverse) - 5'-TGC TCC TCA CAC CAG TTC TTC TC-3', human MAO-B (forward) – 5'-ACT CGT GTG CCT TTG GGT TCA G-3', human MAO-B (reverse) – 5'-TGC TCC TCA CAC CAG TTC TTC TC-3' (same as for MAO-A).

Real-time PCR was carried out on a DNA Engine Opticon® 2 (MJ Research, Inc, Biozym), using the QuantiTect SYBR Green PCR Kit from Qiagen, according to the vendor's protocol. The primer combinations specified above were used and the following PCR protocol was applied: 15 min hot start at 95°C, followed by 40 cycles of denaturation (30 s at 94°C), annealing (30 s at 60°C), and synthesis (30 s at 72°C, total volume of 20 µl). For melting curve analysis, the temperature was elevated slowly from 60°C to 95°C. Data were acquired and analyzed with the Opticon Monitor software (version 2).

### LOX activity assay

For the determination of 15-LOX1 activity, 5–10×10<sup>6</sup> cells were reconstituted in 0.35 ml of PBS containing 0.16 mM arachidonic acid. Following sonication, the lysate was incubated for 20 min at 25°C. The hydroperoxy lipids were reduced to the corresponding hydroxy compounds by addition of 0.1 ml saturated sodium borohydride solution (dry methanol). Following addition of 50 µl glacial acetic acid and 0.5 ml ice-cold methanol, samples were kept on ice for 10 min and protein precipitate was removed by centrifu-

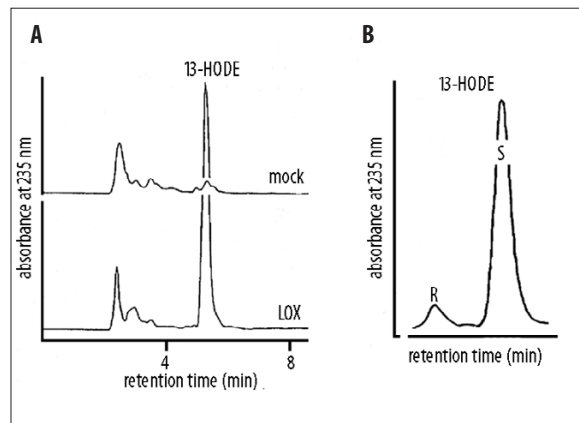
gation. Aliquots of the clear supernatant were analyzed by HPLC. RP-HPLC was performed with a Shimadzu LC-6A liquid chromatograph connected to a Hewlett Packard diode array detector 1040A. Separation of the fatty acid derivatives was performed on a Nucleosil C-18 column (Macherey-Nagel, Düren, Germany; 250×4 mm, 5-µm particle size) and a guard-column (30×4 mm, 5-mm particle size, same vendor). The solvent system was a mixture of methanol/water/acetic acid (80:20:0.2; by vol.) and a flow rate of 1 ml/min was used. The absorbance at 235 nm was recorded.

### Preparation of hydroperoxy fatty acids

13S-HpODE and 15S-HpETE were prepared from the corresponding polyenoic fatty acids (linoleic acid and arachidonic acid, respectively) using soybean LOX-1. Briefly, 1–5 mg of soybean LOX-1 (depending on the specific activity of the enzyme preparation) were dissolved in 50 ml of 0.1 M borate buffer, pH=9.0. Then 1 ml of a methanolic solution of free fatty acid (50 mg/ml) was added drop by drop over a time period of about 5 min with permanent stirring of the incubation mixture. The sample was allowed to incubate for an additional 20 min, the reaction mixture was acidified to pH=3, and the lipids were twice extracted with 50 ml of diethyl ether. The organic extracts were combined, dried over sodium sulfate, and the solvent was evaporated under vacuum. The remaining lipids were reconstituted in 1 ml of methanol and aliquots were injected for RP-HPLC purification using a Shimadzu LC-6A liquid chromatograph connected to a Hewlett Packard diode array detector 1040A. Purification of the oxygenated fatty acid was performed on a Nucleosil C-18 column (Macherey-Nagel, Düren, Germany; 250×4 mm, 5-µm particle size). The solvent system was methanol/water/acetic acid (80:20:0.1; by vol) and a flow rate of 1 ml/min was used. The absorbance at 235 nm was recorded. Since RP-HPLC did not separate all hydroperoxy fatty acid isomers with sufficient effectiveness, additional SP-HPLC was carried out. For this purpose a Nucleosil 100-7 column (Macherey-Nagel, Düren, Germany; 250×4 mm, 5-µm particle size), a solvent system of hexane/2-propanol/acetic acid (99:1:0.1, by vol.), and a flow rate of 1ml/min were used. Chemical identity of the prepared hydroperoxy fatty acids was checked by analytic SP-HPLC, UV-spectroscopy, and gas chromatography/mass spectrometry.

### RESULTS

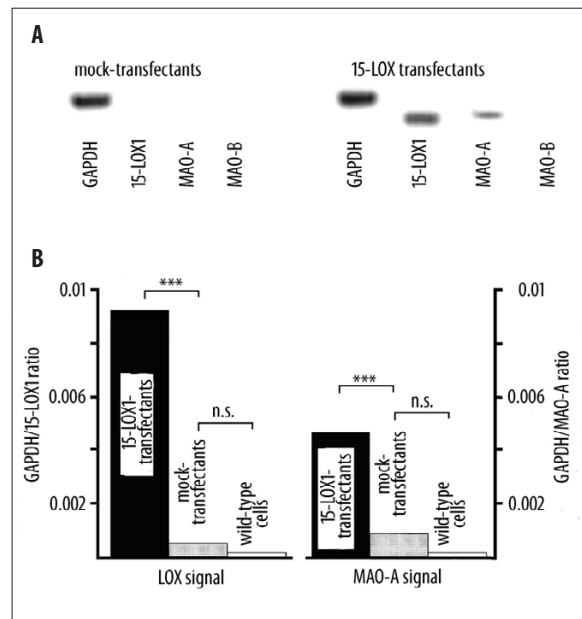
Expression of MAO-A in 15-LOX1-transfected U937 cells: It has recently been reported that treatment of human peripheral monocytes with IL-4 strongly induces upregulation of the expression of MAO-A [43]. A similar upregulation was observed in U937 cells (human monocytic cell line) which were transfected with the rabbit 15-LOX1. Thus, 15-LOX1 appeared to be involved in the signal transduction cascade leading to the upregulation of MAO-A expression [43]. To investigate the mechanism of this regulatory process in more detail, we first assayed the LOX activity of 15-LOX1-transfected U937 cells using linoleic acid as exogenous substrate. For this purpose, 15-LOX1-transfected U937 cells and the corresponding mock transfectants were incubated with linoleic acid (100 µM final concentration) for 15 min at room temperature and the LOX products formed were analyzed by RP-HPLC. From Figure 1A it can



**Figure 1.** Conversion of linoleic acid by 15-LOX1- transfected and mock-transfected U937 cells. Cells were cultured to confluence in culture flasks as described in Materials and Methods. They were harvested by scraping, washed, and reconstituted to a final concentration of about  $10^7$  cells/ml in plain RPMI medium. Then linoleic acid was added as the methanolic stock solution to reach a final concentration of 100  $\mu$ M and activity assays were carried out as described in the Methods section. Formation of 13S-H(p)ODE was quantified by RP-HPLC (panel A) and its enantiomer composition was assayed by chiral phase HPLC on a Chiralcel OD column (panel B) using hexane/2-propanol/acetic acid (100:5:0.1, by vol.) as solvent system and a flow rate of 1 ml/min. This experiment was carried out in triplicate for both 15-LOX1-transfected and mock-transfected cells and almost identical results were obtained. A representative set of chromatograms is shown.

be seen that the 15-LOX1-transfected cells converted exogenous linoleic acid to 13-hydro(pero)xy-9Z,11E-octadecadienoic acid [13-H(p)ODE] and chiral phase HPLC indicated a strong preponderance of the S-enantiomer (Figure 1B). Such a specific product pattern is indicative of an intrinsic LOX activity. The chemical structure of the major product [13S-H(p)ODE] was confirmed by co-chromatography with authentic standards in SP-HPLC, by UV-spectroscopy, and by GC/MS analysis of the hydrogenated derivatives of the silylated methyl esters (data not shown). Quantification of the product formation indicated that the 15-LOX1-transfected cells express the enzyme at a moderate level, which is in the range of murine peritoneal macrophages [44]. Using the corresponding mock transfectants we did not observe the formation of specific LOX products (Figure 1A, upper trace), indicating the absence of 15-LOX1.

To confirm the absence of 15-LOX1 in wild-type and mock-transfected U937 cells and to test the expression of MAO-A and MAO-B in the 15-LOX1-transfected cells we carried out RT-PCR (Figure 2). Initially, semi-quantitative RT-PCR was performed. In these experiments no PCR signals were observed in wild-type cells and mock transfectants, but clear PCR bands were found in the cells transfected with the rabbit 15-LOX1. In contrast, we did not detect MAO-B signals in neither 15-LOX1-transfected cells nor in the corresponding mock transfectants (Figure 2A). Sequence analysis of the PCR fragments confirmed the identity of the bands. For more quantitative data we carried out real-time PCR, and the results are shown in Figure 2B. Here again we observed strong upregulation



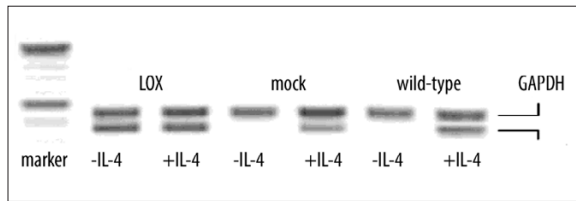
**Figure 2.** Expression analysis of 15-LOX1, MAO-A, and MAO-B in different types of U937 cells. Wild-type U937 cells, 15-LOX1-transfectants, and the corresponding mock controls were cultured to confluence, washed, and total RNA was extracted. Semi-quantitative RT-PCR (panel A) and real-time PCR (panel B) were carried out with the crude RNA preparations as described in Materials and Methods. These experiments were carried out in quadruplicate using four different batches of 15-LOX1-transfected and mock-transfected U937 cells. n.s. not significant, \*\*\*  $p < 0.001$  (Students t-test).

of 15-LOX1 and MAO-A expression in the LOX-transfected cells, whereas no expression of MAO-B was detected.

IL-4-treatment of wild-type U937 cells did also upregulate MAO-A expression: Treatment of human peripheral monocytes with IL-4/IL-13 strongly induced MAO-A expression [43]. Our transfection studies indicated that expression of 15-LOX1 in U937 cells also leads to upregulation of MAO-A, but this effect was independent of IL-4 stimulation. It was reported previously that IL-4 treatment of U937 cells does not augment 15-LOX1 expression [45,46], and thus it was of interest whether or not U937 cells may respond to IL-4 stimulation with increased expression of MAO-A. Cultured macrophages cells express functional IL-4/IL-13 receptors and many constituents of the IL-4/IL-13 signaling cascade [45,46]. For this study we cultured wild-type U937 cells, 15-LOX1 transfectants, and the corresponding mock controls for 3 days in the presence of IL-4 and tested the expression of MAO-A by semi-quantitative RT-PCR. From Figure 3 it can be seen that wild-type cells and mock transfectants did not express MAO-A if the cells were maintained in culture for three days in the absence of IL-4. In contrast, when cultured in the presence of IL-4, MAO-A mRNA was detected. In the 15-LOX1-transfected cells we observed MAO-A expression regardless of whether the cells were maintained for 3 days in the absence or presence of IL-4.

MAO-A expression in U937 cells is upregulated by 15-LOX products: Our transfection studies suggested that the enzy-





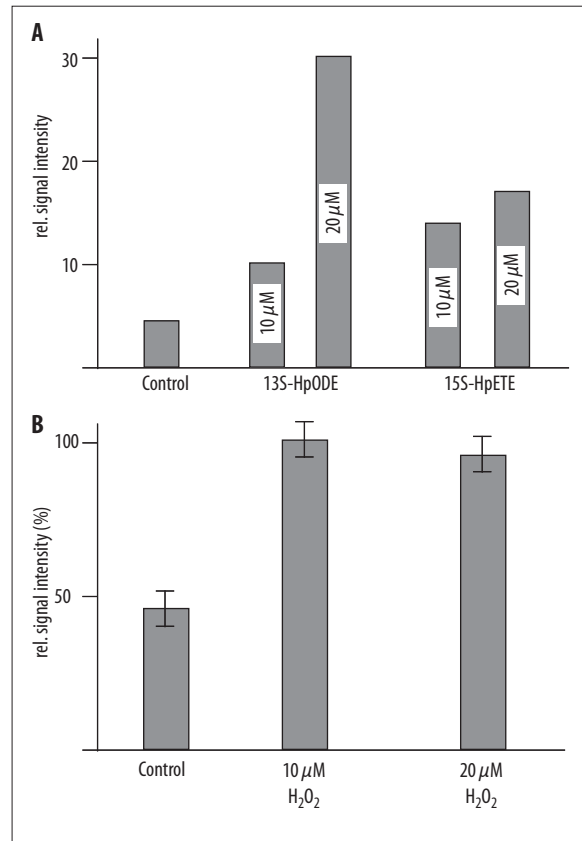
**Figure 3.** Expression of MAO-A in different U937 cells cultured in the absence or presence of IL-4. Different U937 cells were cultured to near confluence in RPMI medium containing 10% FCS and antibiotics. Then IL-4 at a final concentration of 10 ng/ml was added and the cells were kept in culture for additional 3 days. Cells were washed twice, total RNA was extracted, and aliquots were used for semi-quantitative RT-PCR. The experiment was carried out in triplicate and a representative set of data is shown. Densitometric quantification of the band intensities and statistic evaluation of the quantitative data were not performed.

matic activity of 15-LOX might be involved in the MAO-A expression cascade. Since LOX have been implicated in the biosynthesis of lipid mediators, we hypothesized that products of the LOX pathway might mimic the upregulation of MAO-A expression. To test this hypothesis we incubated wild-type U937 cells with the stable end products of the 15-LOX pathway (the hydroxy fatty acids 15S-HETE and 13S-HODE) and checked the expression of MAO-A by semi-quantitative RT-PCR. Unfortunately, we did not observe induction of the enzyme (data not shown). Next, the primary lipoygenase products, the hydroperoxy fatty acids (15S-HpETE and 13S-HpODE), were tested. Here we detected a dose-dependent increase in MAO-A mRNA expression when the PCR signals were related to  $\beta$ -actin (Figure 4A) or GAPDH (data not shown). It should be stressed that the extent of MAO-A upregulation was not as dramatic as in IL-4-treated monocytes or in 15-LOX1-transfected U937 cells. However, it must be kept in mind that in cellular systems, pulses of hydroperoxy fatty acids are rapidly reduced to the corresponding hydroxy derivative and thus the effects observed in our bolus experiments were expected to be much smaller than those observed under continuous formation of endogenous hydroperoxy fatty acids.

Intracellular activity of 15-LOX induces an oxidizing environment since lipid peroxides are formed endogenously [22] and similar effects are introduced when the cells are incubated with exogenous hydroperoxy fatty acids. To test whether oxidative stress may lead to upregulation of MAO-A we incubated U937 cells in the presence of hydrogen peroxide and measured the expression of the MAO-A mRNA by semi-quantitative RT-PCR. As indicated in Figure 4B, we observed an increased MAO-A expression in oxidatively stressed cells. In contrast, no major induction of 15-LOX or MAO-B was observed (not shown).

## DISCUSSION

Recently we reported that the Th2 cytokines IL-4 and IL-13 strongly induced the expression of MAO-A in human peripheral monocytes and in A549 lung carcinoma cells, which normally do not express the enzyme, in tandem with 15-LOX1 [43]. A similar upregulation of MAO-A expression was also observed in the human promyelomonocytic cell line, U937,



**Figure 4.** Impact of hydroperoxides on MAO-A expression. Wild-type U937 cells were treated for 24 hours with different concentrations of hydroperoxides. After washing, total RNA was extracted and the expression of MAO-A mRNA was quantified by semi-quantitative RT-PCR using  $\beta$ -actin as internal standard. PCR conditions are described in Material and Methods. The relative intensities of the RT-PCR signals of MAO-A related to  $\beta$ -actin are plotted. Panel A: 13S-HpODE and 15S-HpETE (primary LOX-products) were used as stimuli. Panel B:  $H_2O_2$  was used as inducer of oxidative stress ( $n=3$ ,  $p<0.01$  between samples and controls).

when transfected with 15-LOX1 [43]. Here we investigated the regulatory mechanism of MAO-A expression in more detail and identified two alternative pathways which may equally contribute to the regulatory effect observed in human monocytes.

i) A 15-LOX1-dependent pathway, which is mediated by 15-LOX1 products (hydroperoxy fatty acids): When wild-type and mock-transfected U937 cells were incubated with primary 15-LOX1 products (15S-HpETE, 13S-HpODE) we observed an increase in the steady-state concentration of MAO-A mRNA (Figure 4A). These data suggest that formation of endogenous lipid hydroperoxides by the transfected 15-LOX1 is one major reason for upregulation of the expression of the MAO-A gene in 15-LOX1-transfected U937 cells. This pathway is clearly IL-4 independent, but the detailed mechanisms involved remain to be investigated. It is an open question as to how endogenous peroxides can actually activate the expression of the MAO-A gene on the molecular level. A search of the putative MAO-A promoter did not reveal clear-cut evidence for the exist-

ence of redox-sensitive *cis*-regulatory elements, but work is in progress in our laboratory to shed light on this important question.

- ii) An IL-4-dependent pathway. When wild-type and mock-transfected U937 cells were cultured for three days in the presence of IL-4 we observed the expression of MAO-A mRNA (Figure 3). In contrast, no PCR signal was seen when the cells were cultured for the same time period in the absence of IL-4. U937 cells are unable to respond to IL-4 stimulation with upregulation of 15-LOX1 expression [45,46], and we confirmed this finding in the present study (data not shown). DNA methylation studies indicated that hyper- and hypomethylation of CpG islands in the 15-LOX1 promoter region is intimately associated with transcriptional repression or activation of the 15-LOX1 gene, respectively. [46]. The 15-LOX1 promoter was exclusively methylated in cells incapable of expressing 15-LOX1 in response to IL-4 stimulation (including U937 cells). In contrast, cells containing an unmethylated promoter, such as peripheral monocytes or A549 cells, respond to IL-4 application with expression of 15-LOX1. Our finding that IL-4-treated U937 cells expressed MAO-A at significant levels although 15-LOX1 was absent suggested the existence of an IL-4-dependent, but 15-LOX1-independent, induction mechanism. In the light of these findings it may be concluded that in human peripheral monocytes, which respond to IL-4 stimulation with an increase in 15-LOX expression, upregulation of MAO-A may follow a dual mechanism, with a 15-LOX1-dependent and a 15-LOX1-independent share.

The biological importance of IL-4-induced upregulation of 15-LOX1 and MAO-A expression for monocyte physiology remains to be investigated. We recently profiled the gene expression pattern of IL-4-treated human peripheral monocytes using the microarray technology and found upregulation of a variety of classical anti-inflammatory gene products and concomitant down-regulation of pro-inflammatory proteins [8]. Thus, IL-4 may switch naive monocytes into cells with dominant anti-inflammatory phenotype, which play a major role in inflammatory resolution. It has been suggested before that 15-LOXs may be involved in the biosynthesis of lipoxins [47,48], which constitute potent anti-inflammatory mediators which are synthesized during the resolution of acute inflammation. The expression profile of various LOX isoforms during the time course of inflammation indicated that polymorphonuclear leukocytes turn on the expression of functional 15-LOX1 when developing from pro-inflammatory to resolving cells [49]. A similar phenotype switch may be induced by IL-4 in human monocytes.

The role of MAO-A in a putative anti-inflammatory response remains to be investigated. Biogenic amines, such as histamine and serotonin, are well-known inflammatory mediators [50–52]. They have also been implicated in neuroimmune interactions and modulation of blood-brain barrier permeability [53]. Since MAO-A preferentially oxidizes serotonin, the enzyme might be involved in the removal of this pro-inflammatory mediator from the site of inflammation. In this respect, IL-4/IL-13-induced upregulation of MAO-A might contribute to switching monocytes from a pro- to an anti-inflammatory phenotype.

## CONCLUSIONS

Resting monocytic cells (peripheral human monocytes, U937 cells) express neither MAO-A nor MAO-B. However, transfection of U937 cells with 15-LOX1, incubation with the 15-LOX1 products 13S-HpODE and 15S-HpETE, or culturing the cells in the presence of the Th2 cytokine IL-4 strongly induced MAO-A expression. In contrast, MAO-B expression remained unaffected. These data suggest that the previously reported IL-4-induced upregulation of MAO-A expression in human peripheral monocytes may proceed via 15-LOX1-dependent and 15-LOX1-independent pathways. Upregulation of MAO-A expression may contribute in switching naive monocytes into a resolving phenotype.

## Acknowledgement

This work contains experimental data that are part of the Ph.D. thesis of Pavlos Chaitidis, which will be submitted to the Faculty of Natural Sciences of the Free University of Berlin.

## REFERENCES:

1. Arai KI, Lee F, Miyajima A et al: Cytokines: coordinators of immune and inflammatory responses. *Annu Rev Biochem*, 1990; 59: 783–36
2. Ward PA, Lentsch AB: The acute inflammatory response and its regulation. *Arch Surg*, 1999; 134: 666–69
3. Ward PA, Lentsch AB: Endogenous regulation of the acute inflammatory response. *Mol Cell Biochem*, 2002; 234–35: 225–28
4. Opal M, DePalo VA: Anti-inflammatory cytokines. *Chest*, 2000; 117: 1162–72
5. Cavaillon JM: Pro- versus anti-inflammatory cytokines: myth or reality. *Cell Mol Biol*, 2001; 47: 695–702
6. de Waal-Malefyt R, Figdor CG, Huijbens R et al: Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes. Comparison with IL-4 and modulation by IFN-gamma or IL-10. *J Immunol*, 1993; 151: 6370–81
7. Conrad DJ, Kühn H, Mulkins M et al: Specific inflammatory cytokines regulate the expression of human monocyte 15-lipoxygenase. *Proc Natl Acad Sci USA*, 1992; 89: 217–21
8. Chaitidis P, O'Donnell VB, Kuban RJ et al: Gene expression alterations of human peripheral monocytes induced by medium-term treatment with the TH2 cytokines interleukin-4 and -13. *Cytokine*, 2004; (submitted)
9. Brash AR: Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. *J Biol Chem*, 1999; 274: 23679–82
10. Funk CD: Prostaglandins and leukotrienes: Advances in eicosanoid biology. *Science*, 2001; 294: 1871–75
11. Rapoport SM, Schewe T, Thiele BJ: Maturation breakdown of mitochondria and other organelles in reticulocytes. *Blood Cell Biochemistry*, 1990; 1: 151–94
12. van Leyen K, Duvoisin RM, Engelhardt H, Wiedmann M: A function for lipoxygenase in programmed organelle degradation. *Nature*, 1998; 395: 392–95
13. Mehrabian M, Allayee H, Wong J et al: Identification of 5-lipoxygenase as a major gene contributing to atherosclerosis susceptibility in mice. *Circ Res*, 2002; 91: 120–26
14. George J, Afek A, Shaish A et al: 12/15-Lipoxygenase gene disruption attenuates atherogenesis in LDL receptor-deficient mice. *Circulation*, 2001; 104: 1646–50
15. Klein RF, Allard J, Avnur Z et al: Regulation of bone mass in mice by the lipoxygenase gene *Alox15*. *Science*, 2004; 303: 229–32
16. Brinckmann R, Topp MS, Zalan I et al: Regulation of 15-lipoxygenase expression in lung epithelial cells by interleukin-4. *Biochem J*, 1996; 318: 305–12
17. Roy B, Cathcart MK: Induction of 15-lipoxygenase expression by IL-13 requires tyrosine phosphorylation of Jak2 and Tyk2 in human monocytes. *J Biol Chem*, 1998; 273: 32023–29

18. Roy B, Bhattacharjee A, Xu HM et al: IL-13 signal transduction in human monocytes: phosphorylation of receptor components, association with Jaks, and phosphorylation/activation of Stats. *J Leukoc Biol*, 2002; 72: 580–89
19. Heydeck D, Thomas H, Schnurr K et al: Interleukin-4 and -13 induced upregulation of the murine macrophage 12/15-lipoxygenase activity: Evidence for the involvement of transcription factor STAT6. *Blood*, 1998; 92: 2503–10
20. Xu B, Bhattacharjee A, Roy B et al: Interleukin-13 induction of 15-lipoxygenase gene expression requires p38 mitogen-activated protein kinase-mediated serine 727 phosphorylation of Stat1 and Stat3. *Mol Cell Biol*, 2003; 23: 3918–28
21. Shankaranarayanan P, Chaitidis P, Kühn H, Nigam S: Acetylation by histone acetyltransferase CBP/p300 of STAT6 is required for transcriptional activation of the 15-lipoxygenase-1 gene. *J Biol Chem*, 2001; 276: 42753–60
22. Schnurr K, Borchert A, Kühn H: Inverse regulation of lipid peroxidizing and hydroperoxy lipids reducing enzymes by interleukins-4 and -13. *FASEB J*, 1999; 13: 143–54
23. Singer TP, Ramsay RR: Flavoprotein structure and mechanism 2. Monoamine oxidases: old friends hold many surprises. *FASEB J*, 1995; 9: 605–10
24. Wouters J: Structural aspects of monoamine oxidase and its reversible inhibition. *Curr Med Chem*, 1998; 5: 137–62
25. Moskvitina TA, Medvedev AE: Different sensitivity of mitochondrial and cytosolic monoamine oxidases to *in vivo* but not *in vitro* inhibition by specific irreversible inhibitors. *Med Sci Monit*, 2001; 7(1): 17–19
26. Denney RM, Fritz RR, Patel NT, Abell CW: Human liver MAO-A and MAO-B separated by immunaffinity chromatography with MAO-B-specific monoclonal antibody. *Science*, 1982; 215: 1400–3
27. Da Prada M, Kettler R, Keller HH et al: From moclobemide to Ro 19-6327 and Ro 41-1049: the development of a new class of reversible, selective MAO-A and MAO-B inhibitors. *J Neural Transm Suppl*, 1990; 29: 279–92
28. Sanz E, Romera M, Bellik L et al: Indolalkylamines derivatives as anti-oxidant and neuroprotective agents in an experimental model of Parkinson's disease. *Med Sci Monit*, 2004; 10(12): BR477–BR484
29. Tsugeno Y, Ito A: A key amino acid responsible for substrate selectivity of monoamine oxidase A and B. *J Biol Chem*, 1997; 272: 14033–36
30. Geha RM, Rebrin I, Chen K, Shih, JC: Substrate and inhibitor specificities for human monoamine oxidase A and B are influenced by a single amino acid. *J Biol Chem*, 2001; 276: 9877–82
31. Grimsby J, Lan NC, Neve R, Chen K, Shih JC: Tissue distribution of human monoamine oxidase A and B mRNA. *J Neurochem*, 1990; 55: 1166–69
32. Sivasubramaniam SD, Finch CC, Rodriguez MJ et al: A comparative study of the expression of monoamine oxidase-A and -B mRNA and protein in non-CNS human tissues. *Cell Tissue Res*, 2003; 31: 291
33. Vitalis T, Fouquet C, Alvarez C et al: Developmental expression of monoamine oxidases A and B in the central and peripheral nervous systems of the mouse. *J Comp Neurol*, 2002; 442: 331–47
34. Bompert G, Copin N, Djouadi F et al: Monoamine oxidase in developing rat renal cortex: effect of dexamethasone treatment. *Eur J Pharmacol*, 2001; 415: 19–26
35. Auda GR, Kirk SH, Billett MA, Billett EE: Localization of monoamine oxidase mRNA in human placenta. *J Histochem Cytochem*, 1998; 46: 1393–400
36. Sivasubramaniam SD, Finch CC, Billett MA et al: Monoamine oxidase expression and activity in human placenta from pre-eclamptic and normotensive pregnancies. *Placenta*, 2002; 23: 163–71
37. Bach AW, Lan NC, Johnson DL et al: cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. *Proc Natl Acad Sci USA*, 1988; 85: 4934–38
38. Chen ZY, Powell JF, Hsu YP et al: Organization of the human monoamine oxidase genes and long-range physical mapping around them. *Genomics*, 1992; 14: 75–82
39. Grimsby J, Chen K, Wang LJ et al: Human monoamine oxidase A and B genes exhibit identical exon-intron organization. *Proc Natl Acad Sci USA*, 1991; 88: 3637–41
40. Shih JC, Zhu QS, Grimsby J, Chen K: 1994. Identification of human monoamine oxidase (MAO) A and B gene promoters. *J Neural Transm Suppl*, 1991; 41: 27–33
41. Zhu QS, Chen K, Shih JC: Bidirectional promoter of human monoamine oxidase A (MAO A) controlled by transcription factor Sp1. *J Neurosci*, 1994; 14: 7393–403
42. Wong WK, Ou XM, Chen K, Shih JC: Activation of human monoamine oxidase B gene expression by a protein kinase C MAPK signal transduction pathway involves c-Jun and Egr-1. *J Biol Chem*, 2002; 277: 22222–30
43. Chaitidis P, Billett EE, O'Donnell VB et al: Th2 response of human peripheral monocytes involves isoform-specific induction of monoamine oxidase-A. *J Immunol*, 2004; 173: 4821–27
44. Sun D, Funk CD: Disruption of 12/15-lipoxygenase expression in peritoneal macrophages. *J Biol Chem*, 1996; 271: 24055–62
45. Brinckmann R, Topp MS, Zalan I et al: Regulation of 15-lipoxygenase expression in lung epithelial cells by interleukin-4. *Biochem J*, 1996; 318: 305–12
46. Liu C, Xu D, Sjöberg J et al: Transcriptional regulation of 15-lipoxygenase expression by promoter methylation. *Exp Cell Res*, 2004; 297: 61–67
47. Serhan CN, Takano T, Gronert K et al: Clish: Lipoxin and aspirin-triggered 15-epi-lipoxin cellular interactions anti-inflammatory lipid mediators. *Clin Chem Lab Med*, 1999; 37: 299–309
48. Goh J, Godson C, Brady HR, MacMathuna P: Lipoxins: pro-resolution lipid mediators in intestinal inflammation. *Gastroenterology*, 2003; 124: 1043–54
49. Levy BD, Clish CB, Schmidt B et al: Lipid mediator class switching during acute inflammation: signals in resolution. *Nature Immunol*, 2001; 2: 612–19
50. Pichler R, Maschek W, Krieglsteiner S et al: Pro-inflammatory role of serotonin and interleukin-6 in arthritis and spondyloarthropathies-measurement of disease activity by bone scan and effect of steroids. *Scand J Rheumatol*, 2002; 31: 41–43
51. Mossner R, Lesch KP: Role of serotonin in the immune system and in neuroimmune interactions. *Brain Behav Immun*, 1998; 12: 249–71
52. Freire-Garabal M, Nunez MJ, Balboa J et al: Serotonin upregulates the activity of phagocytosis through 5-HT1A receptors. *Br J Pharmacol*, 2003; 139: 457–63
53. Abbott NJ: Inflammatory mediators and modulation of blood-brain barrier permeability. *Cell. Mol Neurobiol*, 2000; 20: 131–47