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Th2 Response of Human Peripheral Monocytes Involves Isoform-Specific Induction of Monoamine Oxidase-A1,2

Pavlos Chaitidis,* Ellen E. Billett,† Valerie B. O’Donnell,‡ Alexandra Bermudez Fajardo,‡ Julia Fitzgerald,† Ralf J. Kuban,* Ute Ungetheuem,§ and Hartmut Kühn*∗

Monocyte/macrophage function is critically regulated by specific cytokines and growth factors that they are exposed to at inflammatory sites. IL-4 and IL-13 are multifunctional cytokines generated mainly by Th2 lymphocytes that have important biological activities in allergy and inflammation. The Th2 response of human peripheral monocytes is characterized by complex alterations in the gene expression pattern, which involves dominant expression of CD23 cell surface Ag and lipid-peroxidizing 15-lipoxygenase-1 (15-LOX1). In this study, we report that the classic Th2 cytokines IL-4 and IL-13 strongly up-regulate expression of monoamine oxidase A (MAO-A) with no induction of the closely related isozyme, MAO-B. Real-time PCR indicated a >2000-fold up-regulation of the MAO-A transcripts, and immunohistochemistry revealed coexpression of the enzyme with 15-LOX1 in a major subpopulation of monocytes. MAO-A was also induced in lung carcinoma A549 cells by IL-4 in parallel with 15-LOX1. In promyelomonocytic U937 cells, which neither express 15-LOX1 nor MAO-A in response to IL-4 stimulation, expression of MAO-A was up-regulated following transfection with 15-LOX1. This is the first report indicating expression of MAO-A in human monocytes. Its isoform-specific up-regulation in response to Th2 cytokines suggests involvement of the enzyme in modulation of innate and/or acquired immune system. The Journal of Immunology, 2004, 173: 4821–4827.

Monocytes and macrophages play important roles in both innate and acquired immune functions (1). They respond to nonspecific stimuli, including bacterial LPS and complement factors, but can also interact with the acquired immune response, including Ab-dependent opsonization and Ag presentation. The function of monocytes is profoundly influenced by cytokines, in particular those derived from activated Th cells (2). These mediators have been classified Th1 or Th2 cytokines and IL-4 and IL-13 (IL-4/13) constitute classical Th2 signal transducers (3, 4). Th1 cytokines activate the microbicidal properties of monocyte/macrophages and induce B cells to express IgG, which is effective for opsonizing extracellular pathogens for phagocytosis. In contrast, Th2 cells initiate the humoral immune response by activating naive Ag-specific B cells to produce IgM or IgE (2). In allergic diseases, such as asthma (3) and rhinitis (4), Th2 cytokines are a major cause of leukocyte activation. Similar to lymphocytes, which are categorized into Th1 or Th2 cells, macrophages may exhibit M-1 or M-2 phenotypes (2, 5). Typically, M-2 macrophages are generated in response to IL-4 and show decreased LPS- and IFN-γ-stimulated production of IL-6, IL-8, IL-12, and impaired expression of CD14, FcγRI, FcγRII, and FcγRIII (5–9). In contrast, IL-4 up-regulates expression of scavenger receptors and cell surface Ags and increases their phagocytic potential (5–9).

Lipoxygenases (LOX)4 form a family of pro-oxidative enzymes that oxygenate free and esterified polyenoic fatty acids to the corresponding hydroperoxy derivatives (10). They are cytosolic proteins and have been implicated in the biosynthesis of proinflammatory leukotrienes (11) and anti-inflammatory lipoxins (12), both of which constitute important mediators of the immune response. Up-regulation of 15-LOX1 expression by classical Th2 cytokines has been reported for peripheral human monocytes (13, 14) and human alveolar macrophages (15). Similar effects were also observed during in vitro differentiation of monocytes to dendritic cells (16). 15-LOX1 induction involves functional IL-4/13 cell surface receptors, various protein kinases, and members of the STAT transcription factor family (17–20). Under resting conditions, the STAT-responsive cis-regulatory elements in the 15-LOX1 promoter appear to be blocked by nuclear histones (21). IL-4 activates nuclear acetyltransferases, and histone acetylation may lead to conformational alterations in the nucleosome structure, rendering the STAT-responsive cis-regulatory elements accessible for the trans-acting proteins (21). The detailed functions of 15-LOX1 in immune regulation is not well understood, but the enzyme has been implicated in regulation of actin polymerization and phagocytosis (22), as well as in activation of the peroxisomal proliferator-activating receptor-y, which controls expression of mannose and scavenger receptors in monocytic cells (23, 24).

In other cells, 15-LOX1 has been implicated in maturational degradation of mitochondria during cellular differentiation (25, 26). In vitro incubation of isolated rat liver mitochondria with purified 15-LOX1 induces severe structural membrane alterations. In parallel, inactivation of respiratory enzymes was observed (27).

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1 Abbreviations used in this paper: LOX, lipoxygenase; MAO, monoamine oxidase; 15S-HETE, 15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid.
More recently, we reported functional decline of monoamine oxidase (MAO) activities during in vitro oxygenation of mitochondrial membranes by 15-LOX1 (28). To test whether or not intra-cellular activity of 15-LOX1 may also induce functional inactivation of MAO isoforms, we transfected monocyctic U937 cells with 15-LOX1. Surprisingly, we measured a strong and isoform-specific up-regulation of MAO-A expression. A similar effect was observed when human peripheral monocytes were cultured in the presence of IL-4 or IL-13. Our data indicate for the first time expression of MAO-A in human peripheral monocytes and suggest a specific role of the enzyme in the Th2 response of these cells.

Materials and Methods

Materials

The chemicals used were from the following sources: DMEM, RPMI 1640, penicillin-streptomycin solution, geneticin (G418 sulfate) and L-glutamine from PAA Laboratories (Colbe, Germany); FCS from Biochrom (Berlin, Germany); recombinant human IL-4 and IL-13 from Strathmann Biotech (Hanover, Germany) or Promega (Mannheim, Germany); Moloney murine leukemia virus reverse transcriptase and agarose from Promega; Pyratex (−) DNA polymerase from Qiagen (Hilden, Germany); dNTPs from Carl Roth (Karlsruhe, Germany); DNA m.w. markers (100 bp, 1 kb) from New England Biolabs (Schwalbach, Germany); FuGENE 6 Transfection Reagent from Roche Diagnostics (Mannheim, Germany); recombinant human IL-4 and IL-13 from Strathmann Biotech (Hanover, Germany) or Promega (Mannheim, Germany); arachidonic acid, New England Biolabs (Schwalbach, Germany); FuGENE 6 Transfection Reagent from Roche Diagnostics (Mannheim, Germany), using the QuantiTect SYBR Green PCR kit from Qiagen (Hilden, Germany), using the QuantiTect SYBR Green PCR kit (Qiagen, Hilden, Germany). Total RNA (3 μg) was reverse-transcribed at 37°C for 170 min in 45 μl of 28 mM Tris-HCl buffer (pH 8.3) containing 1.7 mM MgCl2, 42 mM KCl, 5.5 mM DTT, 100 μg/ml BSA, 277 μM dNTPs, 33 ng/μl oligo(dT)18 primer, and 200 U reverse transcriptase. To stop the reaction samples were heated to 94°C for 10 min. The reverse transcriptase 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 GAPDH.

Preparation of human peripheral monocytes and cell culture

Human peripheral monocytes were isolated from buffy-coats by density gradient centrifugation and adherence (13). Cells were cultured for 3 days in the presence or absence of 10 ng/ml IL-4 or IL-13 in RPMI 1640 medium containing 10% (v/v) FCS, L-glutamine, and antibiotics. Cells were harvested by gentle scraping. Cell viability (usually >95%) was determined by trypan blue exclusion. U937 (human promyelocytic monocyte) and HL60 (human myeloblastic) cells were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and A549 lung carcinoma cells were from American Type Culture Collection (Rockville, VA). Cells were maintained in RPMI 1640 or DMEM supplemented with 10% (v/v) FCS containing L-glutamine and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) at 37°C under 5% CO2.

Cell transfection

The eukaryotic expression vector pcDNA 3 (Invitrogen Life Technologies, Leek, The Netherlands) bearing the complete coding region of the rabbit reticulocyte type 15-LOX1 and a corresponding empty vector (mock) were transfected into U937 cells using FuGENE 6, according to the manufacturer’s instructions. Briefly, 2.5 × 105 cells/ml were plated on 35-mm plates and transfected with 2 μg of plasmid using 6 μl of FuGENE 6 reagent. After 72-h exposure, the precipitate was removed and the cells were cultured for 4 wk in selection medium supplemented with 0.5 mg/ml geneticin.

Table 1. Primer pairs used for RT-PCR of the different gene products

<table>
<thead>
<tr>
<th>Gene Product</th>
<th>Primer Sequence</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAPDH (forward)</td>
<td>5’-CCA TCA CCA TCT TCC AGG AGC GA-3’</td>
<td>447</td>
</tr>
<tr>
<td>GAPDH (reverse)</td>
<td>5’-GGG GAA ATC GGG CAT CAG AGC TG-3’</td>
<td>521</td>
</tr>
<tr>
<td>15-LOX1 (forward)</td>
<td>5’-GAG GGA ATC GGG CAT CAG AGC TG-3’</td>
<td>368</td>
</tr>
<tr>
<td>15-LOX1 (reverse)</td>
<td>5’-GGG GAA ATC GGG CAT CAG AGC TG-3’</td>
<td>549</td>
</tr>
<tr>
<td>MAO-A (forward)</td>
<td>5’-GCC GAC ATT CAC TTC AGA AGC CAG GAG-3’</td>
<td>311</td>
</tr>
<tr>
<td>MAO-A (reverse)</td>
<td>5’-TGG TCC TCA CAG CAG TCC TTC GC-3’</td>
<td>330</td>
</tr>
<tr>
<td>MAO-B (forward)</td>
<td>5’-AGA TGG CCA CAC TGT TCG GAA-3’</td>
<td>447</td>
</tr>
<tr>
<td>MAO-B (reverse)</td>
<td>5’-ACT GGT GTC CTT TGG GTC GCA GAT-3’</td>
<td>521</td>
</tr>
<tr>
<td>ALOX3 (forward)</td>
<td>5’-CTA CAT GAT CAT ATG GCC AGG GAC TT-3’</td>
<td>447</td>
</tr>
<tr>
<td>ALOX3 (reverse)</td>
<td>5’-TCC GCA GCG GTA GCA TTT GC-3’</td>
<td>447</td>
</tr>
</tbody>
</table>

* The primer sequences and the size of the PCR products are given.
* Same as for MAO-A.

Enzyme activity assays

For determination of 15-LOX1 activity 5–10 × 106 cells were resuspended 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 of saturated sodium borohydride solution (dry methanol). Following addition of 50 μl of glacial acetic acid and 0.5 ml of ice-cold methanol, samples were kept on ice for 10 min and protein precipitate was removed by centrifugation. Aliquots of the clear supernatant were analyzed by HPLC.

For MAO-A activity measurements (29), cells were resuspended in 20 mM potassium phosphate buffer (pH 7.4) to yield a final protein concentration of ~0.4–1.0 mg/ml. Aliquots (50 μl) were diluted with the same buffer to a final volume of 180 μl and preincubated at 37°C for 5 min. Then the reaction was started by addition of 20 μl (0.01 μCi) of 0.5 mM [3H]-tyramine solution (specific activity of 1 mCi/mmole) and the samples were incubated for 60 min at 37°C. The reaction was terminated by adding 200 μl of 0.5 M HCl, and the radiolabeled reaction products were extracted with 3 ml of 1.0% (v/v) diphenylxazole in toluene/ethylacetate (1/1; by vol.). Radioactivity was quantified by liquid scintillation counting and the MAO-A activities are expressed as radioactivity (cpm) per milligram of cellular protein.
Immunohistochemistry

Human peripheral monocytes were cultured at 37°C in the presence or absence of 10 ng/ml recombinant human IL-4 for 72 h. Cells were harvested by gentle scraping. After washing with PBS, cells were spun down onto glass slides, fixed with ice-cold methanol and permeabilized with 0.1% (v/v) Triton X-100/PBS. Expression of 15-LOX1 was visualized using a guinea pig anti-rabbit 15-LOX1 Ab (diluted 1/1000) and a goat anti-guinea pig IgG-Alexa 488 (1/200 dilution; Molecular Probes, Eugene, Oregon) as secondary Ab. MAO-A expression was probed using a goat anti-human MAO-A Ab (1/500 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and a rabbit anti-goat IgG-Alexa 568 (1/200 dilution). Nuclei were stained with DRAQ5 (Biostatus, Leics, U.K.). Imaging was conducted on an Axiovert 100 inverted microscope connected to a Bio-Rad MRC 1024ES laser scanning system (Bio-Rad Microscience, Hemel Hempstead, U.K.). Images were acquired using a ×40 oil lens, with ex/em 495/519 or 578/603 for Alexa 488 and Alexa 568, respectively. A standard analysis software (Lasersharp 2000; Bio-Rad Microscience) was used and postacquisition processing of the images was conducted using the Adobe Photoshop software package (Adobe Systems, San Jose, CA).

Miscellaneous methods

Native rabbit 15-LOX1 was prepared from the stroma-free supernatant prepared by osmotic hemolysis of a reticulocyte-rich blood cell suspension by sequential ammonium sulfate precipitation and two consecutive steps of fast protein liquid chromatography as described before (30). Measurements of MAO activity of rat liver mitochondria (prepared according to Ref. 31) were conducted oxygraphically following the kinetics of oxygen uptake with a Clark-type electrode after substrate addition. For this purpose, 2 mg/ml mitochondrial protein were preincubated for 4 min at 37°C in 0.1 M phosphate buffer (pH 7.4). Then 2 mM KCN was added to block oxygen uptake due to endogenous respiration. After 2 min of additional preincubation, MAO-A reaction was started by addition of 1 mM hydroxytryptamine, and oxygen uptake was followed. To quantify the impact of LOX treatment on MAO-A activity, mitochondria were preincubated with the pure rabbit 15-LOX1 (variable amounts of the enzyme exhibiting a linoleic acid oxygenase activity that ranges between 1 and 6 nkat/mg mitochondrial protein) for different time periods, and inhibition of MAO-A activity was calculated. MAO-A activity of the mitochondria incubated in the absence of 15-LOX1 was set at 100%. Protein concentrations were determined with the Roti-Quant detection system (Carl Roth). Statistical analysis of all experimental data was conducted with the Microsoft Excel software package (Redmond, WA) using the unpaired Student’s t test.

Results

In vitro oxygenation of mitochondrial membrane by 15-LOX1 inactivates MAO-A

In vitro incubation of rat liver mitochondria with pure rabbit 15-LOX1 resulted in a time-dependent inactivation of MAO-A (Fig. 1). The residual enzyme activity decreased with duration of the incubation period and with increasing LOX concentrations (Fig. 1, inset). These data confirm a previous report on 15-LOX1-induced inactivation of MAO isozymes under comparable experimental conditions (28).

15-LOX1 transfection of monocytic cells induces up-regulation of MAO-A expression

To test whether intracellular 15-LOX1 activity may also inactivate MAO-A in vivo, U937 cells (promyelomonocytic cell line) were stably transfected with the rabbit 15-LOX1. Following transfection, intracellular LOX activity was detected, with the cells being capable of converting exogenous arachidonic acid to the major 15-LOX1 product (15S,5Z,8Z,11Z,13E)-15-hydro(pero)xyeicosanoic acid (15S-LOX1 metabolite). The chemical structure of this compound was confirmed by UV-spectroscopy (Fig. 2, inset A), chiral phase HPLC (Fig. 2, inset B) and gas chromatography/mass spectrometry (data not shown). This compound was not detected with mock-transfected controls (Fig. 2, upper trace) or when the 15-LOX1 transfectants were boiled before activity assay (data not shown).

FIGURE 1. Inactivation of MAO-A during the time course of 15-LOX1-catalyzed oxygenation of mitochondria. Rat liver mitochondria (2 mg protein/ml) were incubated with the pure rabbit 15-LOX1 (1.8 nkat linoleic acid oxygenase activity/mg mitochondrial protein) in 0.1 M phosphate buffer (pH 7.4) at 37°C in an oxygraphic assay chamber (Gilion 5/6H oxigraph; Gilson, Middleton, WI). After the time period indicated, 1 mM salicylhydroxamic acid (15-LOX1 inhibitor) and 2 mM KCN (inhibitor of mitochondrial respiration) were added to prevent endogenous oxygen consumption, and MAO-A reaction was initiated by 1 mM hydroxytryptamine. The reaction rate was quantified measuring the oxygen uptake during the first 30 s of the reaction. Neither 1 mM salicylhydroxamic acid nor 2 mM KCN inhibited MAO-A reaction. Inset, Dependence of MAO-A inhibition of the amounts of 15-LOX1 added (30-min incubation at 37°C).

If, as expected from the above shown in vitro data, MAO-A is inactivated by the intracellular 15-LOX1 activity, one would expect an impaired MAO-A activity in 15-LOX1-transfected cells when compared with the corresponding mock transfecteds. Surprisingly, an almost 10-fold higher MAO-A activity was measured in the 15-LOX1-transfectants (Fig. 3A). To investigate the mechanism of the stimulatory effect and to confirm up-regulation of the enzyme at the mRNA level, semi quantitative RT-PCR was performed. It can be seen from Fig. 3B that under our experimental conditions, no MAO-A mRNA was observed in the mock transfecteds. In contrast, MAO-A mRNA was clearly detected in the corresponding 15-LOX1 transfecteds. It should be stressed that at higher cycle numbers we also observed expression of MAO-A mRNA in the mock-transfected cells, but at a clearly lower level than in 15-LOX1-transfected cells.

IL-4 and IL-13 induce MAO-A in human peripheral monocytes

In human peripheral monocytes, expression of 15-LOX1 is strongly up-regulated when the cells are treated with IL-4 or IL-13 (13, 14) and we confirmed these results in the present study (Fig. 4, A and B). To test whether 15-LOX1 induction is paralleled by augmented MAO-A expression, MAO-A activity was assayed in IL-4-treated monocytes and a strong up-regulation of the enzymatic activity was detected (Fig. 4A). Parallel up-regulation of MAO-A and 15-LOX1 was confirmed at the mRNA level by semi quantitative RT-PCR (Fig. 4B). In contrast, IL-4 treatment did not up-regulate expression of the pharmaco logically most relevant 5-LOX (data not shown). Moreover, the mRNA for MAO-B and ALOX3 were undetectable under our experimental conditions regardless of whether the monocytes were cultured in the presence or absence of IL-4.

For more quantitative information on MAO-A expression, real-time PCR was performed. In the absence of IL-4, 2.2 × 10^3 copies
of MAO-A mRNA were present per 10⁶ copies of GAPDH (Fig. 4C). Following incubation of the cells in the presence of IL-4 for 72 h, the MAO-A mRNA copy number was augmented to 4.6 × 10⁶ copies/10⁶ copies GAPDH, which represents a >2000-fold increase. IL-13 also induced MAO-A mRNA expression although to a lesser extent (3.7 × 10⁵ MAO-A/10⁶ copies of GAPDH, i.e., 160-fold increase). Next, levels of MAO-A or 15-LOX1 mRNA were quantified during the time course of IL-4 treatment. From Fig. 4D it can be seen that induction kinetics of MAO-A and 15-LOX1 are similar. Under our experimental conditions, both enzymes were absent in untreated peripheral monocytes, and after 3 h of IL-4 treatment neither PCR signals were detected. However, after 24 h of IL-4 treatment clear MAO-A and 15-LOX1 bands were observed. These data indicate that MAO-A and 15-LOX1 do not belong to the immediate early genes of IL-4 signaling.

To examine regulation of MAO-A by IL-4 at the protein level, immunohistochemistry was conducted. In the absence of IL-4, neither 15-LOX1 nor MAO-A expression was detected in peripheral human monocytes (Fig. 5, A–H). In contrast, strong staining was observed when the cells were cultured with IL-4 for 72 h (Fig. 5, A–D). As reported before, 15-LOX1 expression was not uniform in IL-4 treated monocytes (13, 16). Under our experimental conditions we found that ~70% of all cells were stained 15-LOX1-positive (moderate to strong staining). This cell population was also MAO-A positive. Evaluating numerous slides we concluded that IL-4-treated monocytes either coexpress 15-LOX1 and MAO-A, or express neither protein. Coexpression of the two enzymes is clearly visualized by immunohistochemistry at higher magnification (Fig. 5, I–L).


deletion MAO-A induction parallels 15-LOX1 expression in A549 cells

To examine whether or not coinduction of MAO-A and 15-LOX1 is restricted to monocytes, we tested the human myeloblastic (HL60) and a nonmonocytic human cell line (A549 lung carcinoma cells). Both cell types express a functional IL-4R and undergo phenotype alterations in response to IL-4 (16). When stimulated with the cytokine expression of 15-LOX1, mRNA is only induced in A549 (17, 32) but not in HL60 cells (Fig. 6A). As shown above for IL-4-treated monocytes (Fig. 4D), induction kinetics of the two mRNA species are rather similar in this cell type (Fig. 6B). Measurements of the MAO-A activity (Fig. 6C) confirmed up-regulation of MAO-A expression in A549 cells. These data suggest that cells, which respond to IL-4 stimulation with induction of 15-LOX1 (monocytes, A549 cells), up-regulate expression of MAO-A. In contrast, in cells, which do not up-regulate 15-LOX1 expression when stimulated with IL-4, MAO-A is apparently not induced. Thus, there appears to be a coupling in expression regulation of 15-LOX1 and MAO-A.


discussion

The Th2 cytokines IL-4 and IL-13 strongly induced expression of MAO-A in a subpopulation of human peripheral monocytes and in A549 lung carcinoma cells in tandem with 15-LOX1. Up-regulation of the two pro-oxidative enzymes creates an intracellular oxidizing environment, which may regulate expression of redox-sensitive genes. A similar up-regulation of MAO-A was also observed in the human promyelomonocytic cell line, U937, when transduced with 15-LOX1, MAO-A, or express neither protein. Coexpression of the two enzymes is clearly visualized by immunohistochemistry at higher magnification (Fig. 5, I–L).


deletion MAO-A induction parallels 15-LOX1 mRNA expression in A549 cells

To examine whether or not coinduction of MAO-A and 15-LOX1 is restricted to monocytes, we tested the human myeloblastic
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FIGURE 4. Tandem induction of MAO-A and 15-LOX1 in human peripheral monocytes by IL-4 and IL-13. Human monocytes were prepared and cultured for 3 days in the presence and absence of 10 ng/ml IL-4 or IL-13, then examined for MAO-A and 15-LOX1 expression. A. Enzyme activities were measured (see Materials and Methods) following IL-4/13 treatment; 100% represents activity in the presence of cytokines (n = 4, p < 0.001 between the two groups for both enzymes). B. Total RNA from monocytes cultured with/without IL-4 or IL-13 was prepared and subjected to semiquantitative RT-PCR (see Materials and Methods). C. Real-time PCR of MAO-A mRNA in IL-4-treated and -untreated monocytes: ●, 10⁶ copies; ▲, 2 × 10⁵ copies; ◆, 5 × 10⁴ copies; ▼, 10⁴ copies; and △, 2 × 10³ copies. D. Time course of MAO-A and 15-LOX1 mRNA expression. Similar experiments were conducted with RNA preparations obtained from monocytes of five different donors and a representative set of data is shown.

FIGURE 5. Immunohistochemistry of 15-LOX1 and MAO-A in human peripheral monocytes. Human peripheral monocytes cultured for 3 days in the presence or absence of 10 ng/ml recombinant human IL-4 (Promega) were fixed and stained as described in Materials and Methods. A–D, IL-4-treated cells; E–H, control cells (no cytokine treatment). A and E, Fluorescence of 15-LOX1 visualized using Alexa 488; B and F, fluorescence of MAO-A visualized using Alexa 588; C and G, nuclei visualized using DRAQ5; D and H, merge of A–C, E–H respectively. I–L, Higher magnification of IL-4-treated monocytes showing colocalization of MAO-A and 15-LOX1.

There are two MAO-isozymes (33), MAO-A and MAO-B, located in the outer mitochondrial membrane. MAO-A deficiency has been associated with impulsive aggressive behavior and inhibitors of this isozyme are used for treatment of affective disorders (34, 35). In contrast, inhibitors of MAO-B are used for treatment of patients with neurological impairment, including Parkinson’s disease (34, 35). The two isoenzymes are encoded for by separate genes (36) and exhibit different substrate and inhibitor specificities (37, 38). MAO-A preferentially oxidizes serotonin and noradrenaline, with MAO-B preferring phenylethylamine. Both isoforms are nonselective for dopamine, tyramine, and tryptamine (37). The gene encoding MAO-A is localized on the X chromosome and has a distinct pattern of cis-regulatory elements in its promoter region when compared with the MAO-B gene (39, 40). Its basic promoter contains four stimulating protein 1 binding sites and reporter gene assays indicated that three of them are functional (40). Although the two MAO isoforms occur in many cells, marked differences in tissue- and development-specific expression patterns have been described (41, 42). However, the underlying mechanisms for differential expression remain to be investigated. Interestingly, expression of neither MAO-isofrom has ever been reported for human monocytes and thus, the role of these enzymes for monocyte physiology has not yet been investigated. Although a role for MAO-B in age-related decline in immune function has previously been suggested (43), little is known about the function of MAO-A in immunocompetent cells in general, and in peripheral monocytes in particular. Some MAO-A substrates, such as serotonin and noradrenaline, may act as vasoactive mediators at inflammatory sites (44, 45). Serotonin, a preferred MAO-A substrate, inhibits generation of TNF-α by macrophages and up-regulates phagocytosis (46). Removal

a functional link in expression regulation between the two pathways.

For the promoter of the 15-LOX1 gene the existence of STAT6-responsive elements has been reported and mechanistic investigations on IL-4-dependent up-regulation of 15-LOX1 expression indicated their functional relevance (19, 21). To investigate whether or not the promoter region of the MAO-A gene does also contain STA6-responsive sequences but their functionality remains unclear. These data suggest that in IL-4/13-treated monocytes induction of MAO-A may proceed at least in part via the conventional IL-4/13 signaling pathway.

MAO isozymes convert biogenic amines to corresponding aldehydes generating ammonia and hydrogen peroxide as byproducts. Interestingly, in U937 cells H₂O₂ at micromolar concentra-
of this mediator from inflammatory sites may induce opposite effects. Unfortunately, the metabolism of biogenic amines in inflammatory sites may induce opposite effects.

PCR was conducted as described in Materials and Methods. A. Comparative time course (RT-PCR) of 15-LOX1 and MAO-A mRNA expression in IL-4-treated cells. The GAPDH/15-LOX1 and GAPDH/MAO-A ratios were calculated from the intensities of the semiquantitative PCR-signals and are used as measure for the expression level of the corresponding enzymes. B. Original RT-PCR data of a more detailed time course. C. Activity assay of IL-4-treated and untreated A549 cells (n = 4, p < 0.001 between the two groups).

FIGURE 6. Expression regulation of 15-LOX1 and MAO-A in IL-4-treated A549 and HL60 cells. Cells were cultured with 10 ng/ml IL-4 for the times indicated. After harvesting, total RNA was extracted and RT-PCR was conducted as described in Materials and Methods. A. Comparative time course (RT-PCR) of 15-LOX1 and MAO-A mRNA expression in IL-4-treated cells. The GAPDH/15-LOX1 and GAPDH/MAO-A ratios were calculated from the intensities of the semiquantitative PCR-signals and are used as measure for the expression level of the corresponding enzymes. B. Original RT-PCR data of a more detailed time course. C. Activity assay of IL-4-treated and untreated A549 cells (n = 4, p < 0.001 between the two groups).

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