5-Hydroxytryptamine modulates cytokine and chemokine production in LPS-primed human monocytes via stimulation of different 5-HTR subtypes

Thorsten Dürk¹, Elisabeth Panther², Tobias Müller¹, Stephan Sorichter¹, Davide Ferrari³, Cinzia Pizzirani³, Francesco Di Virgilio³, Daniel Myrtek¹, Johannes Norgauer⁴ and Marco Idzko¹

¹Department of Pneumology and ²Department of Gastroenterology, University Medical Clinic, University of Freiburg, D-79106 Freiburg i. Br., Germany
³Department of Experimental and Diagnostic Medicine, Section of General Pathology, Interdisciplinary Center for the Study of Inflammation University of Ferrara, I-44100 Ferrara, Italy
⁴Department of Dermatology, University of Jena, D-07740 Jena, Germany

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Abstract
The neurotransmitter 5-hydroxytryptamine (5-HT), commonly known as serotonin, is released at peripheral sites from activated enterochromaffin cells, mast cells and platelets. In this study we analyzed the biological activity and intracellular signaling of 5-HT in human monocytes. By reverse transcription (RT) and PCR, messenger RNA (mRNA) expression of 5-HT receptor 1E (5-HTR1E), 5-HTR2A, 5-HTR4 and 5-HTR7 could be revealed. Functional studies showed that 5-HT modulates the release of IL-1β, IL-6, IL-8/CXCL8, IL-12p40 and tumor necrosis factor-α (TNF-α), while it has no effect on the production of IL-18 and IFN-γ in LPS-stimulated human blood monocytes. Moreover, RT and PCR revealed that 5-HT modulated mRNA levels of IL-6 and IL-8/CXCL8, but did not influence mRNA levels of IL-1β and TNF-α. Pharmacological studies with isotype-selective receptor agonists allowed us to show that 5-HTR3 subtype up-regulates the LPS-induced production of IL-1β, IL-6 and IL-8/CXCL8, while it was not involved in TNF-α and IL-12p40 secretion. Furthermore, activation of the Gs-coupled 5-HTR4 and 5-HTR7 subtypes increased intracellular cyclic AMP (cAMP) and secretion of IL-1β, IL-6, IL-12p40 and IL-8/CXCL8, while, on the contrary, it inhibited LPS-induced TNF-α release. Interestingly, 5-HTR1 and 5-HTR2 agonists did not modulate the LPS-induced cytokine production in human monocytes. Our results point to a new role for 5-HT in inflammation by modulating cytokine production in monocytes via activation of 5-HTR3, 5-HTR4 and 5-HTR7 subtypes.

Introduction
5-Hydroxytryptamine (5-HT), commonly known as serotonin, is a well-characterized neurotransmitter and vasoactive amine. It is involved in the regulation of a large number of physiological functions such as sleep, appetite and behavior (1, 2). In peripheral organs 5-HT is synthesized and released by mast cells, basophils, platelets and enterochromaffin cells. Enhanced extracellular levels of this amine during inflammation and platelet activation are well documented (2–4). Moreover, recent observations point to a role of 5-HT as an immunomodulator. In this context, a broad variety of cell responses such as migration, phagocytosis, superoxide anion generation and cytokine production by 5-HT have been reported (5–9). Moreover, clinical data would indicate a critical role of 5-HT in the pathogenesis of bronchial asthma (10).

The wide variety of 5-HT-mediated cell functions is regulated by expression of different classes of G protein-coupled as well as ionotropic serotoninergic receptors (5-HTR) (9, 11). The 5-HTR1 and 5-HTR2 classes consist of at least five (5-HTR1A, 5-HTR1B, 5-HTR1D, 5-HTR1E and 5-HTR1F) and three (5-HTR2A, 5-HTR2B and 5-HTR2C) G protein-coupled receptors,
respectively. Most of these members are either coupled to pertussis toxin-sensitive $G_{i/o}$ or pertussis toxin-insensitive $G_{s15}$ proteins (9, 11). 5-HTR$_3$ are ligand-gated cation channels, which trigger de-polarization of the plasma membrane through activation of Na$^+$ and K$^+$ fluxes (12). 5-HTR$_4$, 5-HTR$_6$ and 5-HTR$_7$ are linked to $G_s$ protein-mediated stimulation of adenylyl cyclase (9, 11).

Inflammation involves the migration of various types of blood cells, including monocytes into damaged tissues (13). Among innate immune cells, monocytes and the tissue-associated cells, e.g. macrophages, play an essential role in sensing and elimination of invasive microorganisms (13, 14). Binding of cytokines, biogenic amines and microbial products to receptors stimulates monocytes to release cytokines and other effector molecules in order to orchestrate the innate and adaptive immune responses. It is well known that activated monocytes secrete oxidants and cytokines such as IL-1$\beta$, IL-6, IL-8/CXCL8, IL-10, IL-12p40, IL-18 and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (14–19). These molecules essentially contribute to the pathophysiological changes associated with several acute and chronic inflammatory conditions (14–19).

Recently, expression of 5-HTR$_{2A}$ in PBMC has been described and linked to regulation of TNF-$\alpha$ and IL-1$\beta$ release (5). This receptor usually couples to pertussis toxin-sensitive $G_{i/o}$ proteins (9, 11). However, regulatory influences of the $G_{i/o}$-dependent signaling pathway with the cytokine secretory machinery are not common. On the contrary, control functions of $G_s$ proteins in this context are well documented (9, 20, 21). Here we describe that 5-HTR$_3$, 5-HTR$_6$ and 5-HTR$_7$ subtypes regulate the secretion of IL-1$\beta$, IL-6, IL-12p40, IL-8/CXCL8 and TNF-$\alpha$ release in monocytes.

**Methods**

**Reagents**

5-HT, 2-methoxytryptamine (2-MHT), N-methyl-5-hydroxytryptamine (2Me5-HT), R(-)-DOI-hydrochloride (DOI), ketanserin and LPS (Escherichia coli serotype 0111:B4, L-2630) were obtained from Sigma–Aldrich (Deisenhofen, Germany); 5-carboxamidotryptamine maleate, 3-(1-methyl-piperidin-4-yl)-1H-indol-5-ol maleate salt (BRL 54443), 8-hydroxy-DPAT-hydrobromide (8-HDPAT), Pimozide, RS 39604 hydrochloride (RS 39604), SB 269970 hydrochloride (SB 269970), 3-(4-allylpiperazin-1-yl)-2-quinoxalinecarbonitrile maleate (AP) and Y-25130 hydrochloride (Y-25130) were obtained from TOCRIS (Tocris Cookson Ltd, UK).

**Isolation of CD14$^+$ monocytes**

Blood was obtained from healthy human volunteers and peripheral mononuclear cells were separated by centrifugation with Ficoll (Amersham Pharmacia Biotech AB, Uppsala, Sweden) gradients. Cells were further separated with anti-CD14 mAb-coated MicroBeads using Macs single-use separation columns from Mitenyi Biotec (Bergisch Gladbach, Germany) (22). The CD14$^+$ cells were cultured in RPMI 1640 medium, containing 10% FCS, 1% glutamine, 50 IU ml$^{-1}$ penicillin and 50 $\mu$g ml$^{-1}$ streptomycin and maintained at 37°C in a humidified atmosphere with 5% CO$_2$.

**Reverse transcription and PCR**

The messenger RNA (mRNA) was isolated with QiAshredder and RNeasy kits (Qiagen, Hilden, Germany). The mRNA, M-MLV reverse transcriptase and pd(N)$_6$ primers (GIBCO BRL, Gaithersburg, MD, USA) were employed to obtain the cDNA of the different 5-HTR subtypes. Oligonucleotides used as primers in PCR were designed to recognize sequences specific for each target cDNA (9). Primer sequences for 5-HTR and control genes are as follows:

5-HTR$_1A$ (411 bp)—sense: 5'-GCC GCG TCC TCA TCT GTC G-3', anti-sense: 5'-GCC GCG CCA TCG TCA CTA T-3';
5-HTR$_1B$ (460 bp)—sense: 5'-CAG CGC AAA GGA CTA CAT TTA CCA C-3', anti-sense: 5'-TAA GAA GGG CCG CAG CTA GAT AGA-3';
5-HTR$_2A$ (359 bp)—sense: 5'-ACT CGC CGA TGA TAA C TTG TGT TCC CCT-3', anti-sense: 5'-TGA CGG CCA TGA TCT TG TGC TA-3';
5-HTR$_2B$ (416 bp)—sense: 5'-GCC CCC TCC ACC TTC TCT C-3', anti-sense: 5'-TAG CGG TTG AGG TGG TGC GGT T-3';
5-HTR$_3C$ (449 bp)—sense: 5'-GTC CCG CCG CTC GGT GGA TTT CTT TAG ATG TCC TTA-3', anti-sense: 5'-CTC CCC TTC GGC CGT AGT CCT CTG T-3';
5-HTR$_4$ (448 bp)—sense: 5'-CCG GCC GCC CCT CTA TCT T-3', anti-sense: 5'-GCA AAG TAG CCA GCC GAG TCT-3';
5-HTR$_5$ (342 bp)—sense: 5'-GCC GGA CAC ACC AAG AGC AAA AGG-3', anti-sense: 5'-GCG CTA GCC GAT GTC AGT G-3';
5-HTR$_6$ (324 bp)—sense: 5'-GCC GCG CCG ATG CTG AAC G-3', anti-sense: 5'-GCC CGA CGC CAC AAG GAC AAA AGG-3';
5-HTR$_7$ (436 bp)—sense: 5'-GCC GTG GCC GGC GTC TCA-3', anti-sense: 5'-CCT GCC CAA GAG AAA TTA TTA-3';
$\beta$-microglobulin (259 bp)—sense: 5'-CTA GCC GAC ATC CAT TCA-3', anti-sense: 5'-GTT CAC ACG GCA GGC ATA CTG-3'.

Thirty PCR cycles were run at 94°C (denaturation, 1 min), 62°C (annealing, 1 min) and 72°C (extension, 1 min). The generated cDNAs were subjected to electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. Intensity of the different bands in gels was quantified by measuring the optical density with a OneScan computer software package. The cDNA amplification was linear in an amplification range of 24–34 cycles. The identity of the PCR products was confirmed by sequencing after cloning using pCRII vectors. Controls run without reverse transcriptase yielded no PCR products.

**Quantification by real-time PCR**

Total RNA was extracted using the RNeasy kit according to the manufacturer's protocol (Qiagen). Briefly, in DNase I (Invitrogen) treatment, 1 $\mu$g of total RNA from each sample was used as a template for the reverse transcription (RT) reaction. Fifty microliters of cDNA was synthesized using M-MLV reverse transcriptase and pd(N)$_6$ primers (GIBCO BRL). All samples were reverse transcribed under the same conditions.
(25°C for 10 min, 48°C for 30 min) and from the same RT master mix to minimize differences in RT efficiency. All oligonucleotide primers for real-time PCR were designed using Primer 3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/ primer3www.cgi) and synthesized by Invitrogen.

For iCycler reaction, a master mix of the following compounds was prepared to the indicated end concentration: 10 µl SYBR Green master mix (Bio-Rad), 6 µl water, 1 µl sense and 1 µl anti-sense primers (500 nM). This master mix (18 µl) was filled in the iCycler strips and 2 µl cDNA (0.625, 2.5, 10 or 40 ng reverse transcribed total RNA) was added as PCR template. The following iCycler experimental run protocol was used: denaturation (95°C for 9 min), with 40 cycles of amplification and quantification (95°C for 30 s, 60°C for 30 s, 72°C for 30 s) with a melting curve program (60-95°C with a heating rate of 0.1°C s⁻¹). Emitted fluorescence for each reaction was measured during the extension phase. Real-time PCR efficiency (E) was calculated from the given slopes, with the iCycler software, as previously described (9, 25, 26).

The cycle threshold (CT), i.e. the cycle number at which the amount of the amplified gene reaches threshold fluorescence, was determined by using the iCycler software. The relative expression ratio (R) of the different target genes was calculated based on efficiency (E) and CT, deviation of an unknown sample versus a control, and compared with the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase, as previously described (9, 25, 26).

Stimulation of cytokine release
5-HT and 5-HTR agonists were added to cells in the presence or absence of 1 µg ml⁻¹ LPS. After the indicated time point supernatants were collected, and cytokines were measured by ELISA. Secretion of IL-1β, IL-6, IL-8/CXCL8, IL-12p40 and INF-γ was analyzed in supernatants by using matched pair mAbs from R&D Systems (Minneapolis, MN, USA). TNF-α and IL-18 were determined using ELISA kits from Bender-Systems (Vienna, Austria). Samples were assayed in triplicates for each condition.

Measurement of intracellular cAMP
Intracellular cyclic AMP (cAMP) levels were determined by enzyme immunoassay (Amersham Pharmacia Biotech AB) as described previously (20). Forskolin (Sigma) was used as a positive control. The cAMP levels are expressed as an index representing the ratio between values obtained for stimulated cells and cells incubated in control medium.

Results
Human monocytes express the mRNA for different 5-HTR subtypes
Expression of mRNA for the 5-HTR subtypes was analyzed by RT-PCR in blood-derived and LPS-stimulated monocytes. Figure 1 shows that human monocytes express 5-HTR₁E, 5-HTR₂A, 5-HTR₃, 5-HTR₄ and 5-HTR₇ mRNAs. Priming of monocytes with LPS did not significantly modify mRNA expression of the analyzed genes. However, we found no expression of the analyzed genes. However, we found no

transcripts for 5-HTR₁₆, 5-HTR₁₇, 5-HTR₁₂, 5-HTR₁₅, 5-HTR₂₈, 5-HTR₂₆, 5-HTR₉ and 5-HTR₆ in monocytes (data not shown).

Activation of 5-HTR₄ and 5-HTR₇ inhibits TNF-α release
Recent evidence suggests that 5-HT modulates the production of different cytokines in monocyte-derived human dendritic cells (9). Resting monocytes only release small amounts of TNF-α (Fig. 2A). However, 5-HT as well as the selective 5-HTR₄ and 5-HTR₇ agonists, dose dependently decreased this response. The 5-HTR₃ agonist 2Me5-HT, the 5-HTR₁ agonist BRL 54443 and the 5-HTR₂ agonist DOI had no effect on TNF-α release in resting monocytes. TNF-α production in monocytes was strongly induced by LPS (Fig. 2B). In addition, 5-HT added together with LPS dose dependently decreased the release of TNF-α. Half-maximal and maximal effects were seen at 10⁻⁵ and 10⁻³ M, respectively. Stimulation of cells with selective receptor agonists was also performed. The 5-HTR₄ agonist 2-MHT and the 5-HTR₇ agonist 8-HDPAT inhibited TNF-α release in a concentration-dependent manner. Half-maximal and maximal effects were seen at 10⁻⁵ and 10⁻³ M, respectively. Again, the 5-HTR₃ agonist 2Me5-HT, the 5-HTR₁ agonist BRL 54443 and the 5-HTR₂ agonist DOI had no effect on TNF-α release (Fig. 2B).

Figure 2(C) shows that incubation of LPS-primed monocytes with the adenylyl cyclase inhibitor cis-N-(2-phenylcyclopentyl)-azacyclotride-1-en-2-amine (MDL 12330A) resulted in TNF-α-release restoration. These data suggest an involvement of cAMP in this cell response. Moreover, pre-incubation of LPS-stimulated monocytes with the 5-HTR₁ antagonist RS 39604 before stimulation of monocytes with the 5-HTR₄ agonist 2-MHT abrogated the effect on TNF-α release (Table 1). However, the stimulating effect of 2-MHT was not influenced by the 5-HTR₃ antagonists AP and Y-25130 as well as the 5-HTR₇ antagonists Pimozide or SB 269970. Moreover, the effect of the 5-HTR₇ agonist 8-HDPAT was inhibited by the 5-HTR₇ antagonists Pimozide or SB 269970, but not by pre-incubation of monocytes with other 5-HTR antagonists.

In addition, relative mRNA quantification by real-time PCR was performed. However, these experiments showed that expression of TNF-α mRNA was not changed by 5-HT stimulation in resting or LPS-primed monocytes (data not shown).

5-HT stimulates IL-12p40 secretion from human monocytes
We then analyzed the 5-HT-mediated effect on IL-12p40. Figure 3 revealed that addition of 5-HT to LPS-stimulated
monocytes enhanced secretion of IL-12p40 in a concentration-dependent manner. Half-maximal and maximal effects were seen at $10^{-5}$ and $10^{-4}$ M, respectively. Again the 5-HTR4 agonist 2-MHT and the 5-HTR7 agonist 8-HDPAT had an effect similar to 5-HT, whereas the 5-HTR3 agonist 2Me5-HT as well as the 5-HTR1 agonist BRL 54443 or the 5-HTR2 agonist DOI did not affect IL-12p40 release (Fig. 3). Resting monocytes did not release measurable amounts of IL-12p40 (data not shown).

5-HT induces secretion of IL-1β, IL-6 and IL-8/CXCL8

To study in more detail 5-HT-mediated cytokines and chemokines release in monocytes, secretion of IL-6, IL-8/CXCL8, IL-1β, IL-18 and IFN-γ was analyzed. Figures 4 and 5 show that 5-HT, the 5-HTR3 agonist 2Me5-HT, the 5-HTR4 agonist 2-MHT or the 5-HTR7 agonist 8-HDPAT increased the release of IL-6 and IL-8/CXCL8 in resting (A) and LPS-primed (B) monocytes in a concentration-dependent manner. Interestingly, the 5-HTR1 or 5-HTR2 agonists BRL 54443 and DOI did not influence these responses (Fig. 5 and data not shown). Moreover, Table 2 shows that pre-incubation of monocytes with the 5-HTR4 antagonist RS 39604 blocked 2-MHT-induced IL-6 secretion. The 5-HTR7 antagonists Pimozide or SB 269970 inhibited the 8-HDPAT effect and the 5-HTR3 antagonists AP and Y-25130 abrogated the 2Me5-HT-induced response. Identical observations were made by ELISA experiments to detect IL-12p40 and IL-8/CXCL8 (data not shown). In addition, relative mRNA quantification by real-time PCR indicated that 5-HT enhanced IL-6 and IL-8 mRNA levels in LPS-primed monocytes (Figs 4C and 5C). Similar conclusions could be drawn from experiments in resting monocytes (data not shown).

Next the influence of 5-HT was analyzed on IL-1β release. No IL-1β could be detected in the supernatant of resting monocytes after stimulation with or without 5-HT (data not shown). However, 5-HT, the 5-HTR3 agonist 2Me5-HT, the 5-HTR4 agonist 2-MHT or the 5-HTR7 agonist 8-HDPAT increased the release of the inflammatory cytokines IL-1β in LPS-primed monocytes (Fig. 6). Interestingly, relative mRNA quantification by real-time PCR showed that expression of IL-1β mRNA was not changed by 5-HT stimulation in LPS-primed monocytes (data not shown). In addition, 5-HT and all tested 5-HTR agonists had no effect on IL-18 or IFN-γ release (data not shown).

Recent reports indicate that the adenylyl cyclase pathway can modulate the release of several cytokines and chemokines (9, 20, 21). We therefore analyzed cAMP levels in

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**Fig. 2.** 5-HT inhibits the production of TNF-α in monocytes. (A) Resting monocytes were stimulated with the indicated concentrations of 5-HT or isotype receptor agonists. Supernatants were collected 24 h after stimulation and cytokine content was measured by ELISA. Results are given as mean $\pm$ SEM ($n = 4$). (B) LPS-primed monocytes were stimulated with the indicated concentrations of 5-HT or isotype receptor agonists. Supernatants were collected 24 h after stimulation and cytokine content was measured by ELISA. Results are given as mean $\pm$ SEM ($n = 4$). (C) 5-HT-induced inhibition of TNF-α production depends on cAMP. LPS-stimulated monocytes were treated with MDL 12330A ($10^{-6}$ M) for 30 min prior to stimulation with $10^{-3}$ M 5-HT, 2-MHT, 8-HDPAT or medium. Supernatants were collected 24 h after stimulation and were measured by ELISA. Results are given as mean $\pm$ SEM ($n = 4$).
monocytes. Enhanced cAMP levels were detected after the stimulation of resting and LPS-primed monocytes with the 5-HTR4 agonist 2-MHT and the 5-HTR7 agonist 8-HDPAT (Fig. 7A and B), whereas the 5-HTR1, 5-HTR2 and 5-HTR3 agonists did not significantly modify this intracellular second messenger levels (data not shown).

Discussion

In peripheral organs 5-HT is present at high concentrations in mast cells, basophils, platelets and enterochromaffin cells (2–4). It is released at high concentrations during IgE stimulation or platelet aggregation (2–4). Besides its well-characterized function as a neurotransmitter, accumulating evidence has recently emerged on its immune regulatory properties (3–9). Pharmacological and molecular biological studies revealed the existence of several specific 5-HTR. These receptors are either classified as ligand-gated cation channels or belong to the superfamily of G protein-coupled receptors (9, 11). Recently, it has been reported that PBMC express functional 5-HTR2A, regulating TNF-α and IL-1β release (5). In contrast, we reported that 5-HT regulates the release of TNF-α and IL-1β via 5-HTR4 and 5-HTR7 in monocyte-derived dendritic cells (9). Since interactions between pertussis toxin-sensitive Gαi/o protein-coupled receptors such as the 5-HTR2A are not common, we analyzed in deep detail the expression and function of 5-HTR in monocytes.

Here we show by RT and PCR that monocytes express the mRNA of 5-HTR1E, 5-HTR3A, 5-HTR3B, 5-HTR4 and 5-HTR7 subtypes. Moreover, we showed that the mRNA expression pattern of the 5-HTR is not modified by LPS treatment in monocytes. This is different to that seen in monocyte-derived dendritic cells (9). In these cells LPS treatment down-regulated the mRNA levels of the 5-HTR1E and 5-HTR2A subtypes, while it enhanced the expression of 5-HTR4 and 5-HTR7 mRNAs. However, this different behavior might be explained by tissue-specific regulatory mechanisms. The latter might be also supported by the assumption that dendritic cells in comparison to monocytes express 5-HTR1B and 5-HTR2B (9).

To prove functional expression of the 5-HTR subtypes in monocytes, we analyzed in detail the secretion of various monocyte-derived cytokines and their intracellular signaling pathways. Thereby, we can confirm previous observations on 5-HT inhibition of TNF-α release and enhancement of IL-1β secretion in LPS-stimulated monocytes. Moreover, we showed that 5-HT enhanced the release of IL-6, IL-8/CXCL8 and IL-12p40 in these cells. Moreover, data from experiments with RT and PCR suggest that enhanced protein secretion of IL-6 and IL-8/CXCL8 by 5-HT is paralleled also on the mRNA level. In contrast, no influence could be detected by 5-HT on the mRNA levels of IL-1β and TNF-α.

To better define the pharmacology and signaling pathway of 5-HT-mediated responses in monocytes, isotype-selective receptor agonists and antagonists were employed, showing that 5-HTR4 and 5-HTR7 were involved in the modulation of IL-1β, IL-6, IL-8/CXCL8, IL-12p40 and TNF-α secretion; the 5-HTR3 subtype participated in the modulation of IL-6, IL-8/CXCL8 and IL-1β secretion, while it did not influence IL-12p40 and TNF-α production.

Different from what was previously reported in PBMC, we found no evidence for 5-HTR2A involvement in cytokine release in purified CD14+ monocytes (5). Therefore, one could assume that the previously observed effect of the 5-HTR2A on these

Table 1. Effect of the 5-HTR3 antagonists AP and Y-25130, the 5-HTR4 antagonist RS 39604 and the 5-HTR7 antagonists Pimozide and SB 269970 on the TNF-α production of LPS-incubated monocytes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2-MHT (5-HTR4 agonist)</th>
<th>8-HDPAT (5-HTR7 agonist)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6544 ± 243</td>
<td>3900 ± 140**</td>
<td>3702 ± 420**</td>
</tr>
<tr>
<td>RS 39604 (5-HTR4 antagonist)</td>
<td>6290 ± 310</td>
<td>5800 ± 700</td>
<td>4310 ± 410**</td>
</tr>
<tr>
<td>SB 269970 (5-HTR7 antagonist)</td>
<td>5950 ± 512</td>
<td>4800 ± 150*</td>
<td>6320 ± 700</td>
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<tr>
<td>Pimozide (5-HTR7 antagonist)</td>
<td>5800 ± 600</td>
<td>4000 ± 325*</td>
<td>5430 ± 750</td>
</tr>
<tr>
<td>AP (5-HTR3 antagonist)</td>
<td>6600 ± 306</td>
<td>4104 ± 240**</td>
<td>3904 ± 280**</td>
</tr>
<tr>
<td>Y-25130 (5-HTR7 antagonist)</td>
<td>6821 ± 404</td>
<td>4195 ± 355**</td>
<td>3680 ± 315**</td>
</tr>
</tbody>
</table>

LPS-treated monocytes were pre-incubated with 10−7 M of the selective 5-HTR4 antagonist RS 39604 or the 5-HTR7 antagonists Pimozide or SB 269970 or with 10−6 M of the selective 5-HTR3 antagonists AP and Y-25130. Monocytes were then stimulated with 10−4 M 2-MHT or 8-HDPAT. TNF-α production was measured after 24 h. Data are given in pg ml−1 × 200 000 cells and represent means ± SEM (n = 3). Global differences between groups: *P < 0.0001 (analysis of variance); **P < 0.01; *P < 0.05 compared with untreated cells (Tukey's multiple comparison test).
Fig. 4. 5-HT stimulates production of IL-6 in monocytes. Resting monocytes (A) and LPS-incubated monocytes (B) were stimulated with the indicated concentrations of 5-HT or isotype receptor agonists. Supernatants were collected 24 h after stimulation and were measured by ELISA. Results are given as mean ± SEM (n = 4). IL-6 mRNA expression during LPS-primed monocytes was analyzed in the absence or presence of 5-HT (C). Total RNA was isolated from monocytes (1 × 10⁷) stimulated with LPS 1 μg ml⁻¹ in the absence or presence of 10⁻⁴ M 5-HT for 2, 8 and 24 h. IL-6 mRNA expression was quantified as described in Methods. Number of transcripts is normalized to the number of copies of the glyceraldehyde-3-phosphate dehydrogenase ones. Data are means ± SEM (n = 3).

Fig. 5. 5-HT stimulates production of IL-8/CXCL8 in monocytes. Resting monocytes (A) and LPS-incubated monocytes (B) were stimulated with the indicated concentrations of 5-HT or isotype receptor agonists. Supernatants were collected 24 h after stimulation and were measured by ELISA. Results are given as mean ± SEM (n = 4). IL-8 mRNA expression during LPS-primed monocytes was analyzed in the absence or presence of 5-HT (C). Total RNA was isolated from monocytes (1 × 10⁷) stimulated with LPS 1 μg ml⁻¹ in the absence or presence of 10⁻⁴ M 5-HT for 2, 8 and 24 h. IL-8 mRNA expression was quantified as described in Methods. Number of transcripts is normalized to the number of copies of glyceraldehyde-3-phosphate dehydrogenase ones. Data are means ± SEM (n = 3).
cytokine releases might be due to a stimulation of lymphocytes and/or monocyte–lymphocyte interaction. However, our results that the 5-HT-induced modulation of TNF-α production is due to activation of the 5-HTR4 and 5-HTR7 are in accordance with observations on monocyte-derived dendritic cells (9).

By reconstitution experiments, it has been shown that 5-HTR4 and 5-HTR7 couple to Gs protein-mediated stimulation of adenylyl cyclase (9, 11). Our data show that cAMP levels increased upon stimulation of monocytes with 5-HTR4 and 5-HTR7 agonists, pointing to a functional expression of these receptors in human monocytes. In addition, reports on cAMP modulation of IL-1β, IL-6, IL-12p40, IL-8/CXCL8 and TNF-α in other cell types are in good accordance with our data (12, 23, 24). Moreover, it is known that in contrast to IL-12p70 heterodimers, IL-12p40 monomers and homodimers possess inhibitory effects on Th1 cells, leading to the creation of a Th2 environment (27). Therefore, IL-12p40 analyzed in this study has a role similar to that of 5-HT in the modulation of the

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>2-MHT (5-HTR4 agonist)</th>
<th>2Me5HT (5-HTR3 agonist)</th>
<th>8-HDPAT (5-HTR7 agonist)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50 000 ± 4082</td>
<td>90 250 ± 3416**</td>
<td>98 500 ± 3292**</td>
<td>80 000 ± 5292**</td>
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<td>RS 39604 (5-HTR4 antagonist)</td>
<td>48 670 ± 5696</td>
<td>49 330 ± 7965</td>
<td>n.d.</td>
<td>72 400 ± 2906*</td>
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<td>SB 269970 (5-HTR7 antagonist)</td>
<td>49 330 ± 3480</td>
<td>86 330 ± 4380*</td>
<td>n.d.</td>
<td>52 670 ± 2333</td>
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<td>Pimozide (5-HTR7 antagonist)</td>
<td>46 330 ± 4372</td>
<td>81 000 ± 3786*</td>
<td>n.d.</td>
<td>53 670 ± 4055</td>
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<tr>
<td>Y-25130 (5-HTR3 antagonist)</td>
<td>53 400 ± 3600</td>
<td>n.d.</td>
<td>59 350 ± 5350</td>
<td>n.d.</td>
</tr>
<tr>
<td>RS 39604 (5-HTR4 antagonist)</td>
<td>48 201 ± 5135</td>
<td>n.d.</td>
<td>60 000 ± 4200</td>
<td>n.d.</td>
</tr>
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</table>

LPS-stimulated monocytes were pre-incubated with 10^{-7} M of the selective 5-HTR4 agonist RS 39604 or the 5-HTR7 agonists Pimozide and SB 269970 or with 10^{-5} M of the selective 5-HTR3 agonists AP and Y-25130. Monocytes were then stimulated with 10^{-4} M 2-MHT, 2Me5HT or 8-HDPAT. IL-6 production was measured after 24 h. Data are given in pg ml^{-1} × 100 000 cells and represent means ± SEM (n = 3); n.d. = not done. Global differences between groups: P < 0.0001 (analysis of variance); **P < 0.01; *P < 0.05 compared with untreated cells (Tukey’s multiple comparison test).

Fig. 6. 5-HT stimulates production of IL-1β in monocytes. LPS-incubated monocytes were stimulated with the indicated concentrations of 5-HT or isotype receptor agonists. Supernatants were collected 24 h after stimulation and were measured by ELISA. Results are given as mean ± SEM (n = 4).

Fig. 7. On stimulation of monocytes with 5-HT, 5-HTR4 and 5-HTR7 agonists cAMP levels increase in resting (A) and LPS-incubated monocytes (B). Monocytes were stimulated with the indicated concentrations of 5-HT, the 5-HTR4 agonist 2-MHT or the 5-HTR7 agonist 8-HDPAT. The cAMP levels are expressed as index representing the ratio between values obtained for stimulated cells and cells incubated in control medium. Data are means ± SEM (n = 4).
Serotonin and monocytes

immune response toward Th2-dominated responses as seen in asthma (10).

In summary, we showed that 5-HT modulates in human monocytes the release of different cytokines and chemokines, mainly via 5-HTR3, 5-HTR4 and 5-HTR7 activation. These responses might contribute to the development of different inflammatory patterns, pointing to a regulatory role of 5-HT in the immune system.

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AP</td>
<td>3-(4-allylpiperazin-1-yl)-2-quinoxalinecarbonitrile maleate</td>
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<tr>
<td>BRL 54433</td>
<td>3-(1-methyl-piperidin-4-yl)-1H-indol-5-ol maleate salt</td>
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<td>cAMP</td>
<td>cyclic AMP</td>
</tr>
<tr>
<td>CT</td>
<td>cycle threshold</td>
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<tr>
<td>DOI</td>
<td>R-(+-)-DOI-hydrochloride</td>
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<td>8-HDPAT</td>
<td>8-hydroxy-9-(3H)-pyridazino[1,2-c]quinoline hydrobromide</td>
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<td>5-HT</td>
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References