Expression of somatostatin, cortistatin, and somatostatin receptors in human monocytes, macrophages, and dendritic cells

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Dalm, Virgil A. S. H., P. Martin van Hagen, Peter M. van Koetsveld, Sam Achilefu, Adriaan B. Houts Muller, David H. J. Pols, Aart-Jan van der Lely, Steven W. J. Lamberts, and Leo J. Hofland. Expression of somatostatin, cortistatin, and somatostatin receptors in human monocytes, macrophages, and dendritic cells. Am J Physiol Endocrinol Metab 285: E344–E353, 2003. First published May 7, 2003; 10.1152/ajpendo.00048.2003.—Increasing evidence suggests that neuropeptides play a role in the regulatory mechanisms between the neuroendocrine and immune systems. A differential expression of the five known somatostatin (SS) receptors (sst1–5) has been demonstrated in human immune cells and tissues. However, little is known concerning regulation and expression of sst1–5 and the peptide SS. Therefore, we investigated the expression and the time-dependent regulation of sst1–5, SS, and cortistatin (CST), a novel SS-like peptide, in human monocytes (MO), monocyte-derived macrophages (MP), and dendritic cells (DC) in the basal and lipopolysaccharide (LPS)-activated state. MO, MP, and DC selectively expressed sst2 mRNA. SS mRNA was not detectable, whereas all samples expressed CST mRNA. Expression levels of sst2 and CST mRNA showed marked differences and were in the rank order of MP >> DC >> MO. LPS stimulation did not induce expression of SS or sst1,3,4,5. However, sst2 mRNA expression was upregulated significantly by stimulation with LPS. CST mRNA was upregulated as well. During differentiation of MO in MP or DC, time-dependent, significantly increasing sst2 and CST mRNA levels were found. By confocal microscopy, the presence of sst2 receptors was demonstrated on MP, but not on DC. This study demonstrates for the first time a selective and inducible expression of the recently discovered CST, as well as sst2, in human monocyte-derived cells, suggesting a role for a CST-sst2 system rather than a SS-sst2 system in these immune cell types.

cellular differentiation; cellular activation; neuropeptides; messenger ribonucleic acid

SOMATOSTATIN-14 and somatostatin-28 are 14- and 28-amino acid neuropeptides, which are mainly produced in the central nervous system, gastrointestinal tract, and endocrine glands (40, 41). In these systems, somatostatin (SS) has a predominant inhibitory action, especially with regard to the release of mediators, such as hormones (12, 13, 36). SS acts via G protein-coupled seven-transmembrane receptors of which five different subtypes have been cloned, named sst1–5 (35). In addition to their role in endocrine tissues, it is hypothesized that neuropeptides might play a regulatory role in the human immune system as well (30, 32, 48). As a neuropeptide, a role in the immune system might be ascribed to SS and its receptors (30). In contrast to the human immune system, the role of SS and its receptors has been studied extensively in granulomas induced by Schistosoma Mansoni infection in the murine immune system (57). Murine T lymphocytes selectively expressed sst2 (11) and macrophages expressed SS mRNA (56). Treatment of Schistosoma Mansoni-infected mice with SS resulted in granuloma growth inhibition (24). After these studies in the murine immune system, the question was addressed whether SS and sst might play a regulatory role in cells of the human immune system as well. Monocytes and its functionally derived cells, i.e., macrophages and dendritic cells, are important components in different pathways in the human immune system. They play important roles in inflammatory responses by production of both proinflammatory and immunosuppressive cytokines, antigen presentation, and phagocytosis (8, 17, 29, 54). Previous studies have demonstrated binding sites for SS on lymphocytes and monocytes (9, 10), suggesting a role for sst in these cells of the human immune system. Immunohistochemistry studies showed that CD68-positive macrophages in human sarcoid granulomas expressed sst2 (50). Equivocal studies have reported on functions of SS on cells of the monocyte lineage. Both inhibitory (7, 38) and stimulatory (28) effects have been described on cytokine production by macrophages. SS increased cytotoxicity of macrophages against tumor cells as well (37), and SS can act as an antiangiogenic factor by inhibiting endothelial cell growth and monocyte migration (4). However, little is known about the expression and regulation of the different sst subtypes in cells of the monocyte lineage, i.e., monocytes, macrophages, and...
Isolated monocytes were frozen in 10% DMSO medium. Purity gradient centrifugation as described in detail previously for PBMC using a Percoll (density 1.063 g/ml; Pharmacia) centrifugation. Subsequently, monocytes were isolated from a density 1.077 g/ml; Pharmacia, Uppsala, Sweden) gradient by Ficoll mononuclear cells (PBMC) were isolated from buffy coats.

Dendritic cells. Moreover, it is not known whether the natural ligand for the sst, e.g., SS, is expressed itself in cells of the monocyte lineage.

Therefore, we investigated in the present study mRNA expression of sst1-5 and SS in monocytes and monocyte-derived macrophages and dendritic cells. Moreover, we investigated the regulation of the expression of the mRNA encoding an SS-like peptide, cortistatin (CST), that was detected previously in cells and tissues from the human immune system (19) and binds with high affinity to all five sst (26) and, therefore, might serve as an alternative ligand to sst. The sst and CST mRNA levels were studied in monocytes, macrophages, and dendritic cells in both basal and lipopolysaccharide (LPS)-stimulated conditions using a TaqMan assay. To investigate possible regulation of mRNA levels, expression was measured from day 1 to 6 of differentiation of monocytes in both macrophages and dendritic cells. Expression of the sst protein on cell membranes of macrophages and dendritic cells was studied using FITC-labeled octreotide, a fluorescence-labeled sst2-selective agonist, and visualized by confocal microscopy.

MATERIALS AND METHODS

Isolation of blood mononuclear cells. Peripheral blood mononuclear cells (PBMC) were isolated fromuffy coats (Sanquin Blood Bank, Rotterdam, The Netherlands) by Ficoll (density 1.077 g/ml; Pharmacia, Uppsala, Sweden) gradient centrifugation. Subsequently, monocytes were isolated from PBMC using a Percoll (density 1.063 g/ml; Pharmacia) gradient centrifugation as described in detail previously (27). Isolated monocytes were frozen in 10% DMSO medium and stored in −80°C until use.

Cell culture. A frozen suspension of monocytes was thawed rapidly in a water bath at 37°C, and viability was evaluated by Trypan blue exclusion (Life Technologies, Grand Island, NY). Cell viability always exceeded 95%. Cells were seeded at a density of 0.5 × 10^6 cells/cm² in a volume of 1 ml/well in 24-well Nunciele plates (Nalge Nunc International) in RPMI 1640 supplemented with 10% heat-inactivated (30 min for 56°C) FCS (Life Technologies), l-glutamine (2 mM; Life Technologies), and penicillin (1,000 U/ml; Yamanouchi Pharma, Leiderdorp, The Netherlands). Plates were then incubated for 90 min at 37°C to allow adherence of the monocytes to the plate. Thereafter, plates were washed to remove potential contaminating T and B cells. To generate macrophages, fresh medium was added containing granulocyte macrophage colony-stimulating factor (GM-CSF, 500 U/ml; Novartis Pharma, Arnhem, The Netherlands). The cells were cultured for 6 days, with medium refreshment at day 3. To generate dendritic cells, the isolated monocytes were incubated for 6 days with GM-CSF (500 U/ml) and interleukin (IL)-4 (1,000 U/ml), with a medium refreshment at day 3. This method of the generation of macrophages and dendritic cells has been described previously in detail (47).

To study the regulation of sst2, SS, and CST mRNA expression, monocytes, 6-day macrophages, or 6-day dendritic cells were incubated during 24 h without or with LPS (2 μg/ml; Sigma Aldrich, Zwijndrecht, The Netherlands). To study the time course of mRNA expression during differentiation of monocytes into macrophages and dendritic cells, cells were collected at day 1, 2, 3, 4, 5, or 6 of culture without or with a prior 24-h incubation with LPS (2 μg/ml). A schematic overview of the experimental setup is shown in Fig. 1. At the end of each incubation period, cells were collected and used for both PCR and fluorescence-activated cell sorter (FACS) analysis.

FACS analysis. By FACS analysis, evaluating the CD marker pattern of the cells, the phenotypes of the cultured cells were confirmed. Cells were collected using cell scrapers and were centrifuged at 300 g for 5 min. Supernatant was removed, and 25 μl of the diluted monoclonal antibody were added to each cell pellet. The monoclonal antibodies we used included FITC- and phycoerythrin (PE)-labeled antibodies purchased from Becton-Dickinson (San Jose, CA), CD1a-PE (diluted 1:2,50), CD14-PE (1:400), CD26-PE (1:120), CD68-FITC (1:10), CD71-FITC (1:10), CD80-PE (1:50), CD86-FITC (1:100), and human leukocyte antigen class II-PE (1:20). Cells were incubated for 15 min at room temperature. Cells were then washed twice with PBS-0.5% BSA and resuspended in 200 μl PBS-0.5% BSA followed by FACS analysis on a FACSCalibur-FACS (Becton-Dickinson).

RT-PCR studies. RT-PCR was performed as described previously (25). Briefly, poly(A)⁺ mRNA was isolated using Dynabeads oligo(dT)₂₅ (Dynal, Oslo, Norway) from tissue samples and cells. cDNA was synthesized using the poly(A)⁺ mRNA, which was eluted from the beads in 40 μl H₂O for 10 min at 65°C using oligo(dT)₁₂₋₁₈ primer (Life Technologies). One-twentieth of the cDNA library was used for each amplification by PCR using primer sets specific for human SS, sst₁₋₅, CST, and hypoxanthine-phosphoribosyltransferase (HPRT) as a control (see Table 1). The primer set used to detect CST mRNA was adapted from Ejeskar et al. (22). As positive controls for SS, CST, and HPRT, cDNA of human brain RNA (Invitrogen, Groningen, The Netherlands) was used. As positive control for sst₁₋₅, DNA of a B-lymphoblastoid BSM cell line (an epstein-barr virus-transformed B cell line) was used. The PCR reaction was carried out in a DNA thermal cycler with a heated lid (Applied Biosystems, Nieuwerkerk a/d Ijssel, The Netherlands). After an initial denaturation at 94°C for 5 min, the samples were subjected to 40 cycles of denaturation at 94°C for 1 min, annealing for 2 min at 60°C, and extension for 1 min at 72°C. After a final extension for 10 min at 72°C, 10-μl aliquots of the resulting

Fig. 1. Experimental setup of monocyte culture and activation. Isolated monocytes were allowed to differentiate into macrophages and dendritic cells in 6-day cultures. Arrows indicate the different time points at which cells were collected for RT-PCR analysis. In the experiments in which cells were incubated with lipopolysaccharide (LPS), arrows indicate the time points at which cells were collected after a prior incubation for 24 h with LPS. GM-CSF, granulocyte macrophage colony-stimulating factor; II, interleukin.
PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide. The identity of the products was confirmed by direct sequencing using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer’s protocol.

Quantitative PCR. To quantify sst2 and CST mRNAs, a quantitative PCR was performed by TaqMan Gold nucleic assay (Perkin Elmer, Foster City, CA) and the ABI PRISM 7700 sequence Detection System (Perkin Elmer) for real-time amplification, according to the manufacturer’s protocol. Quantitative real-time PCR (Q-PCR) was performed for sst2 only, because no expression of the other subtypes was detected in the cells we investigated. The primer sequences that were used included: sst2 forward, 5'-TATATC-3'; sst2 reverse, 5'-ATGCCAAGATC-3'; CST forward, 5'-GAAGACCATAC-3'; and HPRT reverse, 5'-TCAAATCCAACAAAGTCTGGC-3'.

Table 1. Sequence of primers used for RT-PCR

<table>
<thead>
<tr>
<th>Sequence (5'→3')</th>
<th>Size of PCR Product, bp</th>
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<tbody>
<tr>
<td>Sst1 (forward)</td>
<td>ATGGTGCGCCTCAAGGCCGG</td>
</tr>
<tr>
<td>Sst1 (reverse)</td>
<td>CGCGTGGCGTGAATGGCAA</td>
</tr>
<tr>
<td>Sst2 (forward)</td>
<td>CGCAAGATAGAAGCATACTAC</td>
</tr>
<tr>
<td>Sst2 (reverse)</td>
<td>GATGTCACGTCAGAACGAG</td>
</tr>
<tr>
<td>Sst3 (forward)</td>
<td>TGATCCTGCTGCTGTTACCTG</td>
</tr>
<tr>
<td>Sst3 (reverse)</td>
<td>GCAGCAAGGAGAGAAGCTCCAG</td>
</tr>
<tr>
<td>Sst4 (forward)</td>
<td>ACTCTGCGAGCAAGCAAGCA</td>
</tr>
<tr>
<td>Sst4 (reverse)</td>
<td>ATGAAAGCTGTCAGACAGGA</td>
</tr>
<tr>
<td>Sst5 (forward)</td>
<td>CGCTTCTACATCTACATGACG</td>
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<td>Sst5 (reverse)</td>
<td>GCGGAGGTAGAGATGAGGTA</td>
</tr>
<tr>
<td>Hprt (forward)</td>
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<td>Hprt (reverse)</td>
<td>CAAATCCAAACAGATGGCGC</td>
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<td>Somatostatin (forward)</td>
<td>GATGCTGTGCGGCGCTGAGG</td>
</tr>
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<td>Somatostatin (reverse)</td>
<td>ACAGAGTCTGAAAGGCTCCCA</td>
</tr>
<tr>
<td>Cortistatin (forward)</td>
<td>GCAATATTGCCTTAAAGACGA</td>
</tr>
<tr>
<td>Cortistatin (reverse)</td>
<td>TGGAGGAGGAGGAGGAAGAT</td>
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</tbody>
</table>

Data were analyzed statistically using SPSS for Windows, release 9.0 (SPSS, Chicago, IL). The differences in expression levels of sst2 and CST mRNA in the experiments were determined using one-way ANOVA. When significant overall effects were obtained by ANOVA, multiple comparisons were made using the Newman-Keuls test.

RESULTS

By FACS analysis, the phenotypes of the isolated monocytes, cultured macrophages, and dendritic cells were confirmed. In Table 2, the mean percentages of positively stained cells are shown. Monocytes showed low expression of CD80 compared with macrophages (at day 6) and dendritic cells (at day 6). CD80 is upregulated on dendritic cells in vitro.

Table 2. Expression pattern of CD markers on monocytes, macrophages, and dendritic cells

<table>
<thead>
<tr>
<th>CD14</th>
<th>CD68</th>
<th>CD71</th>
<th>CD26</th>
<th>HLA-DR</th>
<th>CD1a</th>
<th>CD80</th>
<th>CD86</th>
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<td>60</td>
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<td>90</td>
<td>85</td>
<td>95</td>
<td>21</td>
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<td>78</td>
<td>13</td>
<td>14</td>
<td>67</td>
<td>95</td>
<td>83</td>
<td>95</td>
<td>18</td>
</tr>
</tbody>
</table>

Values represent the mean percentage of positively stained cells for the subsequent CD-markers and are the results of 3 independent experiments.
upregulated in macrophages, with a peak at day 3 of culture, and declined thereafter, whereas expression of CD80 in dendritic cells was upregulated and remained stable (47). Dendritic cells express lower CD68, CD71, and CD86 compared with macrophages. This has been described previously (18), although also higher expression levels of CD71 expression have been reported (55). Dendritic cells show a high expression of CD1a, which is known as a dendritic cell marker (45), together with the finding that addition of IL-4 to culture results in cells becoming nonadhesive (47).

By RT-PCR, as presented in Fig. 2, top, a selective expression of sst2 mRNA was found in monocytes and in vitro cultured macrophages and dendritic cells. Activation of these cells with 2 μg/ml LPS did not result in a different expression pattern of the sst subtypes (Fig. 2, bottom). At lower concentrations of LPS (range 10 ng/ml-1 μg/ml), sst2 mRNA was expressed selectively as well (data not shown). The expression of SS mRNA itself could not be detected in any of these cell types, neither in the basal nor in the LPS-activated state (Fig. 3). On the other hand, we detected the expression of the mRNA encoding a recently discovered SS-like peptide, i.e., CST, in monocytes, macrophages, and dendritic cells. As a control, human thymic tissue was used, which expresses both SS and CST mRNA (Fig. 3). To evaluate the quantitative expression of the different mRNAs found by RT-PCR, Q-PCR was used for detection of sst2 and CST mRNA levels. We detected relative low expression levels of sst2 mRNA in cells under basal conditions (macrophages and dendritic cells showed an ~10-fold higher sst2 mRNA expression compared with monocytes, \( P < 0.001 \)), whereas sst2 mRNA expression was significantly upregulated when cells were stimulated with 2 μg/ml LPS (Fig. 4). In experiments using increasing concentrations of LPS (range 10 ng/ml-2 μg/ml), 2 μg/ml LPS was shown to give maximal induction of sst2 mRNA expression (data not shown). As shown, LPS induced an ~40 (\( P < 0.0001 \)), 200 (\( P < 0.0001 \)), and 20 (\( P < 0.001 \))
investigate the upregulation of both sst2 and CST phages, and dendritic cells, respectively. To further measure sst2 and CST mRNA levels. During differentiation of monocytes into macrophages and dendritic cells, using Q-PCR, no expression of sst2 mRNA could be detected in macrophages and dendritic cells until day 6 (data not shown). In the LPS stimulation experiments, sst2 mRNA expression was detected in dendritic cells only at days 5 (P < 0.0001) and 6, whereas sst2 mRNA expression in macrophages was low and relatively constant at days 1–4, with a significant increase at days 5 and 6 (P < 0.0001 vs. day 4). This is shown in Fig. 5. In contrast to the time-dependent basal sst2 mRNA expression, we could detect sst2 mRNA expression in LPS-stimulated macrophages at days 1–5 of culture, although it was very low. However, in LPS-stimulated dendritic cells, we could not detect sst2 mRNA expression at days 1–4. This finding is in accordance with the much lower expression of sst2 mRNA in dendritic cells in both basal (data not shown) and LPS-stimulated conditions (Fig. 5) when compared with macrophages and suggests that sst2 mRNA expression levels in LPS-stimulated dendritic cells are below assay detection limits, rather than dendritic cells do not respond to the LPS stimulation. In unstimulated cells, expression of CST mRNA in macrophages increased after day 1 and reached its maximum already at day 2 (P = 0.01; Fig. 6). Dendritic cells showed maximal expression of CST mRNA absence of LPS.

Whereas RT-PCR demonstrated that SS mRNA was absent after stimulation of the cells with LPS, expression of the CST mRNA as a possible alternative ligand for sst2 in these cells was upregulated upon LPS activation (Table 3). CST mRNA was upregulated significantly by ~2 (P = 0.002), ~3 (P < 0.0001), and 10 (P < 0.0001)-fold in activated monocytes, macrophages, and dendritic cells, respectively. To further investigate the upregulation of both sst2 and CST mRNA by LPS stimulation in monocytes and mature macrophages and dendritic cells, expression and possible regulation of both mRNA levels were studied during differentiation of monocytes into its derived cells. Cells were collected from day 1 to 6 of culture to measure sst2 and CST mRNA levels. During differentiation of monocytes into macrophages and dendritic cells, cells were incubated without or with LPS for 24 h at days 1, 2, 3, 4, 5, or 6 of culture. The expression levels of both sst2 and CST mRNA were measured during this time-dependent differentiation of monocytes into macrophages and dendritic cells. In unstimulated differentiating cells, using Q-PCR, no expression of sst2 mRNA could be detected in macrophages and dendritic cells until day 6 (data not shown). In the LPS stimulation experiments, sst2 mRNA expression was detected in dendritic cells only at days 5 (P < 0.0001) and 6, whereas sst2 mRNA expression in macrophages was low and relatively constant at days 1–4, with a significant increase at days 5 and 6 (P < 0.0001 vs. day 4). This is shown in Fig. 5. In contrast to the time-dependent basal sst2 mRNA expression, we could detect sst2 mRNA expression in LPS-stimulated macrophages at days 1–5 of culture, although it was very low. However, in LPS-stimulated dendritic cells, we could not detect sst2 mRNA expression at days 1–4. This finding is in accordance with the much lower expression of sst2 mRNA in dendritic cells in both basal (data not shown) and LPS-stimulated conditions (Fig. 5) when compared with macrophages and suggests that sst2 mRNA expression levels in LPS-stimulated dendritic cells are below assay detection limits, rather than dendritic cells do not respond to the LPS stimulation. In unstimulated cells, expression of CST mRNA in macrophages increased after day 1 and reached its maximum already at day 2 (P = 0.01; Fig. 6). Dendritic cells showed maximal expression of CST mRNA absence of LPS.

Table 3. Quantitative CST mRNA expression

<table>
<thead>
<tr>
<th>Relative CST mRNA Expression (fold-induction)</th>
<th>Monocytes</th>
<th>Macrophages</th>
<th>Dendritic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS 0.15 ± 0.05</td>
<td>9.6 ± 1.4*</td>
<td>1.7 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td>+ LPS 0.3 ± 0.04†(2.0)</td>
<td>28.9 ± 3.2‡(3.0)</td>
<td>16.3 ± 2.1‡(9.6)</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented as the means ± SD of 3 experiments and represent cortistatin (CST) mRNA expression in cells of the monocyte lineage. Both unstimulated (–) and LPS-stimulated (+) cells were studied, and differences in expression levels were measured.

* P < 0.0001 vs. monocytes, † P = 0.002 and ‡ P < 0.0001 vs. cells without LPS.

Fig. 4. Regulation of sst2 mRNA expression in monocytes, macrophages, and dendritic cells. Monocytes were allowed to differentiate into macrophages or dendritic cells and were incubated for 24 h with LPS at day 6 of culture. Bars represent sst2 mRNA expression in unstimulated (–) and LPS-stimulated (+) cells, given in arbitrary units, relative to a generated standard curve from a Jurkat (T cell) cell line and adjusted for hypoxanthine-phosphoribosyltransferase (HPRT) expression. P < 0.0001 compared with cells in unactivated state (•) and with monocytes (#). Results are the means of 3 independent experiments using cells from 3 different healthy donors.

Fig. 5. Time-dependent increased sst2 mRNA expression in monocytes during differentiation into macrophages and dendritic cells after stimulation with LPS (2 μg/ml) at the different time points. Bars represent sst2 mRNA expression in macrophages (A) and dendritic cells (B) at day 1, 2, 3, 4, 5, or 6 of culture after a prior incubation for 24 h with LPS, given in arbitrary units, relative to a generated standard curve from a Jurkat (T cell) cell line and adjusted for HPRT expression. * P < 0.0001 compared with expression of sst2 at day 5. Results are the means of 3 independent experiments using cells from 3 different healthy donors.
ready at day 1. LPS-induced CST mRNA expression during differentiation of macrophages was already maximal at day 1 of culture. In LPS-activated dendritic cells, CST mRNA expression was low at day 1, two-thirds of maximum at day 2 ($P < 0.01$), and reached its maximum at day 3 (Fig. 6, $P < 0.01$ compared with CST mRNA expression at day 1).

To demonstrate that sst2 receptors were actually expressed on the cultured cells, macrophages and dendritic cells were incubated with the FITC-labeled octreotate and visualized using a LSM410 confocal laser microscope (Carl Zeiss). Unstimulated macrophages and dendritic cells did not show a positive signal when incubated with octreotate-FITC (data not shown). On the other hand, LPS-stimulated macrophages showed binding of the fluorescent SS analog, as shown by the spots in Fig. 7B. During the first minutes of incubation, a positive signal was detected at the cell membrane (data not shown). After the experiment in time, positive signals were also found inside the cells because of internalization of the receptor-ligand complex. When macrophages were incubated with both octreotate-FITC and high amount of unlabeled octreotide ($10^{-6}$ M), no fluorescent signal could be detected (Fig. 7D), demonstrating specificity of binding of octreotate-FITC to the sst2 receptors. LPS-stimulated dendritic cells did not stain positive when incubated with octreotate-FITC. This is in concordance with the data found with Q-PCR in which we described an ~10-fold lower expression level of sst2 mRNA in LPS-stimulated dendritic cells.

**DISCUSSION**

SS-14 and SS-28 are neuropeptides that play an important role in inhibition of hormone release. SS also functions as a neurotransmitter, immunomodulator, and suppressor of angiogenesis and cell proliferation (15, 16, 34, 39–41). Neuropeptides are involved in the interactions that exist between the human neuroendocrine and immune system (32, 48). A role might be ascribed to SS and its receptors in this network as well. The ssts have been demonstrated in various endocrine and lymphoid tissues by classical ligand-binding studies (31, 42, 44). The role of SS and its receptors in the human immune system is still unclear. In contrast to the human immune system, the role of SS and sst in the murine immune system has been studied extensively by Weinstock and Elliott (57). In their studies, the expression and role of SS and sst in a model of mice infected with a Schistosoma Mansoni infection were evaluated. In granulomas formed after this Schistosoma infection, T lymphocytes expressed sst2 (11), whereas macrophages in these granulomas expressed SS mRNA (56). Stimulation of murine splenic macrophages with IFN-γ induced upregulation of the SS mRNA within 4 h (23). Treatment of Schistosoma-infected mice with SS resulted in granuloma growth inhibition by decreasing the IFN-γ production by T cells. Moreover, in rat lymphoid tissues, SS is expressed (2) and rat T and B lymphocytes from the thymus and spleen synthesize and secrete SS (3). In the human immune system, the role of SS and sst and the regulation of their expression still remain unclear. It is not known whether SS plays a regulatory role in the human immune system and whether this is comparable to the situation in the experimental rodent models. Cells of the monocyte lineage, i.e., monocytes, macrophages, and dendritic cells, are known to be an important component in the human immune system (8, 17, 29, 54).

Therefore, in the present study, we investigated by RT-PCR the expression and regulation of the mRNA levels encoding the five known sst and SS in cells of the human monocyte lineage. We detected expression of only sst2 mRNA in monocytes, macrophages, and dendritic cells. Freshly Ficoll density-gradient isolated monocytes showed no expression of sst2 mRNA (Ligtenauer-Kaligis EGR, Dalm VASH, Oomen SPMA,
The regulation of SS, CST, and SST in the monocyte lineage is crucial for immune system function. SST2 plays an important role in granuloma formation in Schistosoma-infected mice, and SS treatment inhibits granuloma growth through binding to SST2 on T cells and subsequent decline of IFN-γ production by these T cells (11, 24). The question is now addressed whether SST2 also plays a role in the human immune system. In previous studies, SST2 expression was detected in granulomatous diseases as sarcoïd granulomas and rheumatoid arthritis (51, 53). Recently, in a preliminary study, 10 patients with sarcoidosis were treated with the long-acting SS analog, octreotide. Significant clinical improvement was found in these patients (33). In one study, two patients with sarcoidosis were treated with octreotide, an SST2-selective SS analog (50). One patient

Mooij DM, van Hagan PM, Lamberts SWJ, and Holland LJ, unpublished observations). When monocytes were subsequently isolated from PBMC by Percoll density-gradient centrifugation, cells were already activated and expressed SST2 mRNA, explaining the expression of SST2 mRNA in our Percoll-Ficoll isolated monocytes. In addition, LPS-activated macrophages and dendritic cells were studied. LPS was chosen as an activating factor because it is known as an activator of dendritic cells, since it induced maturation and migration (21, 46), and LPS effectively activates macrophages and induces tumoricidal activity (5, 14). When cells were activated with LPS, no expression of other SST subtypes could be detected, suggesting a selective role for SST2 in these cells. Because SS itself has a very short half-life, it is expected that SS to which the SST-bearing cells respond is produced locally, probably by the SST-positive cells itself. However, despite expression of SST2, no expression of the SS mRNA itself could be detected in any of the cell samples tested. Even after activation of the cells with LPS, no SS mRNA could be detected, ruling out the possible autocrine role of SS in the human immune system, in contrast to the rodent immune systems. Interestingly, we found the expression of the mRNA encoding CST, a recently discovered SS-like peptide in all three cell types that were evaluated. CST is a 17-amino-acid peptide that shows structural resemblance to SS (20) and has high binding affinity to the five known ssts (26). In one preliminary report, the expression of CST mRNA has been demonstrated in different human tissues, including leukocytes (22). Previously, we demonstrated a selective expression of CST mRNA in cells and tissues of the human immune system, while no expression of SS mRNA was detected (19).

In the present study, we mainly focused on the expression and regulation of the five ssts in cells of the monocyte lineage. As shown, only the ssts mRNA could be detected by RT-PCR, in both activated and nonactivated cells. A previous study showed that SST2 expression in leukocytes was upregulated after phytohemagglutinin stimulation (52). In this study, however, the regulation of SST2 expression in cells of the monocyte lineage was not addressed. To investigate this regulation, monocytes were cultured in macrophages or dendritic cells, as schematically shown in Fig. 1, and expression levels of SST2 mRNA were measured using Q-PCR. Under basal conditions, expression of SST2 was very low. Macrophages and dendritic cells expressed ∼10-fold higher SST2 mRNA levels than monocytes. In LPS-stimulated cells, expression of SST2 mRNA was upregulated significantly, suggesting a potential role of the SST2 in more mature and activated cells. We demonstrated that SST2 mRNA is upregulated relatively late during culture. In this respect, the dramatic rise in SST2 mRNA between days 4 and 5 of culture is obvious. We observed this dramatic rise during maturation of monocytes into dendritic cells, as well as into macrophages. Therefore, we hypothesize that SST2 expression only reaches significant levels after full maturation of monocytes, irrespective of the direction of differentiation. In the murine immune system, it has been demonstrated that SST2 plays an important role in granuloma formation in Schistosoma-infected mice and that SS treatment inhibited granuloma growth through binding to SST2 on T cells and a subsequent decline of IFN-γ production by these T cells (11, 24). The question is now addressed whether SST2 also plays a role in the human immune system. In previous studies, SST2 expression was detected in granulomatous diseases as sarcoïd granulomas and rheumatoid arthritis (51, 53). Recently, in a preliminary study, 10 patients with sarcoïdosis were treated with the long-acting SS analog, octreotide. Significant clinical improvement was found in these patients (33). In one study, two patients with sarcoïdosis were treated with octreotide, an SST2-selective SS analog (50). One patient
responded very well, whereas the second showed no clinical response. These results, in combination with the expression of sst in cells of the monocyte lineage, indicate that SS or its analog octreotide might have clinical significance in diseases affecting the human immune system, acting via the sst2-expressing macrophages or dendritic cells. As we demonstrated expression of sst2 mRNA in cells of the monocyte lineage and no expression of SS mRNA itself, we also investigated the expression of the mRNA encoding CST as a possible autocrine ligand for the sst2, which has been proposed for SS in the rodent immune system (11, 24, 56, 57). As previously reported (19), we found CST mRNA expression in all cell samples. Therefore, we evaluated the regulation of CST mRNA during differentiation of monocytes and by LPS stimulation.

Monocytes showed low expression levels of CST mRNA, which were considerably upregulated in macrophages and dendritic cells. LPS activation of these cells resulted in a significant upregulation of the CST mRNA levels as well. This upregulation during differentiation and by LPS activation suggests a possible role for CST in these human immune cells, especially in the mature cells and in cells in the activated state. CST mRNA expression was also measured during differentiation of monocytes under basal and LPS-stimulated conditions. Overall, CST mRNA expression was upregulated earlier during differentiation, both in basal and LPS-stimulated conditions, than sst2 mRNA expression. Whereas an autocrine SS-sst2 regulatory pathway has been described in the murine immune system, the absence of SS and presence of CST mRNA suggest a more likely autocrine CST-sst2 pathway in the human immune system. However, different lymphoid organs are innervated by nerves of the sympathetic nervous system and by sensory nerves containing neuropeptides such as SS (49). SS from these nerves might interact with the sst present in the human immune tissue, explaining the absence of SS itself in the immune cells. Moreover, CST is expressed in neurons as well. It may not be ruled out that CST as well can reach the sst via this route. The presence of CST mRNA in the immune cells itself, however, suggests an autocrine role of CST and the sst in addition to a possible paracrine role of CST from the neurons. The presence of functional sst2-binding sites on membranes of the cells, both activated and nonactivated, was investigated by confocal laser microscopy using the new compound, FITC-labeled octreotate. By autoradiography, we confirmed that FITC-octreotate was able to displace binding of [125I-Tyr3]octreotide to sst2 in rat brain tissue with relatively high affinity. In LPS-stimulated dendritic cells, no binding of FITC-octreotate to the sst2 could be visualized. These findings are in concordance with the relatively low expression levels of sst2 mRNA in these cells. On the other hand, on LPS-stimulated macrophages, expression of the sst2 protein was visualized, and the binding of FITC-octreotate was displaced successfully when cells were also incubated with an excess amount of unlabeled octreotide, demonstrating specificity of binding. Thus sst2 receptor levels on unstimulated cells and in LPS-activated dendritic cells are probably below the detection level of our assay. Unfortunately, at this moment, we are unable to detect the protein for CST in the cells expressing CST mRNA. However, the expression of the mRNA and, moreover, the regulation of this expression during differentiation and by activation suggest that CST indeed may play a role in the human immune system. Further functional studies should be performed to elucidate the possible effects of CST on these cells.

In conclusion, we investigated the mRNA expression of the five known ssts in cells of the monocyte lineage, as well as its endogenous ligand SS and an alternative ligand CST. In monocytes, macrophages, and dendritic cells, only expression of sst2 mRNA could be detected, whereas no SS mRNA was expressed. Interestingly, CST mRNA was expressed in all cells.

When cells were stimulated with LPS, sst2 expression was upregulated significantly, pointing to a possible role of at least these receptors in the human immune system. These receptors might show interaction with CST in the immune system, but it is also possible that SS reaches the place of inflammation by nerve endings, in which SS is produced. During differentiation of monocytes into macrophages and dendritic cells, both sst2 and CST mRNA levels were upregulated as well, pointing to a more important role of the sst2-CST system in more mature cells. Interestingly, it seemed that CST mRNA was upregulated earlier during differentiation than sst2 mRNA. Finally, the expression of sst2 has been visualized on membranes of LPS-activated macrophages but not on unstimulated cells and LPS-activated dendritic cells, in concordance with the lower expression levels of sst2 mRNA found with Q-PCR. The functional significance of the expression of CST and sst2 in these cells needs to be further investigated.

DISCLOSURES

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REFERENCES

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