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Cortistatin Rather Than Somatostatin as a Potential Endogenous Ligand for Somatostatin Receptors in the Human Immune System

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Cells of the human immune system have been shown to express somatostatin receptors (sst). The expression of sst suggests a functional role of the peptide somatostatin (SS). However, SS expression has not been demonstrated yet in different human immune tissues. Therefore, we investigated by RT-PCR the expression of both SS and cortistatin (CST), a SS-like peptide, in various human lymphoid tissues and immune cells. We detected SS mRNA expression in the human thymus only, while not in thymocytes. CST mRNA was clearly expressed in the immune cells, lymphoid tissues, and bone marrow. Using quantitative RT-PCR, significant differences in expression levels between tissues were demonstrated. Expression of CST mRNA was up-regulated during differentiation of monocytes into macrophages and dendritic cells and could be up-regulated by lipopolysaccharide stimulation. Two differently sized cDNA fragments of CST were detected in the majority of cells and tissues. However, although both fragments were detected in nearly all T-cell lines (7 of 8), most of the B-cell lines expressed the short fragment only (8 of 10). Using autoradiography, we showed that CST displaced [^{125}I-Tyr^{3}]octreotide binding with relatively high affinity on human thymic tissue and sst$_2$-expressing cells. This is the first extensive study demonstrating that human lymphoid tissues and immune cells express different levels of CST mRNA and that its expression can be regulated. On the basis of these observations, we hypothesize a role for CST as an endogenous ligand of at least the sst$_2$ receptor in the human immune system, rather than SS itself. (*J Clin Endocrinol Metab* 88: 270–276, 2003)

SOMATOSTATIN (SS) IS a neuropeptide that is widely distributed throughout the human body. The central nervous system, gastrointestinal tract, and endocrine glands are the major sites of production (1, 2). SS has a predominantly inhibitory action, especially with regard to the release of mediators, such as hormones (3, 4). The action of SS is mediated through G protein-coupled seven transmembrane receptors of which five different receptors were cloned, named sst$_{1-5}$ (5). The receptor subtypes show a specific distribution in different organs, and the natural ligands SS-14 and SS-28 both have high binding affinity to all five receptor subtypes (5). The sst family can be divided into two subclasses, the first consisting of sst$_2$, sst$_3$, and sst$_5$ and the second consisting of sst$_1$ and sst$_4$, on the basis of differential binding affinities of the various ligands (6). Conformationally restricted octapeptide analogs of SS bind with low affinity to sst$_1$ and sst$_4$, whereas these analogs bind with higher affinity to sst$_2$, sst$_3$, and sst$_5$ receptors. Recently, a peptide bearing strong structural resemblance to SS was discovered. This peptide, named cortistatin (CST), has an identical receptor binding domain (7) and binds with high affinity to all five human sst subtypes (8). CST mRNA was first found to be expressed in the human brain, but in further studies it was also found in other tissues such as stomach, kidney, and leukocytes (9). Until now, no specific receptor for CST has been found. Expression studies by RT-PCR showed that with different primer sets, two differently sized cDNA fragments could be detected, a 317-bp fragment in the human brain, kidney, stomach, and leukocytes, and a 701-bp fragment selectively expressed in the human brain (9).

In previous studies, a differential expression of sst in human immune cells and tissues has been observed. This differential expression of sst on immune cells suggests that these receptors play a role in the human immune system (10). Little is known, however, with respect to the expression of SS in the human immune system. In the human thymus, SS mRNA was found to be synthesized in the epithelial and not in the lymphoid component (11). To explore the expression of SS and its related compound CST, we investigated mRNA expression of SS and CST (isoforms) by RT-PCR in different immune tissues, such as thymus, spleen, and bone marrow. In the human thymus, we distinguished thymic epithelial cells (TEC) from thymocytes to investigate whether differences in expression of CST and SS mRNA exist between these cells in this organ. Moreover, a number of human immune cell subsets, *i.e.* peripheral blood leukocytes (PBMC), monocytes, macrophages, dendritic cells, and B- and T-lymphocytes were studied as well. Finally, SS and CST mRNA expression was evaluated in human T- and B-cell lines of different maturation levels. Using quantitative RT-PCR (Q-PCR), differences in expression levels of CST mRNA between immune cells and tissues were investigated. Monocytes and monocyte-derived macrophages, both unstimulated and lipopolysaccharide (LPS)-stimulated, were evaluated to get insight into the regulation of CST mRNA expression during

Abbreviations: CST, Cortistatin; HPRT, hypoxanthine-guanine phosphoribosyl transferase; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; Q-PCR, quantitative RT-PCR; SS, somatostatin; sst, somatostatin receptor(s); TEC, thymic epithelial cells.

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differentiation of monocytes as well as in cells in activated state. In addition to the mRNA studies, we performed receptor autoradiographic studies using [^{125}I-Tyr^3]octreotide on human thymic tissue and sst_2-transfected cells to evaluate whether CST is able to bind to sst receptors in human immune tissue.

Materials and Methods

Isolation of blood mononuclear cells

PBMC were isolated from buffy coats by Ficoll (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden; density, 1.077 g/ml) density gradient centrifugation. Subsequently, B- and T-lymphocytes were separated from PBMC using CD19 and CD3 antibody-coated magnetic beads, respectively, as described previously in detail (11). Monocytes were isolated from the PBMC fraction using a Percoll (Pharmacia & Upjohn Diagnostics AB; density, 1.063 g/ml) density gradient centrifugation as described before (12). Isolated cells were frozen in 10% dimethylsulfoxide medium and stored in −80 C until use.

Cell culture

Monocytes were cultured and allowed to differentiate into macrophages and dendritic cells as described before (13). Fluorescence-activated cell sorting analysis (data not shown) confirmed phenotypes of the cultured cells. Monocytes and macrophages were stimulated for 24 h with LPS (Sigma Aldrich bv, Zwijndrecht, The Netherlands) at a final concentration of 2 μg/ml. Thereafter, cells were collected and stored at −80 C.

T- and B-cell lines

The human cell lines that we used included $CD3^-$ (HSB-2, Molt-3, ALL-1, MT-1), TCRγδ (Peer), or TCRαβ (Molt-16, HuT-78, HPB-ALL) T-cell lines; and precursor (RS4,11, Nalm-1, BV-173, Nalm-6), mature Burkitt (Raji, Daudi, JY, TMM), and plasma (LP-1, L-363) B-cell lines. Total RNA preparations from these cells were isolated as described before (14).

Samples

Normal thymic tissues were obtained from children during cardiovascular surgery. Thymic epithelial cells were collected as described in detail previously (11). Thymocytes were collected using a filter chamber (NPBI, Emmer-Compascuum, The Netherlands). Splenic tissue was obtained from patients suffering from splenic rupture. The protocols were in accordance with the Helsinki Doctrine on Human Experimentation. Informed consent was obtained. Samples were taken directly at operation, quickly frozen on dry ice, and stored at −80 C. Tissue was squeezed and lysed before use as described below. Human bone marrow cells were obtained from healthy donors after informed consent.

RT-PCR studies

RT-PCR was performed as described previously (11). Briefly, poly A^+ mRNA from tissue samples and cells was isolated using Dynabeads Oligo $(dT)_{25}$ (DynAl AS, Oslo, Norway). cDNA was synthesized from the poly A^+ mRNA, which was eluted from the beads in H_2O for 10 min at 65 C, using Oligo $(dT)_{12–18}$ Primer (Life Technologies, Inc., Gaithersburg, MD). Twenty microliters of the cDNA was used for each PCR amplification using primer sets for human SS, CST, and hypoxanthine-guanine phosphoribosyl transferase [(HPRT) as a control; Table 1]. Two different primer sets were used, CST A and B, to detect expected cDNA fragments of 173 bp and 701 bp, respectively (Fig. 1 and Ref. 9). cDNA of human brain RNA (Invitrogen, Groningen, The Netherlands) was used as a positive control. The PCR was performed as described before (11). Identities of the products were confirmed by direct sequencing using an ABI Prism 3100 Genetic Analyzer (PE Applied Biosystems, Utrecht, The Netherlands) according to the manufacturer's protocol.

TABLE 1. Primers used for RT-PCR analysis

Primer	Sequence (5′—3′)	Expected size
HPRT (forward)	-CAGGACTGAACGTCTTGCTC-	413 bp
HPRT (reverse)	-CAAATCCAACAAAGTCTGGC-	
SS (forward)	-GATGCTGTCCTGCCGCCTCCAG-	349 bp
SS (reverse)	-ACAGGATGTGAAAGTCTTCCA-	
CST A (forward)	-GCAAATTCGCTCTAAACACAGGA-	173 bp
CST A (reverse)	-TTGGGAAGGAGGAGAGGAAAGAT-	
CST B (forward)	-CTCCAGTCAGCCCACAAGAT-	701 bp
CST B (reverse)	-CAAGCGAGGAAAGTCAGGAG-	

Primer sets for CST were derived and/or adapted from Ejeskar *et al.* (9).

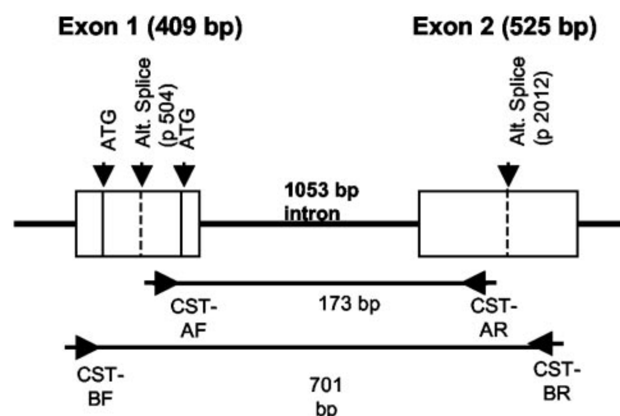

FIG. 1. Overview of the genomic organization of preprocortistatin. Exons are *boxed*. Alternative splicing sites (indicated by p504 and p2012) and primers used for expression studies are indicated. Sequences of different primer sets are listed in Table 1. Overview and primers are adapted from Ejeskar *et al.* (9). CST-AF, Forward primer for CST A mRNA; CST-AR, reverse primer for CST A mRNA; CST-BF, forward primer for CST B mRNA; CST-BR, reverse primer for CST B mRNA.

Q-PCR studies

Total RNA was isolated using either a High Pure RNA Tissue Kit or High Pure RNA Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany) for tissue and cell samples, respectively, according to the manufacturer's protocol. cDNA was synthesized in a reverse transcription reaction as described previously (11), using 2000 ng total RNA per reaction in a total volume of 40 μl. Q-PCR was performed using TaqMan Universal PCR Master Mix (PE Applied Biosystems), 300 nM forward primer, 300 nM reverse primer, 200 nM probe, and 1 μl cDNA template, corresponding to 50 ng total RNA in the reverse transcription reaction, in a total reaction volume of 25 μl. The reactions were performed in an ABI 7700 Sequence Detector (Perkin-Elmer Corp., Oosterhout, The Netherlands). After an initial heating at 95 C for 8 min, samples were subjected to 40 cycles of denaturation at 95 C for 15 sec and annealing for 1 min at 60 C. The primer sequences we used included: CST forward, 5′-GGAGAGAAGCTCCAGTCAGC-3′; and CST reverse, 5′-GGTCCACTCAAACCACCAA-3′. The probe sequence for CST mRNA was 5′-FAM-TATGCTCGCTGTCTCGGCCG-TAMRA-3′. The expression levels of CST mRNA were determined relatively by means of a standard curve generated in each experiment from cDNA isolated from a Raji cell line and are given in arbitrary units.

SS receptor autoradiography

Binding of CST to sst in the human thymus was investigated by autoradiography on unfixed cryosections using [^{125}I-Tyr^3]octreotide, which was prepared as described previously (15). Thymic tissue was collected at operation and immediately frozen, and small parts were embedded in TissueTek (Miles Inc., Elkhart, IN) and processed for cryosectioning. Sections (20 μm) were mounted on gelatin-coated glass slides and stored at −80 C for 3 d to improve adhesion of tissue to the

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slides. Autoradiography was performed as described previously (16) on cryostat sections of human thymic tissue and cell pellets of stably sst_2-transfected CC531 colon adenocarcinoma cells, serving as a control for binding of the different compounds to sst_2 specifically. CC531 cells were originally established from an adenocarcinoma and maintained by serial passage after trypsinization in culture medium (17). The human sst_2 cDNA in pBluescript was a kind gift from Dr. G. I. Bell (Howard Hughes Medical Institute, Chicago, IL). This sst_2 cDNA was excised from pBluescript and inserted into the Nhe-I and Sal-I cloning sites of the retroviral vector pCI-neo (Promega Corp., Madison, WI). After exposure to Kodak Biomax MS film (Eastman Kodak Co., Rochester, NY), the number of pixels was quantified automatically using an AlphaImager 1220 analysis imaging system (Alpha Innotech Corporation, San Leandro, CA). Values are expressed as the percentage of specific binding on control sections without unlabeled peptide.

Results

By RT-PCR studies, we investigated the expression of both SS and CST mRNAs in various tissues and cells of the human immune system. As shown in Fig. 2, panel 2, representing SS mRNA expression, SS was only detected in whole thymic tissue and TEC, not in thymocytes, pointing to an expression of SS only in the epithelial compartment of the human thymus and not in the lymphoid component, as demonstrated previously (11). In addition, no SS mRNA was expressed in human spleen and bone marrow. CST mRNA, however, was clearly expressed in all tissues tested (Fig. 2, panels 3 and 4). The expected 173-bp cDNA fragment of CST is detected in all tissues in which the expected 701-bp cDNA fragment was also found, because the set of primers encoding the short fragment is in between the two primers encoding the longer fragment (Fig. 1). When both cDNA fragments are detected, no statement can be made about whether there might be another isoform of the CST, because the shorter fragment could be part of the longer fragment. However, compared with human brain cDNA, the 701-bp fragment was expressed relatively weakly in the tested samples. In human spleen, we only detected the short, 173-bp cDNA fragment, whereas both fragments were detected in the other three samples, *i.e.* the thymus, thymocytes, and bone marrow. Next to the expression of SS mRNA in human TEC, expression of CST mRNA was detected in these cells as well. As shown in Fig. 2, panel 2, SS mRNA was not detected in monocytes, macrophages, dendritic cells, and PBMC, whereas in all of these cells CST mRNA was clearly detectable. In monocytes, only the short 173-bp fragment could be detected. The results of RT-PCR for the long 701-bp fragment showed a very weak signal, compared with human brain mRNA, and might even be below the detection limit in some cell samples due to its very weak expression. In addition, no SS mRNA expression was detectable in macrophages activated with LPS during 24 and 48 h (data not shown). To further evaluate the expression of SS and the potential two CST isoforms in lymphocytes, we also performed RT-PCR analysis on B- and T-cells in peripheral blood of healthy volunteers and T- and B-cell lines with different levels of maturation. The results of these studies are summarized in Tables 2 and 3. In agreement with the observations in the primary human immune cell types, no expression of SS mRNA could be detected. In B- and T-cells, both fragments of CST were detected (Table 2). Surprisingly, a clear difference was found in the expression of both forms in B- and T-cell lines. Although the 173-bp cDNA CST fragment was found in the majority of the T- (7 of 8) and B- (9

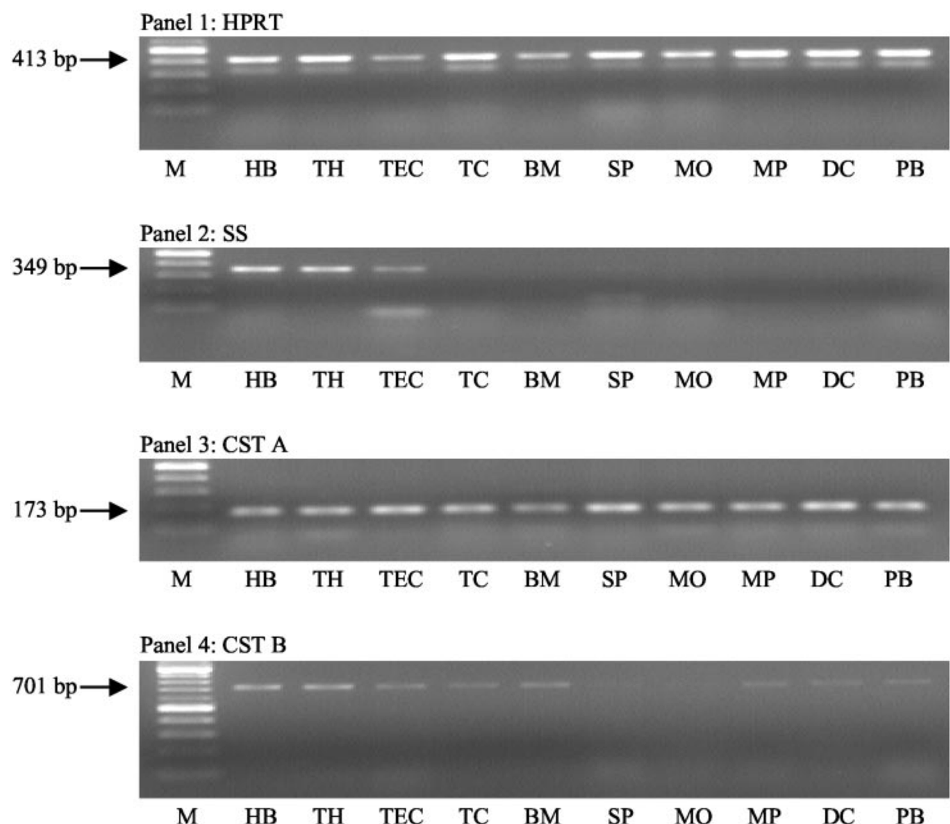

FIG. 2. Expression of SS and CST mRNAs in human immune tissues and cells. Poly-A^+ mRNA was prepared from human brain (HB), thymus (TH), thymic epithelial cells (TEC), thymocytes (TC), bone marrow (BM), spleen (SP), monocytes (MO), macrophages (MP), dendritic cells (DC), and PBMCs (PB). cDNA was synthesized and amplified using primers specific for HPRT (413-bp fragment; panel 1), SS (349-bp fragment; panel 2), and two isoforms of CST (173 and 701 bp, respectively; panels 3 and 4).

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TABLE 2. mRNA expression of SS and CST in different peripheral blood cells

Cell type	HPRT	SS	CST A	CST B
PBMC	+	−	+	+
Monocytes	+	−	+	−
B-lymphocytes	+	−	+	+
T-lymphocytes	+	−	+	+

Data represent mRNA expression of HPRT, SS, the 173-bp fragment of CST (CST A), and the 701-bp fragment of CST (CST B) in different cell subsets of the human peripheral blood.

TABLE 3. mRNA expression in different T- and B-cell lines

Cell line	HPRT	sst_2	SS	CST A	CST B
T-cell lines					
HSB-2	+	+[b]	−	+	+
Molt-3	+	−	−	+	+
ALL-1	+	−	−	+	+
MT-1	+	+[b]	−	+	+
Peer	+	+[a]	−	+	+
Molt-16	+	−	−	+	+
HuT-78	+	−	−	−	−
HPB-ALL	+	+[a]	−	+	+
B-cell lines					
RS4,11	+	−	−	+	−
Nalm-1	+	−	−	+	+
BV-173	+	−	−	+	−
Nalm-6	+	−	−	+	−
Raji	+	−	−	+	+
Daudi	+	−	−	+	−
JY	+	+[b]	−	+	−
TMM	+	+[b]	−	−	−
LP-1	+	−	−	+	−
L-363	+	−	−	+	−

Total mRNA was isolated from CD3- (HSB-2, Molt-3, ALL-1, MT-1), TCR-$\gamma\delta$ (Peer), or TCR-$\alpha\beta$ (Molt-16, HuT-78, HPB-ALL) T-cell lines and precursor (RS4,11, Nalm-1, BV-173, Nalm-6), mature (JY, TMM), Burkitt (Raji, Daudi), and plasma (LP-1, L-363) B-cell lines. On these samples RT-PCR analysis was performed as described in *Materials and Methods*. Specific primer sets for SS and CST [173-bp (CST A) and 701-bp fragment (CST B)] were used to detect SS or CST mRNA.

[a] Data of sst_2 expression are derived from Lichtenauer *et al.* (18).

[b] Data of sst_2 expression are derived from van Hagen *et al.* (19).

of 10) cell lines, the longer fragment was detected in nearly all T-cell lines (7 of 8), whereas only 2 of 10 B-cell lines expressed this isoform (Table 3).

To evaluate differences in expression levels of CST mRNA between the different human immune cells and tissues, Q-PCR was performed. Results of the Q-PCR experiments are summarized in Table 4.

As shown, significant differences in expression levels of CST mRNA were observed between the different cells and tissues. A relatively high level of CST mRNA was measured in the human thymus and TEC, and a lower expression was detected in spleen and human thymocytes, whereas in human bone marrow CST mRNA levels were below detection limit for Q-PCR. In the cell samples, a relatively low expression level of CST mRNA was detected in PBMC and monocytes, whereas expression of CST mRNA was considerably higher in monocyte-derived macrophages and dendritic cells, approximately 60- and 10-fold, respectively, compared with monocytes. Regulation of CST mRNA expression was studied in monocytes and monocyte-derived macrophages

TABLE 4. CST mRNA expression levels in different human immune cells and tissues

Sample	n	Relative amount of CST mRNA	% Relative to TEC
Thymus	3	22 ± 12	71
TEC	3	31 ± 8	100
Thymocytes	2	4.6 ± 0.5	15
Spleen	2	2.8 ± 0.9	9
Bone marrow	2	< Detection limit	
PBMC	3	0.8 ± 0.2	2.5
Monocytes	4	0.15 ± 0.05	0.5
Macrophages	4	9.6 ± 1.4	31
Dendritic cells	4	1.7 ± 0.3	5.5

Data represent the quantified expression levels of CST mRNA in different human immune cells and tissues. Values are presented as arbitrary units, relative to a standard curve generated from a Raji cell line and are results from Q-PCR experiments using 50 ng/μl total RNA for the reverse transcription reaction per sample. n represents the number of samples investigated, and the relative amount of CST mRNA is expressed as the mean ± SD of these samples. In the last column, expression of CST mRNA is shown as a percentage relative to expression of CST mRNA in human TEC, which is set at 100%.

cultured for 6 d. Results of these experiments are shown in Fig. 3.

As described above, resting monocytes showed low expression of CST mRNA. When monocytes differentiated into mature macrophages *in vitro*, CST mRNA levels increased approximately 60-fold. LPS stimulation of both monocytes and macrophages resulted in a 2- and 3-fold increase in CST mRNA, respectively, compared with these cells in unstimulated conditions.

To determine whether CST binds to human endogenously expressed sst, we performed displacement studies using autoradiography on tissue sections of human thymus using [^{125}I-Tyr^3]octreotide. [^{125}I-Tyr^3]octreotide binding was mainly localized in the medullary region of the thymus. Unlabeled octreotide, CST-17, and SS were used to investigate displacement of [^{125}I-Tyr ^{3}]octreotide binding from its receptor, which in the human thymus probably is sst_2, as previously described (11). Binding of [^{125}I-Tyr^3]octreotide was displaced in a dose-dependent manner by unlabeled octreotide, SS, and CST-17. Figure 4, *left panel*, shows that octreotide and SS both displaced [^{125}I-Tyr^3]octreotide binding with high affinity (IC_{50} values of 6.8×10^{-10} M and 1.9×10^{-9} M, respectively). CST-17 displaced [^{125}I-Tyr^3]octreotide binding as well, however, with a slightly lower affinity (IC_{50} value of 2.2×10^{-8} M), compared with SS and octreotide. Binding and displacement of [^{125}I-Tyr^3]octreotide to sst_2 receptors was confirmed on human sst_2-transfected cells as shown in Fig. 4 (*right panel*). The IC_{50} values for displacement of [^{125}I-Tyr^3]octreotide binding to sst_2 receptors were 1.2×10^{-9}, 1.7×10^{-9}, and 4.0×10^{-9} M, respectively, for SS, octreotide, and CST-17.

Discussion

Neuropeptide hormones are involved in a pattern of the complex interactions that exist between the human neuroendocrine and immune system. A place in this network might be hypothesized for SS and its receptors as well. The sst have been demonstrated in various endocrine and lymphoid tissues by classical ligand binding studies (20–22). SS mRNA itself has been detected only in the epithelial component of

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the thymus (11), although not in thymocytes. In addition, it is not known whether the peptide is expressed in other organs of the human hematological and immune systems, like spleen and bone marrow. The presence of sst, however, in these tissues suggests a regulatory role of these receptors in the human immune system. In contrast to the human immune system, evidence has been provided for the expression and functional role of SS in rat (23) and murine (24) lymphoid tissues. In mice, SS mRNA itself has been detected in splenic macrophages but not in splenic lymphocytes (25), whereas rat B- and T-lymphocytes of spleen and thymus synthesize and release SS (26). In the human immune system, however, it is yet unknown whether SS plays a regulatory role and whether this is comparable to the situation in rodents. Moreover, striking differences between human and rodent species have been demonstrated with respect to the expression of sst subtypes in immune cells. In murine immune cells, predominantly sst_2 and sst_4 are expressed, whereas in human immune cells, predominantly sst_2 is expressed and to a lesser extent sst_3 (18). In a previous study, we found that SS mRNA was only expressed in the human thymus and TEC, but not in the thymocyte compartment of the thymus (11). Our present study shows that none of the other investigated tissues, nor lymphoid cell samples, expressed SS mRNA. Therefore, we analyzed whether a potential novel endogenous ligand for the sst in the human immune system, other than SS itself (*i.e.* CST), is expressed. CST is known to have high binding affinity to all different human sst subtypes (8), due to its structural resemblance to SS.

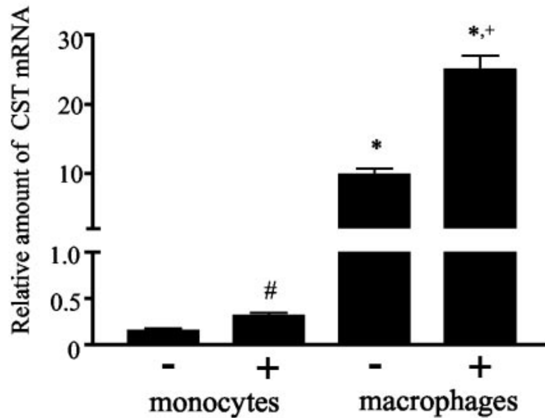

FIG. 3. Regulation of CST mRNA expression in monocytes and macrophages. The *bars* represent expression of CST mRNA in monocytes and macrophages without (−) or with (+) LPS stimulation. The *bars* represent means ± SD of four independent experiments and represent arbitrary units relative to a standard curve generated from a Raji cell line as results of Q-PCR using 50 ng/μl total RNA in the reverse transcription reaction per sample. Monocytes, 0.15 ± 0.05; monocytes + LPS, 0.3 ± 0.04; macrophages, 9.6 ± 1.4; and macrophages + LPS, 28.9 ± 3.2. *, $P < 0.0001$ *vs.* monocytes; #, $P = 0.002$; and +, $P < 0.0001$ *vs.* cells without LPS.

We found the expression of both SS mRNA and CST mRNA in human thymus and also in isolated TEC, whereas squeezed thymocytes expressed CST mRNA only (11). In human spleen and bone marrow, no SS mRNA, but only CST mRNA was detected. Moreover, in the different single cell types tested, *i.e.* human PBMC, T- and B-lymphocytes, monocytes and their functionally derived cells, *i.e.* macrophages and dendritic cells, no SS mRNA was detected, whereas CST mRNA was clearly expressed in all of these cells. These data point to a rather universal expression of CST in immune cells. By Q-PCR, significant differences in CST mRNA expression levels were detected between the human immune cells and tissues. High expression of CST mRNA was detected in thymic tissue and TEC, although we found lower expression in

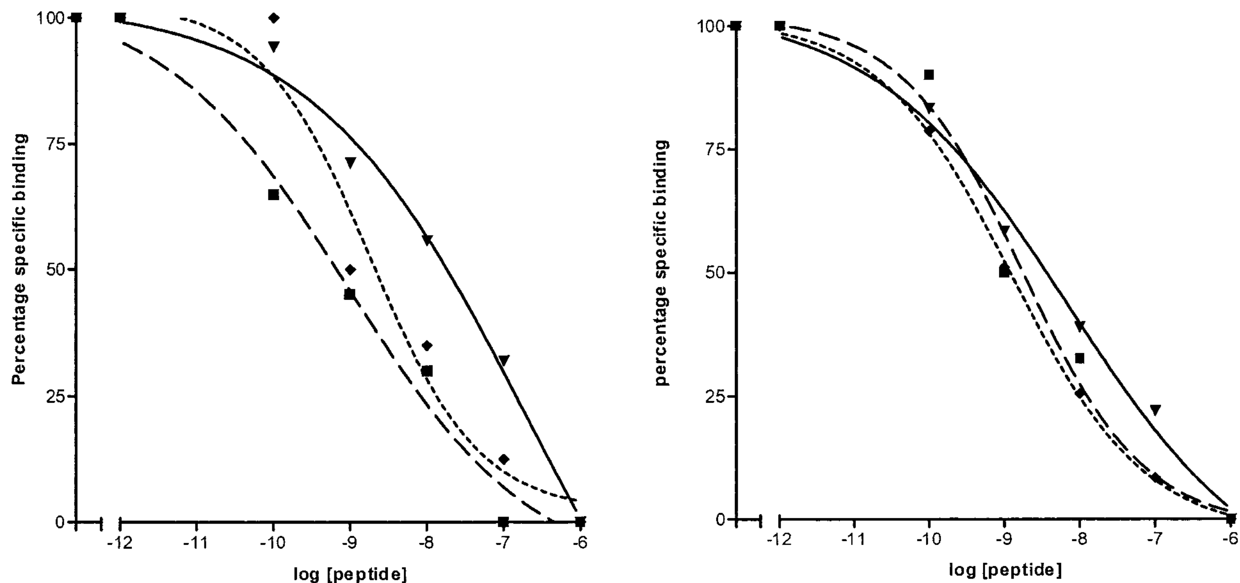

FIG. 4. Displacement of [^{125}I-Tyr^3]octreotide binding on cryostat sections of human thymic tissue (*left*) and cell pellets of human sst_2-expressing cells (*right*). Twenty- and 10-μm sections, respectively, were incubated with [^{125}I-Tyr^3]octreotide without or with increasing concentrations of unlabeled SS, octreotide, or human CST-17. After exposure to Kodak Biomax MS film, the number of pixels was quantified automatically using an AlphaImager 1220 analysis imaging system (Alpha Innotech Corporation, San Leandro, CA). Values are expressed as the percentage of specific binding on control sections without unlabeled peptide. Symbols represent displacement by SS (◆), octreotide (■), or CST-17 (▼).

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thymocytes and splenic tissue, and in bone marrow expression levels were even below detection limit for Q-PCR. The meaning of these differences in expression levels is still unknown, but it might be hypothesized that, although CST mRNA is rather universally expressed in immune cells and tissues, CST plays a more important regulatory role in certain tissues, *i.e*. the tissues in which CST mRNA is highly expressed. Moreover, we evaluated SS mRNA expression levels in TEC (data not shown) and found very low expression levels of SS, whereas high levels of CST mRNA were detected. This difference might indicate that CST plays a more important role in this cell system than SS. However, this hypothesis should be further studied. Interestingly, differences in CST mRNA expression levels were observed between monocytes and their functionally derived cells, *i.e*. macrophages and dendritic cells. Although expression of CST mRNA was relatively low in monocytes, expression in macrophages and dendritic cells was considerably higher (approximately 60- and 10-fold, respectively), pointing to an up-regulatory mechanism for CST mRNA expression during differentiation and maturation of monocytes into both macrophages and dendritic cells and, possibly, a more important role for CST in the mature immune system. In concordance with this hypothesis, we observed in our LPS-stimulation experiments that stimulated monocytes and macrophages expressed approximately 3-fold higher levels of CST mRNA than unstimulated cells did. From these experiments, we hypothesize that CST may have a regulatory role in the human immune system in inflammatory reactions possibly acting via the sst, rather than SS, which is not expressed itself in cells and tissues of the human immune system. However, the exact significance of CST in the human immune system needs to be established.

In the present study, we detected two differently sized fragments of CST mRNA, one 173-bp and one 701-bp fragment (Fig. 1). Because one of these fragments (the 701 bp) was very weakly expressed, compared with its expression in the human brain, this might explain why in a previous study this form was not detectable in leukocytes (9). Although in our study the majority of primary human immune cells and tissues express both isoforms, we observed a clear difference in their expression in human T- and B-cell lines. In contrast to the T-cell lines, B-cell lines expressed mainly one isoform. No clear relationship between the absence and the presence of the expression of the 701-bp fragment and the level of maturation was observed. The exact biological significance of the detection of the two isoforms is still unknown but suggests a differential regulatory mechanism. According to the literature (9), both sets of primers should detect the sequence coding region for the bioactive form of CST, *i.e*. CST-17. Although expression of CST mRNA is abundant in cells and tissues of the human immune system and differences in expression levels have been demonstrated, the question still stands whether the CST mRNA is successfully translated into the CST protein in these cells and tissues. Further studies will be necessary to clarify this issue. However, our observations that CST mRNA expression was up-regulated during differentiation and maturation of monocytes into macrophages and dendritic cells and by LPS stimulation of monocytes and macrophages suggest that CST itself plays at least a role in these cells.

To investigate whether CST was able to bind to sst in human thymus, sst autoradiography was performed. We found that CST concentration dependently was able to displace [^{125}I-Tyr^3]octreotide binding in the human thymus. It is known that [^{125}I-Tyr^3]octreotide binds to sst_2 receptors in the human thymus. These receptors are predominantly localized in the medullary region of this organ (22, 27). Compared with SS and octreotide, CST has a slightly lower affinity for sst_2 in human thymic tissue (approximately 20-fold). These data demonstrate for the first time that CST is able to displace with a relatively high affinity [^{125}I-Tyr^3]octreotide binding from sst endogenously expressed in a human sst-expressing organ. As indicated before, previous studies have already shown that CST has a high affinity for the different human sst subtypes in stably transfected cell lines (8). In agreement with these observations, we also found that CST-17 displaced [^{125}I-Tyr ^{3}]octreotide binding from sst_2 receptors in a transfected cell model with an affinity comparable with that of SS and octreotide. Because no SS mRNA, but only CST mRNA was detected in the samples tested, we hypothesize on the basis of both the expression of CST and the observation that CST is able to bind to human sst_2 receptors, that CST, rather than SS, may act via the sst in the human immune system in an autocrine and/or paracrine function. With regard to the slightly lower binding affinity of CST to sst, the question can be addressed whether a specific CST receptor, for which CST has a higher binding affinity, is present in lymphoid cells and tissues. In brain, evidence for a specific CST receptor has been proposed on the basis of findings that the effects of CST in human brain can be distinct from those found for SS (28). However, studies in rat and mouse brain showed that CST and SS expression is differently regulated by different stimuli (29) and SS and CST both use different signal transduction pathways to generate their effects (29). These findings might explain the different effects of SS and CST in the brain as well, when both peptides would act via the sst.

Functional aspects of CST in the human immune system are unknown at present. There is, however, a striking resemblance between CST/SS and the family of chemokines. The latter are chemotactic cytokines that coordinate development, differentiation, trafficking, and effector functions of leukocytes and their progenitors (30). As CST and SS, chemokines are also cyclic peptides that are characterized by their relative position of the cysteine residues. They all induce cell migration and activation by binding to specific G protein-coupled seven transmembrane receptors on the target cells (30). SS analogs have a predominantly immunosuppressive effect in the human immune system but exert powerful, unique migration-inducing effects on normal and leukemic hematopoietic progenitors (31).

In summary, we described for the first time that no SS mRNA was detectable in different human immune and hematological cell types, whereas in all samples tested CST mRNA was found. Significant differences in expression levels were observed between the different immune cells and tissues investigated. CST mRNA was up-regulated during differentiation of monocytes into both macrophages and

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dendritic cells and by LPS stimulation of monocytes and macrophages, pointing to a regulatory role of CST in the human immune system. Interestingly, human B-cell lines mainly expressed only one of two cDNA fragments, pointing to the existence of two CST isoforms in the human immune system. Considering the important role of dendritic cells and macrophages in autoimmune diseases and the expression of sst_2 receptors on these cell types (16, 32), the functional significance of CST in these cells should be evaluated in further studies. In addition, human T- and B-lymphocytes may express sst, pointing to a potential role of CST in the function of these cells as well.

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