Somatostatin (SS) receptor (sst) scintigraphy is widely used in the visualization of neuroendocrine tumors expressing sst, and radiotherapy using radionuclide-labeled SS analogs has been introduced for treatment of patients with neuroendocrine tumors. Previous sst scintigraphy studies revealed that malignant lymphomas can also be visualized using this technique. The question has been addressed whether lymphomas might also be possible targets for radiotherapy using radionuclide-labeled SS analogs. Therefore, we investigated in vitro the characteristics of lymphoma tissues and lymphoid cell lines to evaluate whether lymphomas can be targets for radiotherapy. Methods: Six orbital lymphomas, 2 Hodgkin’s lymphomas, and 2 non-Hodgkin’s lymphomas from the neck region were collected. Reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative RT-PCR were performed to detect and quantify the expression of sst1, sst2, sst3, and sst5 mRNA. Immunohistochemistry was used to investigate expression of sst2 and sst3. Membrane binding studies and in vitro internalization experiments using [125I-Tyr3]octreotide by lymphoid cell lines (JY, TMM, APD) and primary cells derived from a B-cell–derived chronic lymphocytic leukemia. Results: A selective expression of sst2 and sst3 messenger RNA (mRNA) was demonstrated. By quantitative RT-PCR, expression levels of sst2 and sst3 mRNA were relatively low. Autoradiography studies revealed low binding of [125I-Tyr3]octreotide by lymphoid cell lines (JY, TMM, APD) and primary cells derived from a B-cell–derived chronic lymphocytic leukemia. Conclusion: On the basis of our findings, we conclude that lymphomas do not appear to be candidates for radiotherapy using radionuclide-labeled SS analogs. However, lymphomas are highly radiosensitive tumors and further clinical studies should be performed to evaluate whether the low receptor density is sufficient for targeting treatment in these tumors.
was known about both receptor subtype expression and receptor density of the sst in lymphoma tissues. Both aspects are very important with respect to the possible future application of sst-targeting radiotherapy. Moreover, lymphomas of several patients are not visualized by sst scintigraphy (15,16). Therefore, the question was addressed whether this was due to a relatively low receptor density or to absent or differential sst subtype expression. In this study, the expression of messenger RNAs (mRNAs) encoding the 5 different sst subtypes was investigated in a series of human lymphomas. Sst mRNA expression levels were measured quantitatively, using quantitative polymerase chain reaction (Q-PCR) and compared with sst mRNA levels in growth hormone (GH)-secreting pituitary adenomas, which express high levels of sst mRNA. In a previous study it was shown that no SS mRNA itself was expressed in normal cells and tissues of the human immune system. However, expression of the mRNA encoding cortistatin (CST) (17), a SS-like peptide, was detected. CST is also capable of binding with a SS-like radiolabeled compound, [125I-Tyr3]octreotide, to evaluate internalization of SS-coupled ligand, and membrane binding studies were performed during diagnostic biopsies and surgical intervention, quickly frozen in liquid nitrogen, and stored at −80°C until use or selected on the presence of archived frozen material. Histopathologic classification of the tissues studied is shown in Table 1. The expression of messenger RNAs (mRNAs) encoding the tissues studied is shown in Table 1. The expression of SST IN LYMPHOMAS: RADIOTHERAPY TARGETS? sst 1, sst 2, sst 3, sst 4, sst 5, SS, and CST mRNA was adapted from Ejeskar et al. (18). Informed consent was obtained from all patients involved. In this study we investigated the mRNA expression of both SS and CST in lymphomas. To answer the question of whether radionuclide-labeled SS analogs may also be used in the treatment of lymphomas, next to the Q-PCR studies, receptor–ligand internalization and membrane binding studies were performed with a SS-like radiolabeled compound, [125I-Tyr3]octreotide, to evaluate internalization of SS-coupled radioactivity by different lymphoid cell lines as a model for binding and internalization of the receptors expressed in the lymphoma tissues. By autoradiography, specific binding of [125I-Tyr3]octreotide, which binds with high affinity to sst 2, was evaluated and quantified in a series of lymphoma sections. The expression of sst 2 and sst 3 was also studied by immunohistochemistry. Finally, to exclude possible mutations in the sst 2 receptor, DNA from 4 lymphomas was analyzed by sequencing.

### MATERIALS AND METHODS

#### Tissues

Orbital lymphomas (n = 6), Hodgkin’s lymphomas (n = 2), and non-Hodgkin’s lymphomas from the neck region (n = 2) were collected during diagnostic biopsies and surgical intervention, quickly frozen in liquid nitrogen, and stored at −80°C until use or selected on the presence of archived frozen material. Histopathologic classification of the tissues studied is shown in Table 1. The expression of SST IN LYMPHOMAS: RADIOTHERAPY TARGETS? sst 1, sst 2, sst 3, sst 4, sst 5, SS, and CST mRNA was adapted from Ejeskar et al. (18). Informed consent was obtained from all patients involved. In this study we investigated the mRNA expression of both SS and CST in lymphomas. To answer the question of whether radionuclide-labeled SS analogs may also be used in the treatment of lymphomas, next to the Q-PCR studies, receptor–ligand internalization and membrane binding studies were performed with a SS-like radiolabeled compound, [125I-Tyr3]octreotide, to evaluate internalization of SS-coupled radioactivity by different lymphoid cell lines as a model for binding and internalization of the receptors expressed in the lymphoma tissues. By autoradiography, specific binding of [125I-Tyr3]octreotide, which binds with high affinity to sst 2, was evaluated and quantified in a series of lymphoma sections. The expression of sst 2 and sst 3 was also studied by immunohistochemistry. Finally, to exclude possible mutations in the sst 2 receptor, DNA from 4 lymphomas was analyzed by sequencing.

#### PCR Studies

Reverse transcriptase PCR (RT-PCR) was performed as described (19). Briefly, poly(A)+ mRNA was isolated using Dynabeads Oligo(dT)25 (Dynal AS) from malignant lymphoma samples. Complementary DNA (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, sst 1-5, and hypoxantine-guanine phosphoribosyltransferase (HPRT) as a control (Table 2). As positive controls for SS, CST, and HPRT, cDNA of human brain RNA (Invitrogen) was used. As positive controls for sst 1-5, DNA from a B lymphoblastoid cell line (BLCL)-BSM cell line (an Epstein–Barr virus-transformed B-cell line) was used. The primer set we used for the detection of CST mRNA was adapted from Ejeskar et al. (20). The PCR was performed in a DNA thermal cycler with heated lid (Applied Biosystems). 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 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.

#### TABLE 1

<table>
<thead>
<tr>
<th>No.</th>
<th>Tissue</th>
<th>mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Classification</td>
<td>sst 1</td>
</tr>
<tr>
<td>1</td>
<td>ML-MALT</td>
<td>−</td>
</tr>
<tr>
<td>2</td>
<td>Follicular center lymphoma</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td>Mantle cell lymphoma</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td>Diffuse large B-cell lymphoma</td>
<td>−</td>
</tr>
<tr>
<td>5</td>
<td>Mantle cell lymphoma</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>Follicular center lymphoma</td>
<td>−</td>
</tr>
<tr>
<td>7</td>
<td>Hodgkin’s lymphoma, stage I</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>Hodgkin’s lymphoma, stage II</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>Low-grade non-Hodgkin’s lymphoma</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
<td>Intermediate-grade non-Hodgkin’s lymphoma</td>
<td>−</td>
</tr>
</tbody>
</table>

ML-MALT = extranodal marginal zone B-cell lymphoma (where MALT = mucosa-associated lymphoid tissue).
The unknown cDNA samples, the starting amounts in the cDNA
ing quantity. With these standard curves and the measured Ct of
unknown cDNA samples. A standard curve was constructed by
cDNAs were ampli-

3100 Genetic Analyzer (Applied Biosystems) according to the
manufacturer’s protocol.

To quantify expression of sst mRNAs, a Q-PCR was performed
by the TaqMan Gold nuclease assay (Perkin Elmer Corp.) and the
ABI PRISM 7700 sequence Detection System (Perkin Elmer) for
real-time amplification, according to the manufacturer’s protocol.
Q-PCR was performed for sst2 and sst3 only, because no expres-
sion of the other sst subtypes could be detected in the tissues and
cells we investigated by RT-PCR. The primer sequences used are
summarized in Table 3. In each experiment, standard curves for
each primer set were included. Known amounts of genomic DNA
containing sst2A and sst5 or dilutions of a pool of HPRT-containing
cDNAs were amplified (in duplicate or triplicate) together with the
unknown cDNA samples. A standard curve was constructed by
plotting the threshold-cycle (Ct) versus the logarithm of the start-
ing quantity. With these standard curves and the measured Ct of the
unknown cDNA samples, the starting amounts in the cDNA
samples were determined in duplicate or triplicate. To correct for
differences in the efficiency of RNA isolation and cDNA synthe-
sis, the amounts of sst2A and sst3 were divided by the amount of
HPRT in a given cDNA sample. Since the absolute copy number of
HPRT templates in the control cDNAs used for the HPRT
standard curve is unknown, the starting amounts are given in
arbitrary units. Reaction conditions were optimized until the SD of the
duplicate determinations of the Ct of standard curve samples was
<3%. A linear correlation existed between the logarithm of the start-
ing amount of the template and the Ct in the range of
>300,000 copies down to approximately 30 copies. Below 30
copies, duplicate measurements displayed less accuracy due to
more delays or failures of amplification. Above 30 copies starting
amount, the SD of the copy number calculated by means of the
standard curve ranged from 5% to 20%. The data of Q-PCR are
therefore presented as the ratio of sst2 or sst3 over HPRT, which
makes it possible to compare the different samples. Because the
absolute copy number of HPRT templates in the control cDNAs
for HPRT is unknown, final data are presented as arbitrary units.

### Internalization Studies

Internalization experiments were performed as described in
detail (21) using [125I-Tyr3]octreotide (kindly provided by Dr.
Wout Breeman, Department of Nuclear Medicine, Erasmus
Medical Center, Rotterdam, The Netherlands) (22). In short, 10^6
cells were seeded per well in 24-well plates (Costar Corning)
and incubated with approximately 400,000 cpm/ml [125I-Tyr3]-
ocotreotide with or without an excess of unlabeled peptide. After
different periods of incubation (30, 60, 120 min), cell-surface-
bound radioligand was removed with a 1-mL acid wash for 10 min
(21). Internalized radioligand was measured as acid-resistant
counts in pellets of the acid-washed cells. The cell lines we studied
included 1 myeloid leukemic cell line (TMM), a lymphoid B-cell
line (JY), an EBV-transfected APD cell line, and EBV-immortal-
ized primary B-cells from a patient with a B-cell-derived chronic
lymphatic leukemia (B-CLL). The differentiation state (marker
pattern) of the cell lines we used was comparable with that of solid
lymphomas or leukemias. To our knowledge, no specific orbit
lymphoma cell lines were reported in the literature. As a positive
control for sst2-mediated internalization, stably sst2-transfected
CC531 colon adenocarcinoma cells were used (CC2B). CC531
cells were established from an adenocarcinoma and maintained by
trypsinization and serial passage in culture medium (23). The
human sst2 cDNA in pBluescript was a kind gift from Dr. Graeme
I. Bell (Howard Hughes Medical Institute). This sst2 cDNA was
excised from pBluescript and inserted into the NheI and SalI
cloning sites of the retroviral vector pCI-neo (Promega Corp.).
This vector was used to generate the CC2B cells.

### Binding Studies

Membrane isolation and binding studies were performed as
described (24) on the above indicated cells. In short, membrane
preparations (corresponding to 30–50 μg of protein) were incu-
bated in a total volume of 100 μL at room temperature for 60 min
with increasing concentrations of [125I-Tyr3]octreotide without
and with an excess (1 μmol/L) of unlabeled octreotide in N-
(2-hydroxyethyl)piperazine-N’-(2-ethanesulfonic acid) (HEPES)
buffer (10 mmol/L HEPES, 5 mmol/L MgCl_2, and 0.02 g/L bac-
tracin, pH 7.6) containing 0.2% bovine serum albumin (BSA).
After the incubation, 1 mL ice-cold HEPES buffer was added to
the reaction mixture, and membrane-bound radioactivity was sepa-
rated from unbound radioactivity by centrifugation during 2 min
at 14,000 rpm in an Eppendorf microcentrifuge.

### Table 2

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′→3′)</th>
<th>Size of PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sst2 forward</td>
<td>AGGGTGGCGCTCAAGAAGGCGG</td>
<td>318</td>
</tr>
<tr>
<td>sst2 reverse</td>
<td>GGGGTCGGCTGCTAATGCAAC</td>
<td></td>
</tr>
<tr>
<td>sst3 forward</td>
<td>GCCAAAGTGAAGAAGCATTAC</td>
<td>414</td>
</tr>
<tr>
<td>sst3 reverse</td>
<td>GATGACCTGCTGCTAGACAGA</td>
<td></td>
</tr>
<tr>
<td>sst5 forward</td>
<td>ATUTTTGCCAGACAGACAGAC</td>
<td>323</td>
</tr>
<tr>
<td>sst5 reverse</td>
<td>AGTAAAGCTGGTCTGCAAGA</td>
<td></td>
</tr>
<tr>
<td>SS forward</td>
<td>GATGCTGCTGCTGGCTGGCTGCT</td>
<td>413</td>
</tr>
<tr>
<td>SS reverse</td>
<td>CGGTTCCTATTCAATCTACAGGG</td>
<td></td>
</tr>
<tr>
<td>CST forward</td>
<td>GGAATTGCTTGGCCTAAGAAGCCCTGA</td>
<td>173</td>
</tr>
<tr>
<td>CST reverse</td>
<td>TTGGAAAGGAGGAGGAGGAGAAGAT</td>
<td></td>
</tr>
</tbody>
</table>

bp = base pairs.

### Table 3

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′→3′)</th>
<th>Size of PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sst2 forward</td>
<td>ATGCAAGATGATGAGACGATTAC</td>
<td></td>
</tr>
<tr>
<td>sst2 reverse</td>
<td>TGAGCTATGAGATGAGCTAC</td>
<td></td>
</tr>
<tr>
<td>sst3 forward</td>
<td>CGCGCTACACCTCTGCTTAC</td>
<td></td>
</tr>
<tr>
<td>sst3 reverse</td>
<td>AGGCGGAGTGAGACGATTTA</td>
<td></td>
</tr>
<tr>
<td>sst5 forward</td>
<td>GCGCGGAGGCCTTACGACGAC</td>
<td></td>
</tr>
<tr>
<td>sst5 reverse</td>
<td>TGGCTTCTGTCTGCTGACCTGT</td>
<td></td>
</tr>
<tr>
<td>HPRT forward</td>
<td>TGAGCTGAGCTGACTGCTTAC</td>
<td></td>
</tr>
<tr>
<td>HPRT reverse</td>
<td>TCAATTGCTTGGCTGACCTGT</td>
<td></td>
</tr>
<tr>
<td>HPRT probe</td>
<td>CAAACTTGGCTGAGCTGAGCTG</td>
<td></td>
</tr>
</tbody>
</table>

FAM = carboxyfluorescein; TAMRA = carboxytetracyclamidine.

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The Journal of Nuclear Medicine • Vol. 45 • No. 1 • January 2004
pellet was washed twice in ice-cold HEPES buffer, and the pellet was counted in a γ-counter (1470 Wizard; Wallac). Specific binding was taken to be the total binding minus binding in the presence of 1 μmol/L unlabeled octreotide.

Binding of [125I-Tyr3]octreotide to sst in the malignant lymphoma tissues was investigated by autoradiography on unfixed cryosections. Malignant lymphoma biopsies were taken and immediately frozen, and small parts were embedded in TissueTek (Miles Inc.) and processed for cryosectioning. Twenty-micrometer-thick sections were mounted on gelatin-coated glass slides and stored at −80°C for 3 d to improve adhesion of the tissue sections to the slides. Autoradiography was performed on cryostat sections of malignant lymphoma tissue and sections of neuroendocrine tumors, serving as a positive control for binding to sst2 specifically, as described (25). Binding of [125I-Tyr3]octreotide was displaced by an excess of unlabeled octreotide to show specificity of binding. The number of pixels was quantified automatically using a Phosphor Imager (Amersham Biosciences). Specific binding was defined by >50% displacement of the autoradiographic signal by excess unlabeled octreotide.

Immunohistochemistry was performed on 5-μm-thick sections cut on a cryostat (Jung CM3000; Leica) as described in detail (26). Sections were incubated overnight at 4°C with sst2 (Biotrend) and sst3 (Biotrend) antibodies and 1:2,000 and 1:3,000 dilutions in phosphate-buffered saline (PBS) + 5% BSA, respectively. A standard streptavidin-biotinylated–horseradish peroxidase complex (ABC kit; Biogenex) was used according to the manufacturer’s protocol. Finally, sections were developed with diaminobenzidine and mounted. Paraffin-embedded sections (5 μm) were deparaffinized, rehydrated, exposed to microwave heating (in citric acid buffer, pH 6.0) at 100°C for 15 min, and rinsed in tap water followed by PBS. Subsequent steps were performed as in the protocol for frozen sections. The antibodies were used at dilutions of 1:2,000 in PBS + 5% BSA and sections were incubated overnight at 4°C. Human pancreatic tissue served as a positive control for expression of sst. Finally, to detect possible mutations in the sst2 gene, purified PCR products were sequenced on an ABI Prism 310 Genetic Analyzer, using a BigDye Terminator Cycle Sequencing Ready Reaction DNA sequencing kit (Applied Biosystems).

RESULTS

Results by RT-PCR are summarized in Table 1. In the 2 Hodgkin’s and 2 non-Hodgkin’s lymphomas from the neck region, only expression of sst2 mRNA could be detected. In all orbital lymphomas, sst2 and sst3 mRNA was demonstrated. SS mRNA could not be detected in any of the tissues tested. However, CST mRNA was expressed in all tissue samples. Results of the Q-PCR are shown in Table 4. By Q-PCR, expression levels of sst2 and sst3 mRNA were studied. Sst2 mRNA levels in the lymphoma tissues were compared with the expression of sst3 mRNA in GH-secreting pituitary adenomas, which express high levels of sst2 mRNA. As shown, the expression levels of sst2 and sst3 mRNA in the lymphoma tissues are relatively low, the highest relative expression of sst2 mRNA in the lymphomas being 208 copies when adjusted for HPRT. When compared with GH-secreting pituitary adenomas, these sst2 mRNA levels were 6- to 200-fold lower in the malignant lymphomas.

Although we found positive signals for sst2 and sst3 mRNA in the different tissues using RT-PCR, we showed by Q-PCR that these bands represent only very low expression levels of sst2. Sst3 mRNA expression levels were very low as well, or even below the detection limit in most tissues tested.

Autoradiographic studies were performed to investigate and quantify binding of the sst3-specific agonist, [125I-Tyr3]octreotide, to sst2 in malignant lymphomas. Rat brain and a human GH-secreting pituitary adenoma served as positive controls. Both control samples showed high specific binding of [125I-Tyr3]octreotide. A series of 6 orbital lymphomas (numbers 1-6) were incubated with [125I-Tyr3]octreotide without or with unlabeled octreotide. Specific binding of [125I-Tyr3]octreotide was detected in all orbital lymphomas. However, compared with rat brain and a GH-secreting pituitary adenoma, total binding of [125I-Tyr3]octreotide was much lower in the malignant lymphomas, as shown in Figure 1. The binding of [125I-Tyr3]octreotide was quantified using a Phosphor Imager. The intensity of black spots, which represent binding of [125I-Tyr3]octreotide to sst2, was set at 100% in the GH-secreting pituitary adenoma, and the intensity in the lymphomas was calculated relative to the intensity in the positive control, as shown in Table 5. All malignant lymphomas studied have very low total binding, with the highest being <14% of the binding to the GH-secreting pituitary adenoma and <20% of the binding in rat brain cortex, indicating a very low expression of the sst2 receptors in these tissues. These findings correspond to the relatively low expression levels of sst2 mRNA found by Q-PCR.

By immunohistochemistry using sst2- and sst3-specific polyclonal antibodies, an attempt was made to visualize sst2...

<table>
<thead>
<tr>
<th>Lymphoma no.</th>
<th>sst2/HPRT</th>
<th>sst3/HPRT</th>
<th>GH-secreting pituitary adenoma no.</th>
<th>sst2/HPRT</th>
<th>sst3/HPRT</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>208 †</td>
<td>1</td>
<td>1,714 1,260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>115 †</td>
<td>2</td>
<td>1,020 2,140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>65 †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>32 †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>30 †</td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>28 26</td>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>13 †</td>
<td></td>
<td></td>
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<tr>
<td>6</td>
<td>13 †</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td>8 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>6 †</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values represent arbitrary units, generated to a standard curve. Values are presented as ratio of number of copies of sst over number of copies of HPRT, both relative to a standard curve.

†Quantitative RT-PCR was not performed, as no expression of sst1 or sst3 was found by RT-PCR.

‡Expression was below detection limit of quantitative RT-PCR.
and sst$_3$ in malignant lymphoma tissue. Human pancreas served as a positive control. Sst$_2$ and sst$_3$ immunoreactivity was clearly detected in the pancreatic islets with a pattern of expression as described (6). This immunostaining could be completely abolished by preabsorption of the respective antibodies with 100 nmol/L of the peptide antigen (data not shown). However, under the conditions used, we were unable to detect immunoreactivity for sst$_2$ and sst$_3$ in the different lymphomas. To further evaluate this conclusion, membrane binding and internalization studies were performed to evaluate the amount of radiolabeled octreotide bound and internalized by the sst$_2$ on the cell membranes. For these experiments, we used B-lymphoid and myeloid cell lines as a model for cells of malignant lymphoma tissues. As a positive control, the stably sst$_2$-transfected colon adenocarcinoma cells (CC2B) were used. Q-PCR was performed to investigate the sst$_2$ mRNA expression levels in the cell lines we used. The data are summarized in Table 6, which represents the relative amount of sst$_2$ mRNA expression in different cell lines, calculated relative to a standard curve, generated from a Jurkat cell line and given in arbitrary units as the relative amount of sst mRNA per 750,000 cells isolated. A CC531 cell line was used as the untransfected negative control.

Although all cell lines expressed sst$_2$ mRNA, expression levels were relatively low compared with that of the stably sst$_2$-expressing CC2B cell line. To investigate the expression levels of the sst$_2$ protein in the different cell lines, membrane binding studies were performed. The results are shown in Figure 2. All cells investigated showed high-affinity binding sites for SS; however, the number of receptors was found to be low. To investigate the extent to which these receptors are able to internalize the radionuclide-coupled SS analog, internalization experiments were performed using [$^{125}$I-Tyr$_3$]octreotide.

As shown in Figure 3, the CC2B cells internalized significantly higher amounts of [$^{125}$I-Tyr$_3$]octreotide than the JY, TMM, and APD cell lines. No internalization could be detected in the primary B-CLL cells.

Finally, DNA obtained from lymphoma tissues was used for sequencing to detect possible mutations in the DNA sequence in the sst$_2$ coding region in malignant lymphomas. Four orbital lymphomas were studied (numbers 1, 3, 4, and 6). No mutations could be detected (data not shown).

### TABLE 5

**Autoradiography Experiments on Lymphoma Tissues**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH-producing tumor</td>
<td>100</td>
</tr>
<tr>
<td>Rat brain</td>
<td>72</td>
</tr>
<tr>
<td>Lymphoma no.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>13.8</td>
</tr>
<tr>
<td>1</td>
<td>13.7</td>
</tr>
<tr>
<td>2</td>
<td>9.5</td>
</tr>
<tr>
<td>6</td>
<td>7.7</td>
</tr>
<tr>
<td>3</td>
<td>7.1</td>
</tr>
<tr>
<td>4</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Binding percentages of [$^{125}$I-Tyr$_3$]octreotide in lymphoma tissues are measured using Phosphor Imager, in which number of pixels was quantified. Pixels represent binding of [$^{125}$I-Tyr$_3$]octreotide to tissue. Specific binding of [$^{125}$I-Tyr$_3$]octreotide to GH-producing tumor was set at 100% and amount of binding of radiolabeled compound to different lymphoma tissues is presented relative to that of GH-producing tumor.

### TABLE 6

**Quantitative Expression of sst$_2$ in Different B- and Myeloid Cell Lines**

<table>
<thead>
<tr>
<th>Cells</th>
<th>sst$_2$ per 750,000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>APD</td>
<td>11,770</td>
</tr>
<tr>
<td>JY</td>
<td>11,230</td>
</tr>
<tr>
<td>TMM</td>
<td>8,155</td>
</tr>
<tr>
<td>B-CLL</td>
<td>490</td>
</tr>
<tr>
<td>CC531</td>
<td>*</td>
</tr>
<tr>
<td>CC2B</td>
<td>72,000</td>
</tr>
</tbody>
</table>

*CC531 cell line was used as an untransfected negative control.
DISCUSSION

Previously, it had been demonstrated by sst scintigraphy that malignant lymphomas express sst (10,27). The sensitivity of sst scintigraphy for Hodgkin’s disease lies around 95%–100% (15,16). The sensitivity for non-Hodgkin’s lymphomas is around 80% (15). It was shown that uptake of $^{[111}\text{In-DTPA}]$octreotide in lymphomas was lower compared with the uptake in neuroendocrine tumors (28,29). Because lymphomas of several patients with malignant lymphoma cannot be visualized by sst scintigraphy, we addressed the question of what the cause of this nonvisualization might be. In addition, our studies addressed the question of whether patients with malignant lymphomas might be candidates for radionuclide-labeled SS analog therapy.

In this study, we investigated the expression of the different sst in a series of malignant lymphomas, using both RT-PCR and Q-PCR. By RT-PCR, in 2 Hodgkin’s and 2 non-Hodgkin’s lymphomas from the neck region, expression of $sst_2$ mRNA was detected, whereas the 6 lymphomas originating from the orbital region expressed both $sst_2$ and $sst_3$ mRNA. Until now, only a few studies have demonstrated sst in lymphoma tissues using autoradiography (10,27,30), reporting a differential sst subtype expression in malignant lymphoma tissue. In one study, expression of $sst_2$ mRNA was detected in one Hodgkin’s lymphoma and one non-Hodgkin’s lymphoma, whereas one non-Hodgkin’s lymphoma expressed both $sst_3$ and $sst_4$ mRNA (27). Another study showed that extragastric MALT-type lymphomas (where MALT = mucosa-associated lymphoid tissue) expressed $sst_2$ mRNA, whereas intragastric MALT-type lymphomas expressed $sst_3$ and $sst_4$ mRNA but not $sst_2$ mRNA (30). In our previous studies, we were unable to detect expression of $sst_4$ in the human immune system and in human malignancies.

The expression pattern of the sst subtypes was further studied by Q-PCR, and results were compared with sst expression in 2 GH-secreting pituitary adenomas expressing high levels of sst. We found relatively low levels of $sst_2$ and $sst_3$ mRNA expression in all lymphomas studied compared with that of the pituitary adenomas. Autoradiography and immunohistochemistry further supported these findings. By autoradiography, very low specific binding of $^{[125}]$-Tyr$^3$octreotide on human B and myeloid cell lines: ⋄, B-CLL (dissociation constant $[K_d] = 0.8 \text{ nmol/L, } n = 50 \text{ fmol/mg}$); ■, APD cell line ($K_d = 0.7 \text{ nmol/L, } n = 40 \text{ fmol/mg}$); ●, TMM cell line ($K_d = 0.4 \text{ nmol/L, } n = 100 \text{ fmol/mg}$); ▼, JY cell line ($K_d = 0.4 \text{ nmol/L, } n = 195 \text{ fmol/mg}$); △, CC2B cell line ($K_d = 0.8 \text{ nmol/L, } n = 460 \text{ fmol/mg}$).

FIGURE 2. Membrane binding studies. Scatchard analysis of $^{[125}]$-Tyr$^3$octreotide binding on human B and myeloid cell lines: ⋄, B-CLL (dissociation constant $[K_d] = 0.8 \text{ nmol/L, } n = 50 \text{ fmol/mg}$); ■, APD cell line ($K_d = 0.7 \text{ nmol/L, } n = 40 \text{ fmol/mg}$); ●, TMM cell line ($K_d = 0.4 \text{ nmol/L, } n = 100 \text{ fmol/mg}$); ▼, JY cell line ($K_d = 0.4 \text{ nmol/L, } n = 195 \text{ fmol/mg}$); △, CC2B cell line ($K_d = 0.8 \text{ nmol/L, } n = 460 \text{ fmol/mg}$).

FIGURE 3. Internalization experiment using CC2B, JY, TMM, APD, and B-CLL cells. Cells were incubated either with $^{[125}]$-Tyr$^3$octreotide alone or with $^{[125}]$-Tyr$^3$octreotide and excess of unlabeled octreotide (1 $\mu$mol/L) for 30, 60, and 120 min, respectively. At each time point, internalization was measured. Results are presented as amount of radiolabeled compound internalized by cell in fmol/10$^6$ cells and as degree of internalization for 10$^6$ cells as percentage of administered dose.
Tyroidoctreotide was demonstrated and by immunohistochemistry sst2 or sst3 could not be visualized. This low or absent specific binding can be explained by low receptor levels in these tissues, in agreement with the low sst mRNA levels encoding the receptor proteins found by Q-PCR. It should be taken into consideration that sst2-expressing inflammatory cells infiltrating the lymphomas may account for the sst2 expression levels detected by Q-PCR. However, we found the internalization rate to be <5%. Therefore, the contribution of potential infiltrating cells to sst2 mRNA levels we detected will be very small. Moreover, these infiltrating cells, which may express sst2, will not influence the results and interpretation of the Q-PCR, as the outcome was a very low expression of sst2 mRNA in lymphoma tissues. The variable positive results obtained by sst scintigraphy in malignant lymphomas in previous studies (15,16,29) have been attributed to small lesion sizes (11,16). However, these negative findings might also be explained by very low expression of the receptors on the cell membranes, as demonstrated in our study. To study the internalization of radionuclide-coupled SS analogs, we used several cell lines and one sample of primary B-CLL cells because fresh lymphoma cells were difficult to obtain. Scatchard analysis of these cell lines and B-CLL cells revealed that all cells used showed relatively low levels of high-affinity binding sites and very low internalization, which is in agreement with the low number of SS binding sites. The sst2-expressing CC2B cells internalized significantly higher amounts of the radiolabeled compound. An explanation for the relatively low sst levels and low internalization of radiolabeled SS analogs may be endogenous SS production as has been demonstrated in pheochromocytomas (31). Endogenous SS production may facilitate internalization of the sst2 in an autocrine fashion (31); thus, a lower uptake of radiolabeled SS analogs may be expected in SS-expressing tissues. We evaluated expression of SS mRNA in lymphoma tissues to find an alternative explanation for the low uptake of radionuclide-labeled SS analogs by these cells. No SS mRNA in any of the lymphoma tissues studied was detected, however. Recently, we demonstrated that the SS-like peptide CST is expressed in normal cells of the human immune system (17). CST might act as alternative ligand to sst, rather than SS itself, because of its high binding affinity to all 5 sst (18). Therefore, we also investigated the expression of CST mRNA in the lymphomas. We detected expression in all samples tested instead of the expression of SS mRNA. Although, until now, we could not detect the protein CST, it might be suggested on the basis of the expression of CST mRNA that CST can also facilitate internalization of the sst2 on the cell membrane and cause a lower uptake of radioactively labeled SS analogs as well. Further studies should be performed to elucidate this issue.

To exclude the possibility that mutations in the coding region of the sst2 gene might be responsible for low-efficiency internalization, DNA from 4 malignant lymphomas was sequenced. No mutations in the sst2 coding region could be detected, ruling out that the low internalization rate could be caused by a defect in the receptor. Based on our findings of low sst numbers, low binding, and internalization, lymphomas appear not to be good candidates for radiotherapy using radiolabeled SS analogs. However, recently, advances have been made in gene transfer of sst in defined cancer cells (32–34). It has been demonstrated that the induction of sst2 in both primary and metastatic pancreatic cancer models results in a significant antitumor effect characterized by an increase of apoptosis and an inhibition of cell proliferation (33). In rat colon carcinoma cell lines, induction of sst2 also led to an induced antitumor effect with peptide receptor radionuclide therapy (34). In the sst2-transfected CC2B cells, we indeed found very effective internalization of the radionuclide-coupled SS analog. This finding might be promising with respect to a possible combination of sst gene therapy and SS analog radiotherapy in, for example, lymphoma tissues. Further studies must be performed to elucidate this interesting topic as, in solid tumors, it has been demonstrated that gene therapy using either retroviral or adenoviral vectors with the p53 gene in non–small cell lung cancers resulted in tumor regression or stabilization in most patients (35).

In summary, we investigated the expression of sst, both qualitatively and quantitatively, in different malignant lymphomas, as well as the expression of the natural ligand SS, to answer the question of whether SS or its analogs might play a future role in radiotherapy treatment of these diseases. We demonstrated very low expression levels of sst mRNA and, subsequently, low or absent specific binding to these tissues in our autoradiography and immunohistochemistry experiments. Although previous studies showed expression of sst in different lymphoma tissues (10), it was also clearly demonstrated that lesions were not detected using the sst scintigraphy in all patients (15,29). We hypothesize that these negative results might be due to a very low expression of the sst in malignant lymphomas. In the literature, it was hypothesized that SS or one of its analogs might play a future role in treatment of malignant lymphomas, either as “cold” peptides (36) or as β-emitting radionuclide-coupled peptides (34). In our cell line models, we demonstrated that, despite expression of the sst2, internalization capacity is very low but could be enhanced by overexpression of sst2. These data suggest that lymphomas in vivo might not be a target for radiotherapy with SS or its analogs. However, malignant lymphomas are the human tumors that are most sensitive to radiation (37). In contrast, neuroendocrine tumors, such as carcinoids and gastrinomas, are considered to be rather radioresistant. However, successful tumor shrinkage has been reported in neuroendocrine tumors after 177Lu-DOTA-Tyr6-octreotate administration (38). On the other hand, previous studies demonstrated clearly that the internalization of radionuclide-labeled SS analogs in cell models was time dependent and, thus, increased over time (21,39). In our internalization studies, we also found an increased uptake over time. Therefore, it may
be hypothesized that the use of longer incubation periods may enhance the uptake of radionuclide-labeled SS analogs in lymphoma patients and, thus, the utility of therapy with these analogs. On the other hand, longer incubation periods with radionuclide-labeled SS analogs may cause damage to other sst-expressing organs, such as kidney, spleen, and bone marrow (40). This is a major disadvantage, because of the importance of these organs in the human organism. Therefore, caution should be taken when considering the use of longer incubation periods with radionuclide-labeled SS analogs to treat lymphoma patients. Future clinical trials will show whether, in human lymphomas, the higher radiosensitivity will be sufficient to compensate for low sst numbers and low efficiency of internalization compared with that of neuroendocrine tumors.

CONCLUSION

In this study, we demonstrated that malignant lymphomas expressed relatively low levels of sst, both at mRNA and protein levels. In cell line models, low uptake of radioactively labeled SS analogs was found. Low uptake was not due to mutations in the sst2 gene. Therefore, we conclude that the low uptake is based on low receptor numbers and, thus, malignant lymphomas may not be targets for therapy using radionuclide-labeled SS analogs, as has been suggested previously. However, it should be taken into consideration that lymphomas are highly radiosensitive tumors and relative low uptake might still be efficient in reducing the number of tumor cells. This should be further evaluated in clinical conditions.

ACKNOWLEDGMENTS

We cordially acknowledge Dr. Wout A. Breeman (Department of Nuclear Medicine, Erasmus Medical Center, Rotterdam, The Netherlands) for his contribution to this work and Dr. Rebecca Croxen (Eye Hospital Rotterdam, The Netherlands) for reviewing the English of this manuscript. This work was supported by a grant (903-43-092) from the Dutch Organization for Scientific Research.

REFERENCES


Erratum

In the article “123I-Hippuran Renal Scintigraphy with Evaluation of Single-Kidney Clearance for Predicting Renal Scarring After Acute Urinary Tract Infection: Comparison with 99mTc-DMSA Scanning,” by Imperiale et al. (*J Nucl Med.* 2003;44:1755–1760), the fifth author was listed incorrectly because of a copyediting error. The correct name is Daniela Seracini, MD. We regret the error.