Receptors for the neuropeptide somatostatin (SS) were evaluated in vitro and in vivo in various human lymphatic tissues, i.e., thymus, spleen, and lymph nodes; thymic carcinoids and thymomas were also tested. The receptors were measured in vitro using receptor autoradiography on tissue sections incubated with the SS analog [125I]-[Tyr3]-octreotide or [125I]-[Leu6,D-Trp22,Tyr28]-SS-28. All tissues were SS-receptor positive for either radioligand, except the thymomas. In thymic tissue, the receptors were diffusely located in the medulla, presumably on epithelial cells. In the spleen, the red pulp was strongly labeled. In the lymph nodes, the germinal centers were preferentially labeled. In all tissues, the receptors were of high affinity (kD thymus, 0.84 nmol/L; kD spleen, 1.6 nmol/L; kD lymph node, 0.62 nmol/L) and specific for SS. Displacement by nanomolar concentrations of SS-14, SS-28, and octreotide was observed, as was guanosine triphosphate dependency. The in vivo visualization of somatostatin receptors was performed after injection of [111In-DTPA-octreotide and γ-camera scintigraphy. The spleen, but not thymus or lymph nodes, were visualized. These data suggest an important role for SS in regulating immune functions through SS receptors in thymus, spleen, and lymph nodes. Furthermore, SS may regulate neuroendocrine functions in the thymus.

MATERIALS AND METHODS

Samples. All samples of thymic tissues (listed in Table 1) and of lymph nodes (Table 2) were collected at the University Hospital in Berne and Kantonsspital Lucerne (Switzerland). In addition, five spleens were obtained from the Erasmus University Hospital in Rotterdam. All samples were obtained during surgical intervention performed for diagnostic or therapeutic reasons, with informed consent of all patients or their parents, and in accord with the Helsinki Doctrine on Human Experimentation. With the exception of the Kantonsspital, Lucerne, Switzerland.

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increasing concentrations (10⁻⁷ to 10⁻³ mol/L) of guanosine triphosphate (GTP). The autoradiograms were quantified using a computer-assisted image-processing system previously described. Tissue standards for iodinated compounds (Amersham Laboratories, Little Chalfont, UK) were used for this purpose. A tissue was defined as SS receptor positive when the optical density measured over an area in the total binding section was at least twice the optical density of the nonspecific binding section.

Adjacent sections from selected samples of all tissues tested with ¹²⁵I-[Tyr³]-octreotide were tested for specific binding with the SS-[¹²⁵I]-[Leu⁸,D-Trp²²,Tyr²⁵]-SS-28 radioligand to know if the two ligands identify comparable cellular elements.

SS receptor scintigraphy was performed as described in detail previously. A dose of 7 to 10 μg of ¹¹¹In-DTPA-octreotide corresponding to 185 to 259 MBq of radioactivity was administered as a single intravenous (IV) injection. Planar images were obtained with a large field of view γ camera (Counter-balance 3700 and ROTA II; Siemens, Hoffman Estates, IL). A standard protocol was used to scan the thorax and abdomen region in all patients. All cases had at least one antero-posterior and one postero-anterior scan of the thorax, including the neck region, and of the abdomen 24 hours after tracer injection. The acquisition parameters for planar images were 300,000 and 500,000 preset counts for the neck/chest and abdomen images, respectively.

**RESULTS**

Thymus, spleen, and lymph nodes express SS binding sites. In all three tissues these binding sites can be characterized as being of high affinity, specific for biologically active SS analogs, and GTP dependent (Tables 1, 2, and 3).

**Normal thymus.** All samples of normal thymus contained SS binding sites (Table 1). In young children as well as in adult individuals, the SS binding sites were located in the medulla (Fig 1). Because the medulla appears diffusely and homogeneously labeled and because the present autoradiographic technique does not allow us to assign the label to a given cell, SS binding sites may be located either on vascular epithelial cells, stroma, reticulo-endothelial cells, or on lymphocytes. No significant labeling was observed in the cortex nor in Hassal’s corpuscles. Saturation experiments with increasing concentrations of ¹²⁵I-[Tyr³]-octreotide showed that the SS binding in the thymic medulla from a newborn child is saturable and of high affinity. Scatchard plots of the data showed a dissociation constant (k_d of 0.84 nmol/L and total number of sites (B_max) equivalent to 83 fmol/mg protein (Fig 2). This binding was specific because SS and bioactive SS analogs, ie, octreotide, were able to displace the radioligand in the high-affinity range, whereas the biologically inactive analog SS-28 (1-12) did not dis-

**Table 1. Incidence of SS Binding Sites in Normal and Diseased Human Thymic Tissue**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of Cases</th>
<th>Incidence of Cases With SS Binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal thymus from children</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(age: 0-6 yr)</td>
<td>4</td>
<td>4/4 (100%)</td>
</tr>
<tr>
<td>Normal thymus from adults</td>
<td>8</td>
<td>8/8 (100%)</td>
</tr>
<tr>
<td>Thymomas</td>
<td>4</td>
<td>0/4 (0%)</td>
</tr>
<tr>
<td>Thymic carcinoids</td>
<td>2</td>
<td>2/2 (100%)</td>
</tr>
</tbody>
</table>

**Table 2. Localization, Diagnosis, and In Vitro Presence of SS Binding Sites in 15 Human Lymph Node Biopsies**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Patient/ Age (yr)/Sex</th>
<th>Localization (biopsy)</th>
<th>Diagnosis at Biopsy</th>
<th>SS Binding Sites In Vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LF/43/F</td>
<td>Inguinal</td>
<td>Lymphoid hyperplasia</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>AR/25/M</td>
<td>Axillar</td>
<td>Lymphoid hyperplasia, follicular</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>WS/33/F</td>
<td>Nuchal</td>
<td>Follicular lymphoid hyperplasia</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>KU/52/M</td>
<td>Cervical</td>
<td>Follicular lymphoid hyperplasia</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>SA/35/M</td>
<td>Cervical</td>
<td>Granulomatous lymphadenitis with</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>microabscess†</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>BT/47/M</td>
<td>Axillar</td>
<td>Granulomatous lymphadenitis with</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>microabscess†</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>JC/16/M</td>
<td>Submental</td>
<td>Follicular lymphoid hyperplasia</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>FM/29/M</td>
<td>Cervical</td>
<td>Lymphoiditis, Piringer-Kuchinka type</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>KP/40/F</td>
<td>Cervical</td>
<td>Lymphoid hyperplasia, diffuse</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>ZP/42/F</td>
<td>Axillar</td>
<td>Lymphoid hyperplasia, diffuse</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>KC/19/M</td>
<td>Inguinal</td>
<td>Granulomatous lymphadenitis with</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>microabscess†</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>KC/19/M</td>
<td>Inguinal</td>
<td>Granulomatous lymphadenitis with</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>microabscess†</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>MA/40/F</td>
<td>Mediastinal</td>
<td>Anthracosis, epithelioid cell reaction</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>HA/51/M</td>
<td>Cervical</td>
<td>Lymphoid hyperplasia</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>MM/49/F</td>
<td>Axillar</td>
<td>Lymphoid hyperplasia Piringer-Kuchinka</td>
<td>+</td>
</tr>
</tbody>
</table>

* Detected with receptor autoradiography (see Materials and Methods for details).
† "Cat scratch lymphadenitis."
SOMATOSTATIN RECEPTORS IN LYMPHATIC TISSUE

Tained in child thymus were also obtained in adult thymus. The binding characteristics ob-
gained from a patient with myasthenia gravis was positive for SS-28 (1-12) was biologically inactive (Fig 3), and bioactive SS analogs such as octreotide were able to displace the radioligand in the high affinity range, whereas the biologically inactive SS-28 (1-12) was not (Fig 3). In a case of lymphatic hyperplasia, the binding calculated from Scatchard plot was 0.62 nmol/L and the estimated \( B_{\text{max}} \) was 128 fmol/mg protein. Binding was GTP-dependent (Table 3). Similar binding characteristics of lymph nodes were found in lymphatic hyperplasia and in granulomatous lymphadenitis caused by cat scratch disease.

DISCUSSION

The present study describes for the first time the presence of SS binding sites in human lymphatic tissues such as newborn and adult thymus, spleen, and lymph nodes. This observation relates to a previous study describing SS receptors in the human GALT. As in the GALT, the SS binding sites in the presently investigated tissues may be specific SS receptors because a GTP-dependency could be observed. Moreover, they appear to belong to the SS1 receptor subtype. Therefore, the SS octapeptide octreotide as well as \( [\text{Leu}^8,\text{D-Trp}^{22},\text{Ty}^{25}] \)-SS-28 radioligand. Figure 7 shows an example of a lymph node and a spleen containing binding sites labeled with \( [\text{Leu}^8,\text{D-Trp}^{22},\text{Ty}^{25}] \)-SS-28.

Interestingly, in vivo scintigraphy of more than 250 patients without evidence of lymphatic pathology did not identify lymph nodes labeled with \( [\text{Leu}^8,\text{D-Trp}^{22},\text{Ty}^{25}] \)-SS-28.

Table 3. Inhibition of Specific \( ^{125}\text{I}[-\text{Ty}^3] \)-Octreotide Binding by Various GTP Concentrations in Thymus, Spleen, and Lymph Nodes

<table>
<thead>
<tr>
<th>GTP Concentration</th>
<th>Specific binding (dpm/mg tissue; mean ± SEM; n = 3)*</th>
<th>% Inhibition</th>
<th>Specific binding (dpm/mg tissue; mean ± SEM; n = 3)*</th>
<th>% Inhibition</th>
<th>Specific binding (dpm/mg tissue; mean ± SEM; n = 3)*</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1,008 ± 60</td>
<td>—</td>
<td>2,040 ± 102</td>
<td>—</td>
<td>1,345 ± 77</td>
<td>—</td>
</tr>
<tr>
<td>10^{-1} mol/L</td>
<td>677 ± 44</td>
<td>33</td>
<td>NT</td>
<td>—</td>
<td>682 ± 48</td>
<td>49</td>
</tr>
<tr>
<td>10^{-6} mol/L</td>
<td>351 ± 27</td>
<td>66</td>
<td>1,130 ± 16</td>
<td>44</td>
<td>457 ± 31</td>
<td>66</td>
</tr>
<tr>
<td>10^{-8} mol/L</td>
<td>166 ± 12</td>
<td>84</td>
<td>829 ± 33</td>
<td>59</td>
<td>274 ± 26</td>
<td>79</td>
</tr>
<tr>
<td>Nonspecific binding</td>
<td>128 ± 19</td>
<td>362 ± 14</td>
<td>225 ± 18</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Calculated from optical density values obtained from autoradiograms in successive tissue sections incubated with the ligand \( ^{125}\text{I}[-\text{Ty}^3] \)-octreotide and a corresponding concentration of GTP (see Materials and Methods for details).

In vivo scintigraphy with \( ^{111}\text{In} \)-[DTPA]-octreotide performed in more than 800 patients suffering from various types of mostly neuroendocrine tumors never did identify hot spots in the thymus region. However, one patient with a thymic carcinoid was scanned in vivo and showed accumulation of the radioligand in the primary tumor and its mediastinal metastases.

Thymic neoplasms. Table 1 shows the results of the evaluation of SS binding sites in six human thymic neoplasms. Whereas the four thymomas were negative, the two thymic carcinoids were positive.

In vivo scintigraphy with \( ^{111}\text{In} \)-[DTPA]-octreotide was positive and of high affinity because both SS and bioactive SS analogs such as octreotide were able to displace the radioligand in the nanomolar range, whereas the biologically inactive SS-28 (1-12) was not (Fig 3). In a case of lymphatic hyperplasia, the binding calculated from Scatchard plot was 0.62 nmol/L and the estimated \( B_{\text{max}} \) was 128 fmol/mg protein. Binding was GTP-dependent (Table 3). Similar binding characteristics of lymph nodes were found in lymphatic hyperplasia and in granulomatous lymphadenitis caused by cat scratch disease.

The lymph nodes, thymi, and spleens labeled in vitro with \( ^{125}\text{I}[-\text{Ty}^3] \)-octreotide could also be labeled with \( [\text{Leu}^8,\text{D-Trp}^{22},\text{Ty}^{25}] \)-SS-28 radioligand. Figure 7 shows an example of a lymph node and a spleen containing binding sites labeled with \( [\text{Leu}^8,\text{D-Trp}^{22},\text{Ty}^{25}] \)-SS-28.

Interestingly, in vivo scintigraphy of more than 250 patients without evidence of lymphatic pathology did not identify lymph nodes labeled with \( ^{111}\text{In} \)-[DTPA]-octreotide.

**DISCUSSION**

The present study describes for the first time the presence of SS binding sites in human lymphatic tissues such as newborn and adult thymus, spleen, and lymph nodes. This observation relates to a previous study describing SS receptors in the human GALT. As in the GALT, the SS binding sites in the presently investigated tissues may be specific SS receptors because a GTP-dependency could be observed. Moreover, they appear to belong to the SS1 receptor subtype. Therefore, the SS octapeptide octreotide as well as SS-14 or SS-28 show high-affinity binding to the receptor. Recently, the genes encoding three human SS-receptor subtypes have been cloned and sequenced. One of these, the \( \text{SSTR2} \) type, shows high affinity not only for SS-14 but also for small synthetic SS analogs such as MK 678 or octreotide. Recent data suggest that it is identical to the pharmacologically characterized SS1 receptor subtype. Therefore, lymphatic tissues investigated in the present report probably express the \( \text{SSTR2} \) subtype; however, we cannot completely
Fig 1. SS binding sites in the human thymus from children (A through C) and adults (D through F). (A, D) Hematoxylin-eosin stained sections. Bar = 1 mm. (B, E) Autoradiograms showing total binding of $^{125}$I-[Tyr$^3$]-octreotide. (C, F) Autoradiograms showing nonspecific binding of $^{125}$I-[Tyr$^3$]-octreotide (in presence of $10^{-6}$ mol/L [Tyr$^3$]-octreotide). All sections were incubated with $0.30 \times 10^6$ dpm/mL of tracer. Twelve slices were studied. In both tissues the SS binding sites are located in the medulla.

exclude the possibility that a further subtype, different from SSTR1 and SSTR3, is also expressed.

**Thymus.** The human thymic medulla of both newborn and adult contains high-affinity SS receptors. It is presently not possible to assign these receptors to a defined cell type in the medulla. The very weak staining of the cortex suggests that the cells located predominantly in this area, i.e., lymphatic cells, have no SS receptors. Thus, in the medulla, strong candidates for the expression of SS receptors could be the epithelial cells, densely packed in this region. Some of
these epithelial cells can be labeled with A2B5, a monoclonal antibody (MoAb) directed against a complex neuronal ganglioside found on the cell surface of neurons and neuroendocrine cells. Interestingly, some of the medullary epithelial cells, including A2B5-positive cells, were shown to contain various thymic hormones, strongly suggesting that these cells have neuroendocrine characteristics. Because most normal neuroendocrine tissues, as well as the thymic carcinoids (which originate from neuroendocrine cells), are known to express SS, it is conceivable that these medullary "neuro-endocrine-like" epithelial cells also bear SS receptors. However, because of the diffuse and homogeneous labeling pattern, it is possible that the other nonneuroendocrine types of epithelial cells were also labeled, and it cannot be completely excluded that some mature medullary thymocytes or isolated B lymphocytes in the medulla do contain SS receptors.

In addition to being the primary lymphatic organ generating effectors of cell-mediated immunity, the thymus can be considered a major endocrine gland responsible for the secretion by its epithelial cells of a heterogeneous family of polypeptide hormones such as thymulin, thymosins, and thymopoietin. These peptides not only exert important

Fig 2. Scatchard plot from a saturation experiment using increasing concentrations of 125I-[Tyr3]-octreotide in successive sections of a newborn thymus using receptor autoradiography techniques. \( k_d = 6.94 \text{ nmol/L; number of sites (Bmax): 83 fmol/mg protein.} \)

Fig 3. Effect of peptides on SS binding in human lymphatic tissues. (A) Newborn thymus. Displacement curve of 125I-[Tyr3]-octreotide in tissue sections incubated with 30,000 cpm/100 \( \mu \)L radioligand and increasing concentrations of octreotide (○), SS-14 (△), or 100 nmol/L of the biologically inactive SS analog SS-28 (1–12) (□). Each point represents the mean of triplicate determinations of the optical density. (B) Displacement curve of 125I-[Tyr3]-octreotide in a human spleen using increasing concentrations of [Tyr3]-octreotide (○), SS-28 (△), or 100 nmol/L LHRH (□). The data are averages from triplicate determinations in a single experiment and are representative of three separate experiments. (C) Displacement curve of 125I-[Tyr3]-octreotide in a human lymph node using increasing concentrations of octreotide (○) or SS-14 (△) or 100 nmol/L of SS-28 (1–12) (□). The data are averages from triplicate determinations in a single experiment and are representative of three separate experiments.
regulatory effects within the immune system, such as induction of proliferation and differentiation of T lymphocytes, but also regulate the neuroendocrine system and are themselves subject to control by various hormones (prolactin, growth hormone, thyroid hormones, adrenocorticotropic [ACTH], or opioids) through specific receptors located in the thymus. Thus, these peptides represent an important interface between the immune and neuroendocrine systems.

The functional role of SS and SS receptors should probably be considered under the same point of view, despite the lack of experimental data. In epithelial cells, SS may regulate the secretion of thymic hormones, in a way similar to its well-known inhibition of hormone release in virtually all neuroendocrine cells in the body. Moreover, and in analogy to the described action of SS in other lymphatic tissue, we may speculate that an SS effect relates to lymphopoiesis, perhaps by regulating the proliferation and specific functions of lymphocytes. Endogenous SS should be available locally for that purpose because it was recently shown that the thymic medulla of the rat contains both SS mRNA and immunohistochemically detectable SS.

Thymic neoplasms. Many well-differentiated neoplasms arising from SS receptor-positive tissues contain SS receptors. This principle does not seem to be valid for the four thymomas studied in this report. However, the two thymic carcinoids were SS receptor positive. This is not surprising, because these neoplasms are thought to arise from neuroendocrine cells located in the thymus, and is in keeping with our previous observation that most of the carcinoids, independently of their location, usually express SS receptors. Thymic carcinoids, as their gastrointestinal or bronchial counterparts, may therefore be detected by in

Fig 4. SS binding sites identified with autoradiography in a human spleen. (A) Hematoxylin-eosin–stained section. Bar = 1 mm. (B) Autoradiogram showing total binding of $^{125}$I-[$\text{Tyr}^3$]-octreotide. The red pulp is preferentially labeled. (C) Autoradiogram showing nonspecific binding of $^{125}$I-[$\text{Tyr}^3$]-octreotide (in presence of $10^{-8}$ mol/L [$\text{Tyr}^3$]-octreotide). This example is representative for all five spleens tested, where at least 12 slices per case were studied.

Fig 5. This 62-year-old man had a carcinoid syndrome caused by a metastasizing carcinoid (confirmed by pathology). On the left, $^{111}$In-DTPA–octreotide scanning 20 hours after the IV administration of the radionuclide. Pictures taken in postero-anterior direction of the left side of the body. 1 = spleen; 2 = left kidney. On the right, the results of the procedure repeated after 7 months of octreotide therapy (300 µg/d). The scan was performed without interruption of octreotide administration. Note that the spleen is hardly visible anymore, whereas the appearance of the left kidney has not markedly changed since the start of octreotide therapy. $^{111}$In-DTPA–octreotide is normally excreted through the kidney.
Fig 6. SS binding sites in a human lymph node with follicular lymphoid hyperplasia. (A) Hematoxylin-eosin–stained section showing germinal centers in the lymph node. Bar = 1 mm. (B) Autoradiogram showing total binding of \( ^{125}\)I-Tyr(3)-octreotide. The germinal centers are preferentially labeled. (C) Nonspecific binding.

In the human, most lymphatic tissues express SS receptors. The function of these receptors is not yet established. In analogy to data obtained in animals,\(^{11,17}\) it is tempting to postulate that SS and SS analogs play a significant role in the regulation of the human immune system. However, despite the worldwide use of SS analogs such as octreotide for the treatment of several diseases, the exact role of these receptors in the human remains to be clarified.

CONCLUSIONS

In the human, most lymphatic tissues express SS receptors. The function of these receptors is not yet established. In analogy to data obtained in animals,\(^{11,17}\) it is tempting to postulate that SS and SS analogs play a significant role in the regulation of the human immune system. However, despite the worldwide use of SS analogs such as octreotide for the treatment of several diseases, the exact role of these receptors in the human remains to be clarified.
symptomatic therapy of certain cancers in humans, very few reports on immunologic side effects have appeared. However, preliminary data from one group of investigators support the existence of immunologic reactions to SS; clearly, the present results could stimulate more clinical studies in this direction.

Another conclusion of the present report relates to radiotherapy. The finding of a high number of SS receptors in various tumors recently generated the idea of targeting β-emitting isotope-linked SS ligands to "burn out" selectively such tumors. However, according to our present in vitro data, normal tissues bearing SS receptors, particularly "central" and "peripheral" lymphatic tissues, are likely to be affected by such an approach. Potential functional consequences should be carefully investigated.

ACKNOWLEDGMENT

We thank Dr D. Römer (Sandoz Basel) for the gift of [Tyr³]-octreotide and octreotide.

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In vitro autoradiographic and in vivo scintigraphic localization of somatostatin receptors in human lymphatic tissue

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